Application Checklist

Instructions:

1. **Please check each box below, as appropriate; and**
2. **The completed checklist *must* be submitted as the first page of the CON application.**

☑ **Attached is the CON application filing fee in the form of a certified, cashier or business check made out to the "Treasurer State of Connecticut" in the amount of $500.**

For OHCA Use Only:

Docket No.: 14-31965-CON **Check No.:** 3796
OHCA Verified by: SO **Date:** 11/18/14

☑ **Attached is evidence demonstrating that public notice has been published in a suitable newspaper that relates to the location of the proposal, 3 days in a row, at least 20 days prior to the submission of the CON application to OHCA. (*OHCA requests that the Applicant fax a courtesy copy to OHCA (860) 418-7053, at the time of the publication)***

☑ **Attached is a paginated hard copy of the CON application including a completed affidavit, signed and notarized by the appropriate individuals.**

☑ **Attached are completed Financial Attachments I and II.**

☑ **Submission includes one (1) original and four (4) hard copies with each set placed in 3-ring binders.**

Note: A CON application may be filed with OHCA electronically through email, if the total number of pages submitted is 50 pages or less. In this case, the CON Application must be emailed to ohca@ct.gov.

Important: For CON applications(less than 50 pages) filed electronically through email, the signed affidavit and the check in the amount of $500 must be delivered to OHCA in hardcopy.

☑ **The following have been submitted on a CD**

1. **A scanned copy of each submission in its entirety, including all attachments in Adobe (.pdf) format.**
2. **An electronic copy of the documents in MS Word and MS Excel as appropriate.**

AFFIDAVIT

Applicant: Molecular NeuroImaging, LLC

Project Title: Purchasing a PET CT Scan Camera

I, John Seibyl, M.D., General Partner
(Individual's Name) (Position Title – CEO or CFO)

of Molecular NeuroImaging being duly sworn, depose and state that
(Hospital or Facility Name)

of Molecular NeuroImaging's information submitted in this Certificate of
(Hospital or Facility Name)

Need Application is accurate and correct to the best of my knowledge.

J P Seibyl
Signature

11 Nov 2014
Date

Subscribed and sworn to before me on 11 NOV 2014

Notary Public/Commissioner of Superior Court

My commission expires: 30 APR 2014

I, Kenneth Marek, M.D., General Partner
(Individual's Name) (Position Title – CEO or CFO)

of Molecular NeuroImaging being duly sworn, depose and state that
(Hospital or Facility Name)

of Molecular NeuroImaging's information submitted in this Certificate of
(Hospital or Facility Name)

Need Application is accurate and correct to the best of my knowledge.

Signature Date NOV 11, 2014

Subscribed and sworn to before me on 11 NOV 2014

Notary Public/Commissioner of Superior Court

My commission expires: 30 APR 2014

State of Connecticut
Office of Health Care Access
Certificate of Need Application

Instructions: Please complete all sections of the Certificate of Need ("CON") application. If any section or question is not relevant to your project, a response of "Not Applicable" may be deemed an acceptable answer. If there is more than one applicant, identify the name and all contact information for each applicant. OHCA will assign a Docket Number to the CON application once the application is received by OHCA.

Docket Number:

Applicant: Molecular NeuroImaging, LLC

Applicant's Facility ID*:	**Not Applicable**
Contact Person:	Kimberly Fabrizio
Contact Person's Title:	Sr. Director of Regulatory Affairs and Quality Assurance
Contact Person's Address:	60 Temple Street, New Haven, CT 06510
Contact Person's Phone Number:	203-401-4313
Contact Person's Fax Number:	203-508-1503
Contact Person's Email Address:	kfabrizio@mnimaging.com
Project Town:	New Haven
Project Name:	Purchasing a PET CT Scan Camera
Statute Reference:	Section 19a-638, C.G.S.
Estimated Total Capital Expenditure:	$600,000.00 - $800,000.00

*Please provide either the Medicare, Connecticut Department of Social Services (DSS), or National Provider Identifier (NPI) facility identifier.

1. **Project Description: Acquisition of Equipment**

   a. Please provide a narrative detailing the proposal.

Molecular Neuroimaging, LLC (MNI) is proposing to acquire a PET/CT camera both to meet the increasing demands for PET imaging in our research-dedicated facility and to ensure that we continue to utilize state of the art PET imaging technology to conduct our research. PET imaging technology is rapidly developing and as a leader in PET imaging research it is crucial for MNI to continue to improve its research dedicated equipment.

Molecular NeuroImaging, LLC (MNI) is a neuroimaging services company specializing in the efficient application of scintigraphic biomarkers in drug development and research for neurodegenerative and neuropsychiatric disorders. PET imaging is an essential component of the human research conducted at MNI focused on developing new therapies for unmet medical needs in neurodegenerative conditions such as Alzheimer disease, Parkinson disease and Huntington disease. PET imaging is increasingly supported by NIH to enable research to better understand the causes of neurodegenerative diseases and by the FDA to conduct research required to test new drugs for treatment of neurodegenerative diseases. This creates a growing demand for PET imaging in the research community. PET imaging has become a key component of therapeutic studies and MNI has the opportunity to meet these research needs and accelerate vital ongoing research of these largely untreatable conditions.

MNI is exclusively a research company and does not participate in any health care reimbursement programs and is not a Point of Service provider of any medical services.
MNI is based in New Haven, CT with over 30,000 sq ft. of dedicated research space. MNI's imaging division has extensive experience and proficiency in conducting PET imaging research. MNI is a privately-held, limited liability company incorporated in the state of Connecticut which now boasts over 95 employees in New Haven, CT. MNI was founded in 2001 with 22 staff occupying 12,092 sq ft of space in New Haven. Over the past 14 years the company has expanded to a 32,426 sq ft footprint. MNI has completed 51 research studies involving PET image acquisition and estimates the addition of a second, technologically more sophisticated and efficient camera will significantly enhance the research by expanding the number of PET slots available to the CT research community.

MNI staff has leadership roles in numerous research PET imaging trials, research consortia and international research organizations focused on neuroscience and nuclear medicine research with the overarching goal of advancing knowledge of the etiology of neurodegenerative and neuropsychiatric diseases and developing more effective treatments for these conditions. MNI offers a fusion of internationally-recognized expertise in neuroscience and imaging research. MNI efficiently develops and executes PET and SPECT imaging studies to support early drug development and investigational trials, emphasizing imaging outcome measures for evaluating disease progression. During the past fourteen years MNI has greatly expanded in size and reputation. MNI has developed a number of strategic alliances, corporate partnerships and research contracts, which

include the top 30 global pharmaceutical companies as well as many small and mid-size biotech companies throughout the US and the world.

MNI was founded in 2000 by Drs. Marek and Seibyl and with an initial 22 employees created a unique research company that combines knowledge in neurology and neuropsychiatry with state-of-art imaging using custom radiopharmaceuticals for investigating neurodegenerative and neuropsychiatric disorders. In addition to founding MNI, Drs. Seibyl and Marek established a not-for-profit entity, the Institute for Neurodegenerative Disorders (IND), to serve as a sister organization to allow the advancement of grant funded research.

Our current PET camera is a Siemens HR+ and dates from 2001. Given its age, this camera requires a significant amount of continued maintenance. By investing in a more state-of-the-art camera, MNI will increase the number of research scans we can conduct add to our current technology, and create a business continuity scenario in the event the existing camera requires maintenance.

With the advancement of technology, recent PET cameras utilize a combination of computed tomography (CT) with PET to acquire the necessary transmission scan required for quantitation of radiopharmaceutical uptake. These newer generation PET/CT cameras represent the state-of-the-art in positron emission imaging, affording several advantages over the previous generation of PET scanners: a) the CT-based transmission scan can be obtained in seconds instead of minutes, so that the time on the imaging bed is minimized for research subject comfort; particularly for whole body studies, where 8-10 bed positions are required, the total imaging time can be reduced by 45-60 minutes, b) the research subject's x-ray based transmission scan (in addition to being used for attenuation correction) provides an improved image of the human anatomy, which plays a key role in analyzing the PET image, c) the CT scan allows for several advanced image processing methods to be applied to the PET images, most notably, the ability to use the CT image for a highly accurate registration to the research subject's available MRI scan. The registered MRI image affords ultra-high resolution images of the research subject's anatomy. Particularly for brain studies, the MRI can contribute to the important role of delineating gray versus white matter in the PET functional scans. The addition of this second, state-of-the-art PET/CT camera will make the scan acquisition process much more efficient from both a research subject and business perspective. Research imaging has become a critical tool to advance medical knowledge of devastating diseases such as Alzheimer and Parkinson disease. With the addition of a PET/CT camera at its Connecticut based facility, MNI will have the capacity to increase the number and quality of the scans acquired to the advancement of medical research. MNI expects to continue its growth in the state of Connecticut with the addition of the new camera, which in turn should yield approximately 11 new jobs at MNI within the first two years of its implementation.

b. Provide letters that have been received in support of the proposal.
Please see the enclosed Letters of Support from the following person(s)/ organizations:

The following Letters of Support have been attached to this application.

Toni N. Harp –	**Mayor of New Haven**
Samuel Markind, MD –	**Associated Neurologists, P.C.**
Alan P. Siegal, MD –	**Geriatric and Adult Psychiatry, G.A.P Clinical Care & Research**
Danna Jennings, MD –	**Institute for Neurodegenerative Disorders**

c. Provide the Manufacturer, Model, Number of slices/tesla strength of the proposed scanner (as appropriate to each piece of equipment).

The proposed PET/CT camera will be a camera manufactured by Siemens with a model of Biograph 6. The camera is a 6 slice CT and 64 slice PET instrument.

d. List each of the Applicant's sites and the imaging modalities and other services currently offered by location.

MNI is located at a single location in New Haven, CT as a research company dedicated to advancing science in the neuroscience and imaging arena. Currently MNI has one PET camera and one SPECT camera onsite at 60 Temple Street, New Haven, CT. MNI does not offer any point of service care or any medical services to patients.

As a research-dedicated company, MNI provides PET services across clinical spectrum including radioligand development, radioligand production, design and implementation of Phase I to Phase IV clinical imaging trials, and customized clinical imaging center coordination and management in multi-center imaging studies. MNI has two related business areas:

1) Translational Research - development and application of radioligands for both early drug development and/or to investigate disease pathophysiology. MNI's translation research in PET imaging identifies the most valuable radioligands that can then be utilized in large clinical studies to support large worldwide studies by MNI's imaging core lab Note that the proposed PET/CT camera will be used to support MNI's growing Translational research work. This translational work is conducted in Connecticut.

2) Clinical multi-center imaging Core Lab services to apply radioligands in multi-center studies for subject eligibility and/or disease monitoring. MNI core lab services provide a comprehensive, coordinated, clinical research strategy for the application of imaging tracers in multi-center clinical trials throughout the world. MNI core lab services provide imaging oversight to the largest Alzheimer and Parkinson's disease PET and SPECT imaging trials conducted. These studies utilize the expertise we have developed in New Haven to work with more than 400 sites worldwide to collect PET images in large muti-

center studies. These research imaging sites acquire PET images locally and transfer imaging data to MNI for quality control and analysis.

2. Clear Public Need

Explain why there is a clear public need for the proposed equipment. Provide evidence that demonstrates this need.

MNI proposes that the purchase of a technologically advanced PET imaging camera will improve the quality and scope of our research and further meet the increasing demands for investigational PET imaging studies being performed here in Connecticut as a critical part of the evaluation of new therapies for neurodegenerative disorders. MNI seeks to continue to provide PET imaging services for local research investigators in addition to advancing PET analysis techniques for worldwide studies. As a leader in managing multi-center PET research, MNI must maintain state- of the-art technology locally to develop imaging protocols and analyses ultimately utilized in these larger studies.

MNI's PET imaging research directly contribute to the development of new therapies for Alzheimer disease, Parkinson disease, Huntington disease and other neurologic and psychiatric disorders. Developing more effective therapies for neurodegenerative diseases including Alzheimer disease, Parkinson disease, Huntington disease and many others is also a focus of numerous researchers participating in clinical studies in the state of Connecticut. MNI works with many neurologists and geriatric psychiatrists across the state to provide imaging services required by these research studies. It is essential for these investigators to have access to technically advanced PET imaging to conduct this research. NIH and pharmaceutical research sponsors will require state-of-the-art brain PET imaging for their numerous planned clinical studies and local researchers will need to demonstrate availability of PET imaging to be considered as clinical sites for these studies.

The PET imaging research conducted at MNI directly leads to the use of PET imaging radioligands in large clinical studies in our core lab imaging studies. The development of novel therapeutics for Alzheimer disease and other neurodegenerative disease relies on large multi-center studies aimed to evaluate the efficacy of these drugs. The availability of PET imaging biomarkers is crucial in these large clinical studies to ensure an accurate diagnosis of study participants in addition to its use in monitoring disease progression. As a worldwide leader in PET imaging research MNI must update its on-site PET imaging camera capability to include currently available state-of-the-art technology.

a. Complete **Table 1** for each piece of equipment of the type proposed currently operated by the Applicant at each of the Applicant's sites.

MNI does not currently operate any PET/CT scanners; however we have included information regarding our existing PET camera (Siemens HR+) and SPECT camera (Pickler International, Model: 3000XP, Serial: 289).

TABLE 1
EXISTING EQUIPMENT OPERATED BY THE APPLICANT

Provider Name/Address	Service	Days/Hours of Operation	Utilization
MNI/ 60 Temple Street, New Haven, CT	PET (64 slices)	M-F; 8:30-5:30	Research/ 366 scans from 9/2013 to 9/2014
MNI/ 60 Temple Street, New Haven, CT	SPECT(Image matrix (64x64, 128x128 or 256x256)	M-F; 8:30-5:30	Research/ 191 scans from 9/2013 to 9/2014

b. Provide the following regarding the proposal's location:

i. The rationale for locating the proposed equipment at the proposed site;

The location for the PET/CT camera proposed will be at the MNI facility in New Haven, CT. MNI has a specialized chemistry laboratory to produce radioligands used for the PET imaging studies also located at 60 Temple Street in New Haven, CT. Given that these radioligands must be administered and the subject imaged a very short time after production because of the rapid radioactive decay of the radioligand, it is crucial to have the PET camera in close proximity to the radioligand production site. In addition, the MNI facility is centrally located in the City of New Haven, with easy access off Interstates 91 and 95 making it convenient for participants to visit throughout the state.

ii. The population to be served, including specific evidence such as incidence, prevalence, or other demographic data that demonstrates need;

The current and target population to complete PET imaging at MNI are study participants involved in research trials of new therapies for neurodegenerative disorders. Depending on the protocol and the research question that is being addressed, participants would include healthy volunteers and individuals either at risk for, or carrying a diagnosis of Alzheimer disease, Parkinson disease, Huntington disease, multiple sclerosis or other neurodegenerative disorders. Alzheimer disease and Parkinson disease are the two most common and rapidly expanding neurodegenerative disorders with and estimated 4 million people in the US being affected by AD and 1 million people affected by Parkinson disease. It is estimated that over 50 million people will have some form of dementing illness by 2020 worldwide.

All research participants recruited for the studies conducted at MNI will provide written informed consent in accordance with clear guidelines issued by the Department for Health and Human Services (http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html#46.116). In most cases participants are compensated for their time as volunteers of the research.

As a research-dedicated imaging center, all individuals undergoing PET imaging at our facility do so as a participant in a research study. Under no circumstances does MNI or its

physicians provide fee for service imaging or participate in any health care reimbursement programs or clinical care to the population being served.

iii. How and where the proposed patient population is currently being served;

The proposed subject populations are individuals with neurologic disorders (including but not limited to Alzheimer disease, Parkinson disease, Huntington disease) and healthy control volunteers who are enrolled in research studies at 60 Temple Street, New Haven CT 06510. The PET/CT camera would advance the technology at MNI to the level that is currently accepted to serve as an imaging center for actively recruiting research studies.

Currently MNI has a PET camera (Siemens HR+) that has limited efficiencies. MNI is often approached by it sponsors to perform multiple PET studies. Due to the logistics involved with use of radiopharmaceuticals MNI must often delay the initiation of new studies until existing studies are completed. With the addition of a PET/CT camera, MNI will have advanced its technology capabilities and it will have the capability for performing more studies simultaneously, helping meet the growing demand for PET imaging as research tool.

In our experience, researchers involved in studying these populations typically wait for access to our current PET camera or in some cases the sponsor may take its research business to a facility outside of Connecticut.

Patients are not served using the imaging cameras at MNI; the proposed camera is for research only.

iv. Identify the name and location (name, facility ID, address, service, hours of operation) of existing providers in the service area and within close proximity, provide the utilization of these services for the most recently completed year;

TABLE 2
EXISTING SERVICE PROVIDERS

Note: The following list of PET imaging providers was pulled from a search on the CMS website and includes facilities that offer fee for service PET imaging to serve the patient population seeking care. It should be noted that the use of the PET/CT camera at MNI will be limited entirely to research purposes and therefore is not directly comparable. The PET/CT camera at MNI will be used exclusively for research purposes and will not receive reimbursements for its use from CMS.

Facility Name	Facility ID*	Facility Address	Services	Utilization*	Days/Hours of Operation
Advanced Radiology Consultants	C02747	Trumbull CT 06611	PET Imaging	Medical	UNK
Bridgeport Hospital	70010	267 Grant Street, Bridgeport CT 06610	PET Imaging	Medical	UNK
Bristol Hospital	70029	41 Brewster Road, Bristol CT 06011	PET Imaging	Medical	UNK
Connecticut Oncology & Hematology	C00633	220 Kennedy Drive , Torrington CT 06790	PET Imaging	Medical	UNK
Danbury Hospital	70033	111 Osborne Street, Danbury, CT 06810	PET Imaging	Medical, Research	UNK
Greenwich Hospital	70018	5 Perryridge Road, Greenwich CT 06830	PET Imaging	Medical	UNK
Harold Leever Regional Cancer	470000025	1075 Chase Parkway, Waterbury CT 06708	PET Imaging	Medical	UNK
Imaging Center of Hartford Hospital	70025	80 Seymour Street, PO Box 5037, Hartford, CN 06102	PET Imaging	Medical, Research	UNK
Lawrence & Memorial Hospital	70007	365 Motauk Avenue, New London, CT 06320	PET Imaging	Medical	UNK
Robert D. Russo, & Associates Radiology, PC	C02013	PO Box 6128, Bridgeport CT 06606	PET Imaging	Medical	UNK
St. Vincent's Medical Center	70028	2800 Main Street, Bridgeport CT 06606	PET Imaging	Medical	UNK
The Hospital of Central Connecticut	70035	100 Grand Street, New Britain CT 06050	PET Imaging	Medical, Research	UNK
The Stamford Health System	70006	Shelbourn Road & West Broad Street, Stamford CT 06904	PET Imaging	Medical	UNK
University of Connecticut Health Center	300001399	263 Farmington Avenue, Farmington CT 06030	PET Imaging	Medical, Research	UNK
Yale-New Haven Hospital	1336139500	20 York Street, New Haven CT 06504	PET Imaging	Medical, Research	UNK
YNHH- St. Raphael Campus	70022	1450 Chapel Street, New Haven, CT 05611	PET Imaging	Medical, Research	UNK
Yale University	NA	New Haven, CT 05611	PET/PET CT	Research	UNK
GE Discovery PET/CT scanner	NA	New Haven, CT 05611	PET/ PET CT	Research	UNK

** If research was added under utilization, the facility was listed as a research site on a PET Imaging clinical study on clinicaltrials.gov.*

v. The effect of the proposal on existing providers; and

Area providers of commercial clinical PET imaging would in no way be affected by the addition of a research-dedicated PET/CT camera at MNI. Given the marked increase in volume for PET imaging research as it relates to research trials, there is an increased need for research-dedicated PET camera availability.

vi. If the proposal involves a new site of service, identify the service area towns and the basis for their selection.

Not Applicable (N/A) - The proposal does not involve a new site of service.

c. Explain why the proposal will not result in an unnecessary duplication of existing or approved health care services.

The proposed PET/CT camera will be used for research imaging rather than for health care services. Given that camera will be a research-dedicated device, it will not result in any unnecessary duplication of existing or approved health care services.

3. Actual and Projected Volume

a. Complete the following tables for the past three fiscal years ("FY"), current fiscal year ("CFY"), and first three projected FYs of the proposal, for each of the Applicant's existing and proposed pieces of equipment (of the type proposed, at the proposed location only). In Table 4a, report the units of service by piece of equipment, and in Table 4b, report the units of service by type of exam (e.g. if specializing in orthopedic, neurosurgery, or if there are scans that can be performed on the proposed scanner that the Applicant is unable to perform on its existing scanners).

TABLE 4A
HISTORICAL, CURRENT, AND PROJECTED VOLUME, BY EQUIPMENT UNIT

Equipment	Actual Volume (Last 3 Completed FYs)			CFY Volume*	Projected Volume (First 3 Full Operational FYs)***			
	FY 2011	FY 2012	FY 2013	FY 2014	FY 2015**	FY 2016	FY 2017	FY 2018
SPECT	**256**	**166**	**187**	**165**	170	170	170	170
PET	**109**	**150**	**210**	**270**	280	285	290	290
PET CT	**0**	**0**	**0**	**0**	80	192	225	250
Total PET	**109**	**150**	**210**	**270**	**360**	**477**	**515**	**540**

* Annualized for 2014 based on actual scans January through August 2014. Full year estimate based on a straight line extrapolation for the full year 2014 estimate.
**First year is a partial year for May – December period
*** Period covered by estimate is for MNI annual FY, which is a calendar year.

TABLE 4B
HISTORICAL, CURRENT, AND PROJECTED VOLUME, BY TYPE OF SCAN/EXAM

Service	Actual Volume (Last 3 Completed FYs)			CFY Volume*	Projected Volume (First 3 Full Operational FYs)***			
	FY 2011	FY 2012	FY 2013	FY 2014	FY 2015**	FY 2016	FY 2017	FY 2018
PET Dosimetry	0	3	10	8	10	12	12	12
PET Dynamic	13	40	60	70	80	80	85	85
PET Dynamic and Blood Draw	19	31	76	85	85	85	87	87
PET No Dynamic	77	74	64	107	105	108	106	106
Total PET	109	148	210	270	280	285	290	290
PET CT	0	0	0	0	80	192	225	250
Total	109	148	210	270	360	477	515	540

*Annualized for 2014 based on actual scans January through August 2014 and a straight line extrapolation for the full 2014 estimate.
** First year of estimate is a partial year for May to December period.
***Period covered by estimate is for MNI annual FY, which is a calendar year.

b. Provide a detailed explanation of all assumptions used in the derivation/ calculation of the projected volume by scanner and scan type.

Pertaining to the proposed PET/ CT camera, 2015 will represent a partial year. After receiving the CON application approval, MNI will proceed with the camera purchase, installation and acceptance testing. Assuming CON approval is received by March 2015, MNI estimates the following timetable:

a. Purchase, delivery, installation/setup of camera – March 2015
b. Validation (acceptance) testing completed – April 2015
c. Begin research imaging – May 2015. During the remainder of 2015, we would be contracting for new studies and begin acquiring scans on the camera; with an average of approximately 12 scans per month (Nov and Dec are both short months).

MNI will see growth in 2016 and future years as a result of an increase in the number of PET scans performed in the context of neurodegenerative and neuropsychiatric research efforts, given that the addition of a PET/CT would allow us to take on additional studies. The growth expected as a result of the new PET/CT camera should be similar to that realized utilizing our current camera -- around 35%-50% per year cumulatively. Utilizing the new PET/CT camera, we anticipate a growth of about 38% over the first full year of 2016.

c. Explain any increases and/or decreases in volume seen in the tables above.

MNI has seen a steady increase in the number of research imaging studies conducted in our New Haven facility during the past three years, which is reflective in the continued increase in number of scans performed. There was a decrease in the number of PET dosimetry scans completed in 2013 from 2012, which is primarily due to use of radioligands in our

more current studies that were further advanced in development and did require dosimetry assessments to understand the biodistribution of the radioligands being studied.

d. Provide a breakdown, by town, of the volumes provided in Table 4a for the most recently completed FY.

TABLE 5
Utilization by Town

Town	Equipment*	Utilization FY 2014 (1/2014 – 8/2014)
New Haven (research only)	PET (64 slices)	182 scans
New Haven (research only)	SPECT (Image matrix (64x64, 128x128 or 256x256)	118 scans

e. Describe existing referral patterns in the area to be served by the proposal.

Referrals to MNI occur entirely in the context of research studies. Investigators throughout CT refer research participants to complete their PET imaging studies at MNI. Research-dedicated PET imaging resources in the state are limited and referrals to our center are made based entirely to conduct PET imaging research.

f. Explain how the existing referral patterns will be affected by the proposal.

We would not anticipate a change in referral patterns, though we would expect an overall increase in the number of imaging studies in the areas of neurodegenerative and neuropsychiatric disorders and therefore an overall increase in demand for brain PET imaging for these research studies.

g. Provide a copy of any articles, studies, or reports that support the need to acquire the proposed scanner, along with a brief explanation regarding the relevance of the selected articles.

MNI has conducted research independently at its New Haven facility, as well as in collaboration with pharmaceutical companies and research organizations, such as the Michael J Fox Foundation, National Institute of Health (NIH), Department of Defense. During the past twelve years the mission of MNI is to provide imaging research data to support medical advances in debilitating diseases like Parkinson disease , Alzheimer disease, Huntington disease and other neurologic and psychiatric disorders including, Schizophrenia, Depression, Multiple Sclerosis, and Fragile X syndrome. During the past three years alone, MNI has been able to conduct over 746 PET scans that have contributed to research developing new therapies for these disorders. The addition of a PET/CT camera is necessary for MNI to support additional research, as well utilize the advanced technology of the newer camera to further bring state-of-the-art PET imaging analysis to our collaborative and independent research studies.

Well over 33 journal articles and/or abstracts have been published regarding the research to which MNI has contributed. MNI looks forward to the ability to continue to provide imaging services to support such research initiatives. Appendix I contains a list of

abstracts and journal articles that have been published by MNI, as well as a list of research studies conducted at MNI in New Haven over the past three years.

A list of abstracts/ journal articles have been included in Appendix I as a representation of the published research MNI has conducted. MNI will provide copies of any of the publications listed per request.

4. **Quality Measures**

   a. Submit a list of all key professional, administrative, clinical, and direct service personnel related to the proposal. Attach a copy of their Curriculum Vitae.

MNI has attached a copy of the curriculum vitae (CV) for all employed staff where their job function relates to this proposal. The following CVs have been included:

Andrea Perez, CNMT: Nuclear Medicine Technologist
Candace Cotto, RN: Clinical Research Nurse
Cheryl Riordan, RN: Clinical Research Nurse
Cristian Constantinescu, PhD: Associate Director, Imaging Translational Research
Danna Jennings, MD: Vice President and Sr. Director of Clinical Research
David Russell, MD: Associate Director of Clinical Research
Gina Nicoletti, CNMT: Nuclear Medicine Technologist
Jennifer Madonia, MS, PA-C: Associate Director of Clinical Research
John Seibyl, MD: Nuclear Medicine Physician and General Partner
Kenneth Marek, MD: General Partner
Meghan Pajonas, RN: Clinical Research Nurse
Nicholas Sandella, CNMT: Imaging Technical Quality Control & Processing Specialist
Ricardo Hidalgo, CNMT, NCT, RT: Nuclear Medicine Research Technologist
Scott Vogel, RN: Clinical Research Nurse

   b. Explain how the proposal will improve quality, accessibility and cost effectiveness of health care delivery in the region, including but not limited to, (1) provision of or any change in the access to services for Medicaid recipients and indigent persons, and (2) the impact upon the cost effectiveness of providing access to services provided under the Medicaid program.

MNI's proposal is to conduct PET imaging research so the work will not have any immediate impact on the quality of health care delivery in the region. However, the research conducted at MNI has already and is expected to continue to contribute to the development of new therapies for unmet medical needs for patients with neurologic and psychiatric illnesses. PET technology is crucial to developing better strategies for diagnosis and treatment. Continuing to provide state-of-the-art PET imaging research will ultimately lead to important new diagnostic tools and therapies as this research is translated to clinical utility.

5. Organizational and Financial Information

a. Identify the Applicant's ownership type(s) (e.g. Corporation, PC, LLC, etc.).
LLC

b. Does the Applicant have non-profit status?
☐ Yes (Provide documentation) ☒ No

c. Provide a copy of the State of Connecticut, Department of Public Health license(s) currently held by the Applicant and indicate any additional licensure categories being sought in relation to the proposal.

MNI has enclosed the State of Connecticut, Department of Public Health licenses held by the two Applicants: John Seibyl, MD and Kenneth Marek, MD.

d. Financial Statements

i. If the Applicant is a Connecticut hospital: Pursuant to Section 19a-644, C.G.S., each hospital licensed by the Department of Public Health is required to file with OHCA copies of the hospital's audited financial statements. If the hospital has filed its most recently completed fiscal year audited financial statements, the hospital may reference that filing for this proposal.
N/A

ii. If the Applicant is not a Connecticut hospital (other health care facilities): Audited financial statements for the most recently completed fiscal year. If audited financial statements do not exist, in lieu of audited financial statements, provide other financial documentation (e.g. unaudited balance sheet, statement of operations, tax return, or other set of books.)
N/A

e. Submit a final version of all capital expenditures/costs as follows:

TABLE 6
TOTAL PROPOSAL CAPITAL EXPENDITURE

Purchase/Lease	Cost
Equipment (Medical, Non-medical Imaging)	$465,000
Land/Building Purchase*	$0
Construction/Renovation**	$124,149*
Land/Building Purchase*	$0
Other (specify)	
Total Capital Expenditure (TCE)	**$589,149**
Lease (Medical, Non-medical Imaging)***	**$0**
Total Capital Cost (TCO)	**$0**
Total Project Cost (TCE+TCO)	**$589,149**

*See Appendix E for a detailed cost breakdown
***Please see Appendix K for a vender quote for the purchase of the proposed PET CT camera.

f. List all funding or financing sources for the proposal and the dollar amount of each. Provide applicable details such as interest rate; term; monthly payment; pledges and funds received to date; letter of interest or approval from a lending institution.

Equipment funding provided by:	**Bank of America**
Equipment Line of Credit Maximum:	**$750,000**
Term:	**5 years from funding date.**
Interest Rate Type:	**Fixed**
Interest Rate:	**Set at funding date.**
PET CT monthly Camera Payment @ 4.05% est. interest rate	**$8,574.00**

g. Demonstrate how this proposal will impact the financial strength of the health care system in the state or that the proposal is financially feasible for the applicant.

The proposal will not impact the financial strength of the health care system in the state, since MNI only performs research and not point of service care. The proposal would therefore have no financial impact on any other providers of point of service care in the state.

The proposal is financially feasible for MNI because the added capacity for research scans at MNI coupled with a reduced price per scan provided to sponsors will allow MNI to secure additional research studies with a resulting significant increase in projected research study revenue. MNI will be able to provide a reduced price per scan because of the efficiencies yielded from having a 2nd camera, since the PET research scan volume can be doubled with only a modest increase in costs for non-camera resources such as staffing.

6. Patient Population Mix: Current and Projected

a. Provide the current and projected volume (and corresponding percentages) by patient population mix; including, but not limited to, access to services by Medicaid recipients and indigent persons for the proposed program.

N/A

TABLE 7
APPLICANT'S CURRENT & PROJECTED PAYER MIX

Payer	Most Recently Completed FY2013		Projected					
			FY2014		FY2015		FY2016	
	Volume	%	Volume	%	Volume	%	Volume	%
Medicare*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Medicaid*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CHAMPUS & TriCare	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Government	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Commercial Insurers	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Uninsured	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Workers Compensation	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Non-Government	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Payer Mix	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

b. Provide the basis for/assumptions used to project the patient population mix.
N/A

c. For the Medicaid population only, provide the assumptions and actual calculation used to determine the projected patient volume.
N/A

d. If the proposal fails to provide or reduces access to services by Medicaid recipients or indigent persons, provide explanation for good cause for doing so. *Note: good cause shall not be demonstrated solely on the basis of differences in reimbursement rates between Medicaid and other health care payers.*
N/A

7. **Financial Attachment I**
   a. Provide a summary of revenue, expense, and volume statistics, without the CON project, incremental to the CON project, and with the CON project. **Complete Financial Attachment I.** (Note that the actual results for the fiscal year reported in the first column must agree with the Applicant's audited financial statements.) The projections must include the first three full fiscal years of the project.

Please see the attached spreadsheets in Appendix E.

   b. Provide the assumptions utilized in developing **Financial Attachment I** (e.g., full-time equivalents, volume statistics, other expenses, revenue and expense % increases, project commencement of operation date, etc.).

Please see the attached spreadsheets in Appendix E.

   c. Provide the minimum number of units required to show an incremental gain from operations for each fiscal year.

Please see the attached spreadsheets in Appendix E.

   d. Explain any projected incremental losses from operations contained in the financial projections that result from the implementation and operation of the CON proposal.

No Loses are anticipated in operations resulting from the implementation and operation of the CON proposal.

   e. Describe how this proposal is cost effective.

The additional PET/CT camera will allow MNI to double the volume of PET research scans at a modest investment of other resources such as staff time. This then reduces the research cost of a PET scan. A reduced cost per scan will allow MNI to pass savings onto our sponsors, which will encourage and generate an increase in the number of research studies. This increase in the overall number of research studies will reduce the amount of time required to learn important new scientific knowledge about diseases including Alzheimer and Parkinson, and hopefully hasten improved treatments for these devastating diseases.

List of Appendices

Appendix A: Letters of Support
Appendix B: List of Key Personnel Curriculums Vitae and Job Descriptions
Appendix C: DPH Licenses issued to applicants
Appendix D: 2011, 2012 and 2013Audited Financial Statements
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Appendix F: Copies of Public Notice
Appendix G: List MNI Human Volunteer Research Studies from Past 3 Years
Appendix H: Initial Architectural Plans
Appendix I: Supporting Research Papers Published by MNI/ MNI Staff
Appendix J: Projected Timelines
Appendix K: Quote for Equipment (PET CT Camera)

Appendix A: Letters of Support

Toni N. Harp, Mayor of New Haven
Alan P. Siegal, M.D., Geriatric and Adult Psychiatry
Danna Jennings, M.D., Institute for Neurodegenerative Disorders
Samuel Markind, M.D., Associated Neurologists, P.C.

CITY OF NEW HAVEN

TONI N. HARP, MAYOR

165 Church Street
New Haven, Connecticut 06510
T: 203.946.8200 F: 203.946.7683
www.CityofNewHaven.com

SETTLED 1638

All-America City
SINCE 1958

September 22, 2014

Dr. John Seibyl, President
Molecular Neurolmaging, L.L.C.
60 Temple Street, Suite 8A
New Haven, CT 06510

Dear Dr. Seibyl:

As the Mayor of New Haven, I'd like to take this opportunity to indicate my support for the acquisition of a PET/CT camera by the New Haven based biotech research company, Molecular Neurolmaging, LLC.

Molecular Neurolmaging's work researching Alzheimer, Parkinson and other disorders plays a key role in advancing the search for cures for these devastating diseases. In addition, MNI's growth has yielded many high quality jobs in Connecticut, and the new camera will add more. Finally, MNI is a leader in brain imaging medical research supporting the Biotech industry, and thereby contributes towards Connecticut's goal and developing a vibrant Biotech Hub.

Sincerely,

Toni N. Harp
Mayor

Geriatric and Adult Psychiatry

G.A.P. Clinical Care & Research Center
60 Washington Ave., Suite 203
Hamden, CT 06518

Phone: (203) 288-0414 Fax: (203) 288-3655

PARTNERS
Alan P. Siegal, M.D.
Jeanne Jackson, M.D.

STAFF
Lauren Mercer, M.D.
Catherine Forrest, A.P.R.N.
Julie Flood, A.P.R.N.
Lyndsie Ryalls, A.P.R.N.
Heidi Sward, L.C.S.W.
Tracy S. Morales, L.C.S.W.
Andrea DeClement, Research Director

September 15, 2014

Division of Office of Health Care Access
Connecticut Department of Public health
410 Capitol Avenue
Hartford, CT 06134

RE: PET Camera at Molecular NeuroImaging

To Whom It May Concern:

I write this letter to express my strong support for Molecular NeuroImaging (MNI) acquiring a second Positron Emission Tomography (PET) camera to be located in its imaging facility in New Haven, CT. The research-dedicated imaging facility at MNI provides state-of-the-art nuclear medicine imaging and has served as a resource for Alzheimer's disease research participants to take part in amyloid brain PET imaging, which has become a critical outcome measure for nearly all of the current therapeutic trials for Alzheimer's disease.

The success of moving forward to develop improved treatments for Alzheimer's disease requires the timely completion of the necessary clinical trials to understand the efficacy of new potential disease modifying agents. Our group, Geriatric and Psychiatry Clinical Care and Research Center, is actively involved in Alzheimer's disease clinical research studies. Expanding the camera availability will be a major benefit as it provides an increase the time slots offered for our participants thus allowing us to meet the demanding timelines set forth in these studies.

Thank you for your consideration.

Sincerely yours,

Alan Siegal, MD
GAP Clinical Care and Research Center
Hamden, CT

IND
Institute for Neurodegenerative Disorders
60 Temple Street • Suite 8B • New Haven, Connecticut 06510 • Phone: 203.401.4300 • Fax: 203.401.4301 • www.indd.org

September 15, 2014

Division of Office of Health Care Access
Connecticut Department of Public health
410 Capitol Avenue
Hartford, CT 06134

RE: PET Camera at Molecular NeuroImaging

To whom it may concern,

As a neurologist and the principal investigator of more than 45 imaging studies conducted at Molecular NeuroImaging (MNI) over the past several years, I fully support MNI's Certificate of Need application to acquire a second research-dedicated Positron Emission Tomography (PET) camera. MNI is a research dedicated facility and as such provides a unique resource to the neurological research community in Connecticut. Neurologists throughout the state refer their patients to the MNI imaging research program to participate in imaging trials. I have conducted trials at MNI collaboratively with industry partners that have lead to critical drug development go/no-go decisions. Studies for which I have served as the principal investigator and have worked jointly with the MNI imaging research team have contributed significant data to the ultimate approval of the two amyloid imaging agents that are commercially available. While MNI provides a local resource for individuals with neurological conditions to participate in trials using the most technologically advanced imaging procedures, MNI also has a core lab research group that brings this technology forward to studies conducted worldwide.

The addition of a second camera to the MNI imaging suite provides the opportunity to expand the studies offered to individuals with neurological conditions including Alzheimer's disease, Parkinson's disease, Huntington's disease, traumatic brain disorders and multiple sclerosis. The new PET camera will have the added benefit of a computed tomography component making it more efficient to analyze whole body dosimetry for novel tracers in the early developmental stages.

I appreciate your thoughtful review of MNI's CON application.

Sincerely yours,

Danna Jennings, MD
New Haven, CT

Associated Neurologists, P.C.

69 Sand Pit Road, Suite 300 · Danbury, Connecticut 06810 · Tel: (203) 748-2551 · Fax: (203) 790-6375
1566 W. Main Street, Suite 212 · Waterbury, Connecticut 06708 · Tel: (203) 755-7367 · Fax: (203) 573-1534
1 Old Park Lane, Unit 3 · New Milford, Connecticut 06776 · Tel: (860) 350-3151 · Fax: (860) 350-3119
22 Old Waterbury Road, Suite 106 · Southbury, Connecticut 06488 · Tel: (203) 262-4270 · Fax: (203) 790-6375

Adult Neurology

Jan Meshman, M.D.
Diane Wirz, M.D.
Samuel Markind, M.D.
Neil Culligan, M.D.
David Greco, M.D.
Robert Bonwetsch, M.D.
Behzad Habibi, M.D.
Michelle Lavallee, M.D.
Loraine Richter, PA-C
Courtney Kennedy, PA-C
Margaret Cavino, PA-C
Lee Hamfe, APRN
Melisa Pellikan, RN

Pediatric Neurology

William Yorns, Jr, D.O.

Neuropsychology

Erin Lasher, Psy.D.
Jonathan Woodhouse, Psy.D.
Marybeth Bellar, Psy.D.
Jennifer Denkin, Ph.D
Lori Wagner, Psy.D.

Neurophysiology

Rachelle Christie, R EEGT
JoAnn Miles R EEGT

Physical Therapy

Lisa Dransfield, P.T., D.P.T., M.A.
Cynthia Gehr, P.T.
Michelle DiBona, P.T., M.A.
Kimberly Down, MS, OTR/L
Debra Nash, P.T.
Karen Nall, Licensed PTA
Nicole Saviano, Licensed PTA
Diane Yandow, P.T.
Karen Olencki, MT, MBA

Administration

Arlene Bona
Wendy White

September 15, 2014

Division of Office of Health Care Access
Connecticut Department of Public health
410 Capitol Avenue
Hartford, CT 06134

RE: Molecular NeuroImaging PET camera acquisition

Dear Office of Health Care Access,

As an active member of the neurologic research community in the state of Connecticut, I am writing to support Molecular NeuroImaging's (MNI) Certificate of Need application to acquire an additional Positron Emission Tomography (PET) imaging camera. Molecular NeuroImaging is a research-dedicated facility located in New Haven, CT and provides an excellent resource for nuclear medicine imaging to the neurological research community. Over the past several years, I have utilized MNI's imaging research facility as several of my patients are active participants in the research studies conducted at the Molecular NeuroImaging imaging facility in New Haven, CT. The imaging facility at MNI provides highly technical nuclear medicine imaging services, which is rapidly becoming a requirement for most of the clinical trials evaluating therapeutics for neurodegenerative disorders including: Alzheimer's disease, Parkinson's disease, Huntington's disease and multiple sclerosis.

Given the recent increase in the volume of studies developing additional biomarkers and evaluating the safety and efficacy of novel immunotherapies in Alzheimer's disease, there is a critical need to expand the imaging resource at MNI to accommodate this increase in demand. The CT neurological research community relies heavily on this research oriented-facility to continue to collaboratively develop more effective treatments for those suffering with neurodegenerative conditions.

Sincerely yours,

Samuel Markind, MD
Associated Neurologists, PC
Danbury, CT

Appendix B: List of Key Personnel Curriculums Vitae and Job Descriptions

Andrea Perez, CNMT: Nuclear Medicine Technologist
Candace Cotto, RN: Clinical Research Nurse
Cheryl Riordan, RN: Clinical Research Nurse
Cristian Constantinescu, PhD: Associate Director, Imaging Translational Research
Danna Jennings, MD: Vice President and Sr. Director of Clinical Research
David Russell, MD: Associate Director of Clinical Research
Gina Nicoletti, CNMT: Nuclear Medicine Technologist
Jennifer Madonia, MS, PA-C: Associate Director of Clinical Research
John Seibyl, MD: Nuclear Medicine Physician and General Partner
Kenneth Marek, MD: General Partner
Meghan Pajonas, RN: Clinical Research Nurse
Nicholas Sandella, CNMT: Imaging Technical Quality Control & Processing Specialist
Ricardo Hidalgo, CNMT, NCT, RT: Nuclear Medicine Research Technologist
Scott Vogel, RN: Clinical Research Nurse

1/11/12
2/25/13

ANDREA PEREZ

[redacted]

Education

Bachelor of Science Degree in Public Health
Specialization:
Community Health, Southern Connecticut State University, New Haven, Connecticut, December 2000.
- Grade Point Average 3.49

Associate of Science Degree Nuclear Medicine Technologist
Gateway Community Technical College, New Haven, Connecticut, May 1997.
- Honors: Dean's List, all semesters, PSI BETA ® (Association of College Honor Society).

Student Presentation 28th annual Spring Symposium, New England Chapter, Technologist section, April 24, 1997.

Certification of Community Mediator
Specialization:
Educator on Conflict Resolution and Substance Abuse, Community Mediation Inc. New Haven, Connecticut, August 1995.

Professional experience

2001 – Present **Nuclear Medicine Research Technologist**
Molecular NeuroImaging L.L.C./Institute for Neurodegenerative Disorders, New Haven, CT.
- Provide imaging responsibilities for protocol studies
- Provide imaging reconstruction and data conversion for projects
- Perform data and image analysis on a variety of computers
- Maintain quality control on the camera and equipment
- Patient scheduling
- Organizational meetings
- Draft papers or portion of papers for publications and/or publications
- Serve on organizations, comities and council at the national level in the Society of Nuclear Medicine.
- Translation of source documents for multi-center trials

1995 – Present **Volunteer Community Mediator**
Community Mediation Inc. New Haven, CT., August 1995

1997 – 2001 Nuclear Medicine Research Technologist.
Rose L. Hoffer NeuroSPECT Center, Yale University, School of Medicine, Department of Diagnostic Radiology, New Haven, CT.
- Responsible for the coordination of patient flow
- Patient scheduling,
- Organizational meetings,
- Patient data-base and patient image analysis.

1994 – 2000 **Café Supervisor**
Atticus Café and Book Store, New Haven, CT.

1999 **Volunteer Coordinator**
Por la Vida Colombia Relief Fund, Latino Youth Development Inc. New Haven, CT.
Responding to emergency relief needs on Colombia.

Internships:
Hospital of Saint Raphael, New Haven, CT., 1994-1997.
Yale-New Haven Hospital, New Haven, CT., 1994-1997.
VA Medical Center, West Haven, CT., 1994-1997.

2/25/13

Professional memberships

Society of Nuclear Medicine (SNM), Technologist section.

Skills

Bilingual:
Spanish and English, fluent in both languages.
Understand and able to speak Portuguese.

Personal Competencies:
Consider myself an extremely reliable, responsible and self-motivated individual, always able to get things done. I have excellent creative thinking skills and leadership skill. I am a very honest and self-disciplined individual. I consider myself a thoughtful individual with great interpersonal skills.

Computer:
Macintosh and PC literate, Windows 98/00/XP, Creating Websites, Microsoft Office, File Maker Pro, Power Point, Odessey Computer, System Sun System.

Public Health Competencies:
Conduct research investigations, understand and perform statistical analysis, understand medical terminology, program planning, program implementation, program evaluation, knowledge of grant writing process, knowledge of epidemiological methods and techniques.

Accreditations

Certified Nuclear Medicine Technologist (CNMT); BLS Certified.

Publications and Presentations

- Abstract publication in The Journal of Nuclear Medicine Technology, May 2012.
- Abstract publication in The Journal of Nuclear Medicine Technology, May 2011.
- Poster presentation at the Society of Nuclear Medicine, 56th annual Meeting, June 2011.
- Abstract publication in The Journal of Nuclear Medicine Technology, May 2009.
- Poster presentation at the Society of Nuclear Medicine, 56th annual Meeting, June 2009.
- Abstract publication in The Journal of Nuclear Medicine Technology, May 2008.
- Oral presentation at the Society of Nuclear Medicine, 55th annual Meeting, June 2008.
- Abstract publication in The Journal of Nuclear Medicine Technology, May 2007.
- Oral presentation at the Society of Nuclear Medicine, 54th annual Meeting, June 2007.
- Abstract publication in The Journal of Nuclear Medicine Technology, May 2005, page 109.
- Oral presentation at the Society of Nuclear Medicine, 52nd annual Meeting, June 2005.
- Abstract Submission to the Society of Nuclear Medicine, January 2003.
- Abstract publication in The Journal of Nuclear Medicine Technology, June 2001, page 111.
- Oral presentation at the Society of Nuclear Medicine, 48th annual Meeting, June 2001.
- Abstract publication in The Journal of Nuclear Medicine Technology, June 1999.
- Poster presentation at the Society of Nuclear Medicine, 45th annual Meeting, June 1999.

Awards received

- First place technologist on abstract and oral presentation at the Society of Nuclear Medicine, 48th annual Meeting, July 2001.
- Third place on abstract and poster presentation at the Society of Nuclear Medicine, 45th annual Meeting, June 1999.
- Second place on abstract and poster presentation at the Society of Nuclear Medicine, 56th annual Meeting, June 2009.

Volunteer experience

Hospital of Saint Raphael, New Haven CT., March 1994.

Yale New Haven Hospital, New Haven CT., January 1994.

References

Available upon request.

CANDACE L. COTTO
776 Evergreen Avenue
Hamden, CT 06518
203-287-9147
Candace.cotto@yahoo.com

SUMMARY: Thirty four year diverse nursing experience, most recently as a Clinical Research Nurse Coordinator.

EDUCATION:

CONCORD HOSPITAL SCHOOL OF NURSING
330 Pleasant Street
Concord, New Hampshire, 03301

QUALIFICATIONS:

- Experienced nurse with excellent clinical skills.
- Excellent leadership skills, with the ability to build consensus for positive change.
- Outstanding organizational and interpersonal skills used to build collaborative working relationships.
- Experienced in clinical trial data collection.
- Strong sense of ownership and accountability for results combined with a commitment of performance excellence and self-improvement.
- Excellent IV skills and experience in obtaining, packaging and sending blood and/or tissue samples.
- Excellent clinical decision making and nursing intervention.
- Working knowledge of determining patient eligibility, patient enrollment and obtaining informed consent in multiple clinical trials.
- Demonstrates effective written, verbal and training communications skills.
- Professional interactive ability to work as an effective team member in a multi-specialty arena sharing mission and goals.
- Strong computer skills. Experience with EXCEL and WORD.
- Demonstrates ability to coordinate multiple activities among multi-disciplinary groups.
- Thorough working knowledge of protocol development and use within multi-specialty clinical trials.

WORK EXPERIENCE:

2008-present

MOLECULAR NEUROIMAGING, LLC
INSTITUTE for NEURODEGENERATIVE DISORDERS
60 Temple Street, Suite 8B
New Haven, Connecticut, 06510

Clinical Research Nurse

- **Study Coordinator: BAN2401-G000-201- A Placebo-controlled, Double-blind, Parallel-group, Bayesian Adaptive Randomization Design and Dose Regimen-finding Study to Evaluate Safety, Tolerability, and Efficacy of BAN2401 in Subjects with Early Alzheimer's Disease.**

- **Study Coordinator: MNI-420-02-DO. An open-label, single oral dose study to compare brain A2a occupancy and in vivo potencies for A2a receptor binding of SCH 900800 and preladenant using Single Photon Emission Computed Tomography (SPECT) with [123I] MNI-420 in healthy male subjects.**

- **Study Coordinator: A Phase II a, 12 Week, Multicentre, Double-Blind, Randomized, Placebo controlled, Parallel-Group Study to Assess the safety and Tolerability of Oral AZD3241 in Patients with Parkinson's Disease.**

- **Study Coordinator: PO5664- A Phase 3, Double-blind, Double-Dummy, Placebo and Active-Controlled Dose Range Finding Efficacy and Safety of Preladenant in Subjects with Early Parkinson's Disease.**

- **Study Coordinator: SURE-PD-Randomized, double blind, placebo controlled, dose range finding trial of oral Inosine to assess safety and ability to elevate urate in early Parkinson's disease.**

- **Study Coordinator: MOTION-27918- Phase 3, double blind, placebo controlled, randomized trial to determine efficacy and safety of low dose and high dose of Safinamide as add-on therapy, in subjects with early PD treated with a stable dose of a single dopamine agonist. (May2010-closed)**
- **Study Coordinator: SETTLE-27919 (closed)**

- **Study Coordinator: MOTION extension-27938- Extension study to the MOTION 27918 study (February 2011-closed)**

- **Study Coordinator: LZAN-AD-Effect of Passive Immunization on the Progression of Alzheimer's Disease: Solanezumab vs. Placebo (November 2009-closed)**

- **Study Coordinator: LZAO-Open label study to follow LZAN**

- **Study Coordinator: PO5664-Preladenant-Phase 3, Double Dummy, Placebo and Active-controlled Dose Range finding, Efficacy and Safety Study of Preladenant in subjects with Early Parkinson's Disease. (December 2009-present)**

- **Study Coordinator: PHAROS-HD (closed)**

- **Study Coordinator: COHORT-HD –Cooperative Huntington's Disease Observational Research Trial (closed)**
- **Study Coordinator: SPIN-PD (closed)**
- **Study Coordinator: (PostCEPT) A Longitudinal Observational Follow-up of the PRECEPT Study cohort. (closed)**
- **Study Coordinator: (PROBE) Blood α-Synuclein, Gene Expression, and Smell Testing as Diagnostic and Prognostic Biomarkers in Parkinson's disease.(closed)**
- **Study Coordinator: Parkinson Associated Risk Factor Study (PARS): evaluating potential screening tools for Parkinson's disease. (July 2008-2010)**
- **Study Coordinator: (QE3)-A Phase III, multi-center, randomized, double-blind placebo-controlled, parallel group study of the effects of Coenzyme Q^{10} in Parkinson's disease. (closed)**
- **Study Coordinator: (APLIED)- A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of Three Doses of Aplindore MR, in Patients with Early Parkinson's Disease. (Aplindore-211) (closed)**

- **Works independently to coordinate subjects and staff for imaging research trials evaluating individuals with PD, AD and healthy control subjects.**
- **Clinical study coordination to include:**
  - **Conducting clinical trial visits including completion of relevant clinical instruments, phlebotomy and EKG's.**
  - **Maintaining case report forms and source documents in an organized fashion.**
- **Works closely with the regulatory affairs staff to ensure timely regulatory approval status and GCP compliance.**
- **Participates in subject recruitment and subject consenting.**
- **Meets with investigators on a regular basis to review progress of the trials**

May 2006-July 2008

YALE UNIVERSITY SCHOOL OF MEDICINE
333 Cedar Street, New Haven, CT. 06520

Research Nurse Coordinator for Hematological Malignancies

- **Coordinated the preparation, evaluation, submission and continuation of all hematological malignancy clinical trials protocols. Developed working templates and procedures consistent with regulatory and agency requirements.**
- **Coordinated activities of a multi-disciplinary team, including collaborating clinicians, researchers, sponsor representatives, and service providers at Yale and other institutions. Served as the primary liaison with the pharmaceutical companies, clinics, supporting departments and other researchers to facilitate progress.**
- **Independently determined patient eligibility, coordinated patient enrollment, and obtained informed consent.**
- **Assessed and monitored research subject's response to study participation. Communicated findings to study physicians and implemented appropriate actions. Managed and coordinated routine follow-up care for study participants.**
- **With thorough working knowledge of regulatory and clinical nursing guidelines, developed, implemented and delivered orientations and continuous training to all**

members of the clinical trials team. Maintained training records and assured that training content met all regulatory specifications and the needs of clinical trials sponsors. Trained and mentored less senior staff.

- Performed nursing duties that were necessary in order to provide optimal clinical research care.

October 2005 – April 2006 — YALE NEW HAVEN HOSPITAL,
Clinical Manager for Solid Organ Transplantation and Unit based Educator

- Ensured that desired patient outcomes were achieved through safe and efficient utilization of resources.
- Achieved seamless delivery of service by appropriately and effectively communicating with staff, Patient Service Manager and other hospital leaders, colleagues and physicians.
- Monitored staff compliance with YNHH policies, procedures and regulatory requirements, as well as with systems and processes developed to enhance patient care and satisfaction.
- Collaborated in developing a highly skilled multidisciplinary team who were utilized effectively.
- Monitored continuous performance improvement efforts that form a basis for unit activity.
- Upheld and communicated practices and procedures that were consistent with institutional policies and procedures.
- Monitored systems to ensure effective utilization and availability of equipment and supplies.
- Held staff accountable for achieving high levels of performance consistent with evidence based practice.

2003-October 2005 — YALE NEW HAVEN HOSPITAL
Clinical Coordinator of Phototherapy

- Effectively interacted with a diverse population of dermatologic patients.
- Provided direct patient care.
- Clinical consultation with staff in their assessment of clinical problems, for nursing interventions, and evaluations of the outcomes of care provided.
- Effectively treated a large number of patients who were treated with combinations of Photopheresis and Phototherapy.
- Collaborated with other nursing staff on numerous occasions in developing educational programs for Connecticut, Massachusetts, and New York dermatology nurses.
- Collaborated with Nurse Manager and billing office to devise an effective system for obtaining Medicare reimbursement for Phototherapy patients.

1990-2003 — YALE NEW HAVEN HOSPITAL
Staff Nurse - Phototherapy

- Participated fully as a member of the Photopheresis staff.
- Worked collaboratively with both medical staff and nursing staff.
- Participated in numerous clinical trials, from concept to completion.
- Directly involved with the primary investigators of multiple clinical trials.
- Assisted with the coordination of patient enrollment and eligibility.
- Monitored patients for safety and protocol compliance.
- Documented proper protocol related events in a timely manner.

1988-1990 **YALE NEW HAVEN HOSPITAL**
Staff Nurse - Apheresis

- Participated as part of a multidisciplinary Apheresis/transfusion team.
- Participated in clinical trials involving bone marrow transplant patients.
- Actively participated in numerous oncological research protocols.
- Worked in collaboration with the director of the blood bank.

1986-1988 **YALE NEW HAVEN HOSPITAL**
20 York Street, New Haven, CT. 05610
Staff Nurse - PACU

- Participated fully as a critical care nurse in the post anesthesia recovery room.
- Worked collaboratively with surgical, anesthesia and nursing staff.
- Ensured that desired patient outcomes were achieved through safe and efficient practice.

1986-1988 **ST. CLARE'S HOSPITAL**
Denville, New Jersey
Assistant Nurse Manager- ICU, CCU, Telemetry

- Directly responsible for the patient care of a 14 bed ICU-CCU and telemetry unit.
- Provided input into the performance review of individual staff members.
- Assisted the Nurse Manager and the Administrative Director in communicating and interpreting changes in nursing practice activities and standards of care to staff members.
- Collaborated with other staff members to define, reduce and eliminate barriers within the unit which may have negatively impacted patient care.

1985-1986 **PAOLI HOSPITAL**
Paoli, Pennsylvania
Staff Nurse, ICU, Emergency Room

1981-1984 **ALEXANDRIA HOSPITAL**
Alexandria, Virginia
IV Team, Staff Nurse ICU-CCU, Module Leader, Progressive Care/Dialysis

1979-1981 **CENTRAL VERMONT MEDICAL CENTER**
Berlin, Vermont
Assistant Nurse Manager – Medical Surgical Unit

ADVANCED CLINICAL TRAINING:

February 12, 2013 ADAS-Cog Qualification

uary 31, 2013 CDR Qualification

January 25, 2013 INFORM 4.6 to 5.0 Delta Course

September 7, 2010	UPDRS and Modified Hoehn and Yahr Training and Certification
ıe 24, 2010	INFORM 4.6 Training
June 21, 2010	Administration of the Columbia-Suicide Severity Rating Scale, MOCA, BDI-II, ESS, EQ-5D, QUIP-RS, SAQ & PDQUAL Training.
May 26, 2010	Eli 250 ECG Training
May 26, 2010	INFORM 4.5 Training
April 2010-present	IATA training
June 11, 2009	eCTS Training for Lilly Speak Treatment Study and Dispensing Study
April 20, 2009	Shipping of Infectious substances and Dry Ice Training
April 2009	Intralinks Clinical Reviewer Training
August 2008	Shipping of Infectious Substances and Dry Ice Training IATA
August 2008	eResearch Community and eData Entry Training CTCC
August 2008	"CDR Rater" Certification Alzheimer's Disease Research Center Memory and Aging Project On Line Training program
August 2008	"Protecting Human Research Participants" NIH Web-based training
July 2006	Certified- Chemotherapy Administration
April 2003	THERAKOS UVAR-XTS Advanced Training Program
May 2001	Advance IV therapy and Access Devices
1979-present	Basic Cardiac Life Support
December 2000	THERAKOS UVAR-XTS Advanced Program
May 2000	Advanced EKG Interpretation
1990	YALE NEW HAVEN HOSPITAL Clinical Advancement Seminar
1991	Certified-Dermatology Core Curriculum
8	Certified-Advanced Aphaeresis Training
1985-1986	Certified-Critical Care

1985	Certified-IV Therapy
'82	Certified-Chemotherapy Administration
1983	Critical Care Medication Course

PROFESSIONAL DEVELOPMENT:

January 29-31 2013	BAN2401-G000-201 Investigator Meeting Charlotte, NC
June 10, 2010	Multiple Sclerosis and Parkinson's Disease Clinical Perspectives and Interventions The Study at YALE
June 2010	PO5664 Investigator/Coordinator Meeting Dallas, TX
May 2010	PSG Annual Investigator/Coordinator Meeting Dallas, TX
December 2009	MOTION and SETTLE Investigator/Coordinator Meeting Orlando, Florida
May 2009	PSG Annual Investigator/ Coordinator Meeting Speaker for QE3 Enrollment San Diego, CA
March 2009	APLIED Investigator/ Coordinator Meeting Philadelphia, PA
November 2008	HSG Annual Convention St. Pete's Beach Island, FL
September 2008	SURE-PD Investigator/Coordinator Meeting Boston, MA.
November 2005	Excellence in Management Training Course- Yale New Haven Hospital
September 2003	Clinical Nurse III Recognition
October 2002	State of the Art on Cutaneous Lymphomas Cambridge, Massachusetts
September 2000	NY Academy of Sciences New York, New York
March 2000	Dermatology Nurse Association-National Convention San Francisco, California Participant Group Lecture "What is new in CTCL?"
.rch 1999	Dermatology Nurse Association-National Convention New Orleans, Louisiana

March 1998	Dermatology Nurse Association-National Convention Orlando, Florida Program Moderator
...arch 1995	Dermatology Nurse Association-National Convention New Orleans, Louisiana
December 1993	Dermatology Nurse Association-National Convention Washington, D.C.
December 1992	Dermatology Nurse Association-National Convention San Francisco, California
December 1991	Dermatology Nurse Association-National Convention Dallas, Texas
December 1990	Dermatology Burse Association-National Convention Atlanta, Georgia
October 1990	New York Academy of Sciences New York, New York
1990	American Academy of Blood Bank Seminar Farmington, Connecticut
1990	"*From Novice to Expert.*" Patricia Benner Yale New Haven Hospital
..d8	Health Fair Yale New Haven Hospital

PUBLICATIONS: *Impaired Olfaction and Other Prodromal Features in the Parkinson At-Risk Syndrome Study*
Movement Disorders, Vol 27, No. 3, 2012

COMMITTEES: YALE NEW HAVEN HOSPITAL
October 2005-2007

- SFE Nursing Leadership Group
- SFE Practice Group
- SFE Documentation Group
- SCM "super user" Documentation Group
- Pain Documentation/Policy Development
- Pain Audit Committee
- Nursing Policy and Procedures

ALEXANDRIA HOSPITAL

1984	Progressive Care/Dialysis
1982	Clinical Ladder Development

...OFESSIONAL ORGANIZATIONS

1991-2005	Dermatology Nurse Association

15 MAR 13

Cheryl Riordan
29 MAY 2014

Cheryl B. Riordan 203-401-4390
60 Temple Street, Ste. 8B, New Haven, CT 06510 criordan@indd.org

Professional Summary

Registered Nurse with diverse experience, currently working as a Clinical Research Coordinator.

Core Qualifications

- Licensed in Connecticut
- Works well independently as well as collaboratively
- HIPPA compliant
- Patient and Family Focused
- Strong clinical judgment and skills
- Organized and detail oriented
- Excellent communication skills
- Proficient in Excel, Word, Power Point, Access, Mysis ,Cerner, EPIC

Professional Experience

Institute for Neurodegenerative Disorders
Molecular NeuroImaging, LLC
New Haven, CT
June 2011-
Clinical Research Nurse

Study Coordinator- TauRx237-005,Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, 18-Month Safety and Efficacy Study of Leuco-methylthionine bis(hydromethanesulfonate) in Subjects with Mild Alzheimer's Disease

Unblinded Pharmacist- BAN2401, Placebo-controlled, Double-blind, Parallel-group, Bayesian Adaptive Randomization Design and Dose Regimen-finding Study to Evaluate Safety,Tolerability and Efficacy of BAN2401 in Subjects With Early Alzheimer's Disease

Study Coordinator- The Parkinson's Progression Markers Initiative (PPMI)

Back-up Coordinator- H8A-MC-LZAX, Effect of Passive Immunization on the Progression of Mild Alzheimer's Disease: Solanezumab (LY2062430) Versus Placebo

Study Coordinator- NS43128 (NET-PD), A Multicenter, Double-Blind, Parallel Group, Placebo Controlled Study of Creatine in Subjects with Treated Parkinson's Disease (PD). Longterm Study – 1 (LS-1)

Study Coordinator- SEP432-102, A Phase 1, Open-Label, Single-photon Emission Computed Tomography(SPECT) Study to Evaluate Serotonin and Dopamine Transporter Occupancy After Multiple Dose Administration of SEP-228432 to Achieve Steady State in Healthy Subjects(closed)

Unblinded Pharmacist-3133K1-3000-US, A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Efficacy and Safety Trial of Bapineuzumab (AAB-001, ELN115727) in SubjectsWith Mild to Moderate Alzheimer Disease Who Are Apolipoprotein E ε4 Non-Carriers(closed)

Study Coordinator-28849, Open-Label Escalating Dose Study Using 123I □-CIT Single Photon Emission Computerized Tomography (SPECT) to Evaluate Dopamine and Serotonin Transporter Occupancy by Safinamide in Parkinson's Disease Patients(closed)

Study Coordinator -MPPF-01, Phase I evaluation of [18F]MPPF as a brain tracer of serotonin receptor 5HT1a in subjects with Parkinson disease and healthy subjects(closed)

Study Coordinator- NEU-02, Safety Assessment of Neuroptix SAPPHIRE II System to Aid in the Diagnosis of Probable Alzheimer's disease in five normal subjects and five patients with probable Alzheimer's disease.(closed)

- Ensure studies are conducted in compliance with GCP, company SOP's and regulatory requirements
- Participate in study initiation, interim study maintenance and close-out process
- Develop and maintain source documents and tracking logs relevant to the imaging study

- Completion of study Case Report Form (CRF)
- Responsible for recruiting and scheduling of study participant's visits and projected dates of return as well as overseeing all data management within the study
- Completes the consenting process and inclusion/exclusion criteria to determine eligibility of study participant
- Completes study visit activities including EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs
- Completes follow up activities with both the site and the participant after the participant has departed from the facility.
- Communicates all protocol violations, deviations and any study results that are not within normal ranges to the PI and/or the Senior Clinical Research Nurse
- Responsible for study medications accounting, dispensing and destruction as required by studies and procedures
- Work collaboratively with other study coordinators and sites
- Assists with lumbar punctures, arterial lines, starts and maintains intravenous study medication

Bridgeport Hospital
Women's Care Center
May 2008-
Registered Nurse

- Responsible for the care of patients with multiple diagnoses including, post operative care of gynecological surgeries and mastectomies, high risk antepartum care, postpartum care and newborn care.
- Perform assessments and developed individualized care plans
- Implement care plan while maintaining patient safety
- Collaborate with multidisciplinary team to ensure optimal patient outcomes
- Evaluate clinical findings and patient responses to interventions.
- Provide discharge planning and education.
- Document thoroughly all elements of patient care
- Orient new nurses to Women's Care Center and assist in the training of student nurses from a variety of local nursing schools.
- Assume responsibility of charge nurse, handling staffing issues, floor assignments and insuring the satisfaction of patients and their visitors.

New England Retina Associates, Hamden, CT
August 1997-May 2008
Credentialing Coordinator for Multi-Physician Ophthalmology Practice

- Developed training manual for new employees.
- Supported Clinical Research Department with subject scheduling, CRF completion and all credentialing issues.
- Created Access Data base of Retina Specialists worldwide along with society affiliations
- Responsible for credentialing Physicians, Physician Assistants, and Fellows for licensure, hospital privileges, insurance participation and professional memberships
- Communicated regularly with Medical Staff Offices at eleven hospitals in Connecticut, New York and Rhode Island
- Researched appropriate continuing education courses and maintained continuing education records for all medical personnel
- Obtained approval for accreditation from local hospitals for CME credits to be given at all Practice sponsored lecture series
- Organized yearly CME approved lectures conducted by New England Retina Associates

Education

Bridgeport Hospital School of Nursing, Bridgeport, CT
Diploma in Nursing
May 2008

New York University, New York, NY
Bachelor of Arts, Humanities
October 1990

Certifications

Connecticut License #085131
Basic Life Support
Advance Cardiac Life Support
Neonatal Advanced Life Support
Electronic Fetal Monitoring-C
CDR Rater
Columbia Suicide Severity Rating Scale Administration

Continuing Education

NIH Protecting Human Research Participants
IATA Training and Blood Borne Pathogens
IV insertion
Phlebotomy
Glucose monitoring
EKG
Informed Consent Process
Blood Product Safety II: Administration
Preventing Central Line Associated Blood Stream Infection
Rh Immune Globulin Review
Genentec Stroke Module 6: Stroke Centers
HIPPA Data Safety
Infection Control
RCRA Regulated Pharmaceutical Waste
Back Safety
YNHHS Code of Conduct
Developmentally Appropriate Care of the Adult Patient
Developmentally Appropriate Care of the Pediatric Patient
Breastfeeding

Cristian C Constantinescu

Profile

Biomedical engineer specialized in molecular imaging modalities PET and CT with excellent analytical skills demonstrated by many years of experience.

Skills

- PET and CT image processing
- PET kinetic modeling
- PET/CT physics
- Presentation skills
- Project management
- Software/algorithm design and development
- PMOD
- The Mathworks MATLAB
- C programming

Professional Experience

1/2014 - Present Molecular NeuroImaging, LLC New Haven, CT
Associate Director, Imaging Translational Research

- Manage PET and SPECT acquisition protocols for pre-clinical and early clinical studies
- Conduct image-processing, analysis and modeling for existing or novel radiotracers
- Provide guidance on selection of radiotracers for further development and clinical study
- Assess the acquisition and processing of image data in support of validation of quantitative outcome measures
- Install and evaluate available software packages for image processing
- Research and assess new development in image processing or data modeling
- Develop needed software, methods and modeling when not already available
- Lead studies to investigate human and animal disease, preventive methods, and treatments
- Write publications and reports

9/2007-12/2014 University of California Irvine Irvine, CA
Associate Project Scientist

- Designed PET and CT preclinical imaging protocols and workflows
- Planned preclinical imaging experiments
- Independently evaluated the performance of Inveon dedicated PET scanner
- Supervised calibration and quality control for Inveon PET and CT scanners, Siemens HR+ clinical scanners
- Designed imaging protocol
- Developed methods for in vivo dosimetry and biodistribution of radiotracers in mice and extrapolation to human
- Administered preclinical PET/CT imaging lab computer system and data design flow for preclinical lab
- Performed data backup and established recovery policies
- Wrote scientific papers and research grant applications

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Cristian C Constantinescu

5/2002 – 8/2007 Indiana University School of Medicine Indianapolis, IN
Graduate Research Assistant

- Developed models of neurotransmitter kinetics using dynamic PET
- Designed MATLAB and C software programs for image analysis

1/1999 – 5/2000 Purdue University, Department of Physics West Lafayette, IN
Graduate Research Assistant

- Developed models Analyzed nuclear spectroscopy data (Gammasphere, Argonne National Lab) from fission of neutron rich nuclei

Education and Training

2007 Purdue University West Lafayette, IN
Ph.D., Biomedical Engineering

1997 University of Bucharest Bucharest, Romania
M.S., Applied Nuclear Physics

1996 University of Bucharest Bucharest, Romania
B.S., Technological Physics

Affiliations

Institute of Electrical and Electronics Engineers, Inc. (IEEE)

Journal Publications

1. N. Tahara, J. Mukherjee, H.J. de Haas, A.D. Petrov, A. Tawakol, N. Haider, A. Tahara, C.C. Constantinescu, J. Zhou, H.H. Boersma, T. Imaizumi, M. Nakano, A. Finn, Z. Fayad, R. Virmani, V. Fuster, L. Bosca, and J. Narula, " 2-deoxy-2-[18F]fluoro-D-mannose positron emission tomography imaging in atherosclerosis," Nat Med, vol. 20, no.2, pp. 215-219, Jan. 2014.
2. M. R. Mirbolooki, S.K. Upadhyay, C. C. Constantinescu, M. L. Pan, and J. Mukherjee, " Adrenergic pathway activation enhances brown adipose tissue metabolism: A [(18)F]FDG PET/CT study in mice," Nucl Med Biol, vol. 41, no. 1, pp. 10-16, Jan.2014.
3. E. D. Morris, S. J. Kim, J. M. Sullivan, S. Wang, M. D. Normandin, C. C. Constantinescu, and K. P. Cosgrove, "Creating Dynamic Images of Short-lived Dopamine Fluctuations with lp-ntPET: Dopamine Movies of Cigarette Smoking," J Vis Exp, vol. 78, Jan.2013.
4. N. Saigal, A. K. Bajwa, S. S. Faheem, R. A. Coleman, S. K. Pandey, C. C. Constantinescu, V. Fong, and J. Mukherjee, "Evaluation of serotonin 5-HT receptors in rodent models using [18F]mefway PET," Synapse, vol. 67, no. 9, pp. 596-608. Sep.2013.

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5. C. C. Constantinescu, E. Sevrioukov, A. Garcia, M. L. Pan, and J. Mukherjee, "Evaluation of [18F]Mefway Biodistribution and Dosimetry Based on Whole-Body PET Imaging of Mice,"Mol Imaging Biol, vol. 15, no. 2, pp. 222-229, Apr.2013.
6. M. R. Mirbolooki, C. C. Constantinescu, M. L. Pan, and J. Mukherjee, "Targeting presynaptic norepinephrine transporter in brown adipose tissue: A novel imaging approach and potential treatment for diabetes and obesity," Synapse, vol. 67, no. 2, pp. 79-93, Feb.2013.
7. C. C. Constantinescu, E. Sevrioukov, A. Garcia, M. L. Pan, and J. Mukherjee, "Evaluation of [18F]Nifene Biodistribution and Dosimetry Based on Whole-Body PET Imaging of Mice," Nucl Med Biol, vol. 40, no. 2, pp. 289-294, Feb.2013.
8. K. M. Bieszczad, R. Kant, C. C. Constantinescu, S. K. Pandey, H. D. Kawai, R. Metherate, N.M. Weinberger, and J. Mukherjee, "Nicotinic acetylcholine receptors in rat forebrain that bind (18) F-nifene: Relating PET imaging, autoradiography, and behavior," Synapse, vol. 66, no. 5, pp. 418-434, May2012.
9. C. Reist, J. C. Wu, Y. Lilja, J. Mukherjee, D. Gripeos, C. Constantinescu, M. A. Raggi, L. Mercolini, and V. Ozdemir, "Ketoconazole-associated preferential increase in dopamine D2 receptor occupancy in striatum compared to pituitary in vivo: role for drug transporters?," J. Clin. Psychopharmacol., vol. 32, no. 1, pp. 110-113, Feb.2012.
10. M. R. Mirbolooki, C. C. Constantinescu, M. L. Pan, and J. Mukherjee, "Quantitative Assessment of Brown Adipose Tissue Metabolic Activity and Volume Using 18F-FDG PET/CT and Adrenergic Activation," Eur. J. Nucl. Med. Mol. Imaging Research, vol. 1, no. 30, Dec.2011
11. C. C. Constantinescu, R. A. Coleman, M. L. Pan, and J. Mukherjee, "Striatal and extrastriatal microPET imaging of D2/D3 dopamine receptors in rat brain with [(18) F]fallypride and [(18)F]desmethoxyfallypride," Synapse, vol. 65, no. 8, pp. 778-787, Aug.2011
12. A. Garcia, M. R. Mirbolooki, C. Constantinescu, M. L. Pan, E. Sevrioukov, N. Milne, P. H. Wang, J. Lakey, K. G. Chandy, and J. Mukherjee, "18F-Fallypride PET of Pancreatic Islets: In Vitro and In Vivo Rodent Studies," J Nucl Med, vol. 52, no. 7, pp. 1125-1132, July2011.
13. R. Kant, C. C. Constantinescu, P. Parekh, S. K. Pandey, M.-L. Pan, B. Easwaramoorthy, and J. Mukherjee, "Evaluation of 18F-nifene binding to 42 nicotinic receptors in the rat brain using microPET imaging," Eur. J. Nucl. Med. Mol. Imaging Research, vol. 1, no. 6 June2011.
14. E. D. Morris, C. C. Constantinescu, J. M. Sullivan, M. D. Normandin, and L. A. Christopher, "Noninvasive Visualization of Human Dopamine Dynamics from PET Images," Neuroimage, vol. 49, no. 4 2010.
15. C.C. Constantinescu, J Mukherjee, "Performance Evaluation of an Inveon PET preclinical scanner," Phys. Med. Biol., 54 (2009), 2885-2899.
16. K. K. Yoder, E. D. Morris, C. C. Constantinescu, T. E. Cheng, M. D. Normandin, S. J. O'Connor, and D. A. Kareken, "When What You See Isn't What You Get: alcohol Cues, alcohol Administration, prediction Error, and human striatal dopamine," Alcohol Clin.Exp.Res., vol. 33, no. 1, pp. 139-149, Jan.2009.
17. C. C. Constantinescu, K. K. Yoder, D. A. Kareken, S. J. O'Connor, C. A. Bouman, M. D. Normandin, T. Cheng, and E. D. Morris, "Estimation from PET data of transient changes in dopamine concentration induced by

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alcohol : support for a non-parametric signal estimation method," Phys. Med. Biol., 53 (2008) 1353-1367.
18. C. C. Constantinescu, C. Bouman, and E. D. Morris, "Nonparametric Extraction of Transient Changes in Neurotransmitter Concentration from Dynamic PET Data," IEEE Trans. Med. Imaging, vol. 26, no. 3, pp. 359-373, Mar.2007.
19. K. K. Yoder, C. C. Constantinescu, D.A. Kareken, M. D. Normandin, T. Cheng, S. O'Connor, and E. D. Morris, "Heterogenous effects of alcohol on dopamine release in the striatum : a PET study," Alcohol Clin. Exp. Res., vol 31, no.6, pp 1-9 June2007.
20. S. K. Saha, C. Constantinescu, P. J. Daly, P. Bhattacharyya, C. T. Zhang, Z. W. Grabowski, B. Fornal, R. Broda, I. Ahmad, D. Seweryniak, I. Wiedenhver, M. P. Carpenter, R. V. F. Janssens, T. L. Khoo, T. Lauritsen, C. J. Lister, and P. Reiter, "Excitations two- and three-valence-proton nuclei 134Te and 135I," Physical Review C, vol. 65, no 1, Jan. 2002.

Conference Abstracts

1. M.R. Mirbolooki, C. Constantinescu, M.L. Pan, and J. Mukherjee, " 18F-FDG PET imaging of brown adipose tissue to evaluate β3-adrenoreceptor agonist efficacy in vivo", J. NUCL. Med. MEETING ABSTRACTS, vol. 54, p. 1216, May 2013.
2. J. Mukherjee, M.L. Pan, C. Constantinescu, M.R. Mirbolooki, A. Baranwal, and C. Liang, "Norepinephrine uptake imaging in rodent brain and brown adipose tissue", J. NUCL. Med. MEETING ABSTRACTS, vol. 54, p. 1119, May 2013.
3. A. Baranwal, C. Liang, M.-L. Pan, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "18F-Fluorodeoxyglycosylamine: Maillard reaction of 18F-FDG with biological amines", J NUCL MED MEETING ABSTRACTS, vol. 54, p. 1071, May2013.
4. C. C. Constantinescu, M. L. Pan, B. Patel, M. Mukherjee, H. Patel, C. Liang, M. R. Mirbolooki, and J. Mukherjee, "Preliminary evaluation of in vivo NET binding in the rat brain with [11C]Dalene and [11C]TAZA using PET imaging," Journal of Cerebral Blood Flow and Metabolism, vol. 32, no. S141 Aug.2012.
5. M. R. Mirbolooki, M. L. Pan, B. Patel, C. Liang, H. Patel, M. Mukherjee, C. Constantinescu, and J. Mukherjee, "Imaging norepinephrine transporter and glucose metabolism in rat brown adipose tissue," Journal of Cerebral Blood Flow and Metabolism, vol. 32, no. S89 Aug.2012.
6. M. L. Pan, M. Mukherjee, B. Patel, H. Patel, C. Liang, V. Vasilevko, C. Constantinescu, M. R. Mirbolooki, and J. MPanukherjee, " 11C-TAZA and 11C-Dalene: new PET imaging agents for human brain senile plaques also bind to norepinephrine transporter," Journal of Cerebral Blood Flow and Metabolism, vol. 32, no. S88 Aug.2012.
7. C. C. Constantinescu, E. Borrelli, M. L. Pan, M. R. Mirbolooki, C. Liang, and J. Mukherjee, "Evaluation of D3 versus D2 receptor binding in rats and D2R knockout mice using in vivo PET imaging with [18F]desmetoxyfallypride and [18F]fallypride," Journal of Cerebral Blood Flow and Metabolism, vol. 32, no. S47 Aug.2012.
8. J. Mukherjee, M.L. Pan, C. Liang, M. Mukherjee, V. Vasilevko, H. Patel, C. Constantinescu and M. R. Mirbolooki, "Norepinephrine binds to 11C-PIB

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labeled β-amyloid plaques in post-mortem Alzheimer's disease brain," Journal of Cerebral Blood Flow and Metabolism, vol. 32, no. S43 Aug.2012.

9. V. Bui, M. L. Pan, C. Liang, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "Schiff base PET imaging analogs for human Alzheimer's disease (AD) senile plaque," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1626, May2012.
10. C. Constantinescu, A. Corches, M. R. Mirbolooki, M. L. Pan, and J. Mukherjee, "Effect of acetylcholinesterase inhibitor, donepezil, on in vivo binding of nicotinic α4β2 receptor agonist [18F]nifene in the rat brain," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1891, May2012.
11. C. Constantinescu, M. R. Mirbolooki, B. Vikram, M. L. Pan, E. Borrelli, and J. Mukherjee, "Probing the D3R system using D2R knockout mice imaging with selective D3 agonist [18F]FHXPAT and D2/D3 antagonist [18F]fallypride," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 358, May2012.
12. C. Liang, M. L. Pan, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "Synthesis and analysis of [18F]-4-fluoromethylcyclohexanoic acid, a metabolite of 18F-Mefway," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1625, May2012.
13. D. Majji, M. L. Pan, C. Constantinescu, M. R. Mirbolooki, and J. Mukherjee, "Rodent PET evaluation of fluorine-18 labeled dopamine D3 receptor agonist, 18F-7-OH-FHXPAT," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1634, May2012.
14. M. R. Mirbolooki, C. Constantinescu, S. A. Naqvi, N. Thorosian, M. L. Pan, and J. Mukherjee, "Enhancement of brown adipose tissue metabolism through adrenergic system activation quantifiable with 18F-FDG PET/CT in mice," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1776, May2012.
15. M. R. Mirbolooki, C. Constantinescu, M. L. Pan, and J. Mukherjee, "Imaging dopamine receptors in brown adipose tissue using 18F-fallypride and 18F-FHXPAT," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 407, May2012.
16. M. Mukherjee, M. L. Pan, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "11C-TAZA, a new PET imaging agent for Aβ-amyloid plaques in the human brain," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1631, May2012.
17. B. Patel, M. L. Pan, C. Liang, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "11C-Dalene, a new agent for beta amyloid senile plaques in the human brain," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1604, May2012.
18. J. Thio, C. Liang, M. L. Pan, M. R. Mirbolooki, C. Constantinescu, and J. Mukherjee, "3-18F-Mefway, an isomeric analog of mefway for imaging serotonin 5HT1a receptors," J NUCL MED MEETING ABSTRACTS, vol. 53, no. 1_MeetingAbstracts, p. 1611, May2012.
19. C. Constantinescu, A. Garcia, E. Sevrioukov, M. L. Pan, and J. Mukherjee, "Evaluation of [18F]Nifene biodistribution based on whole-body microPET imaging of mice," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1468, May2011.
20. C. Constantinescu, A. Garcia, E. Sevrioukov, M. L. Pan, and J. Mukherjee, "Evaluation of [18F]Mefway biodistribution based on whole-body microPET

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imaging of mice," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1462, May2011.
21. V. Fong, S. Faheem, E. Sevrioukov, C. Constantinescu, M. L. Pan, N. Saigal, and J. Mukherjee, "18F-Mefway microPET imaging in rat brains," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1571, May2011.
22. V. Galitovskiy, E. Sevrioukov, A. Corches, M. L. Pan, C. Constantinescu, S. Grando, and J. Mukherjee, "18F-Nifene PET for early diagnosis of lung cancer," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1578, May2011.
23. A. Garcia, S. Grewal, N. Milne, M. R. Mirbolooki, C. Constantinescu, M. L. Pan, P. Wang, K. G. Chandy, and J. Mukherjee, "18F-Fallypride PET/CT scanning in type 1 diabetic patients," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 222, May2011.
24. A. Kharajian, C. Constantinescu, R. Coleman, M. L. Pan, and J. Mukherjee, "Comparative PET imaging of D2/D3 receptors in the rodent spinal cord with [18F]fallypride and [11C]fallypride," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1193, May2011.
25. M. R. Mirbolooki, E. Sevrioukov, A. Garcia, C. Constantinescu, and J. Mukherjee, "Imaging brown adipose tissue activation using β3-adrenoceptor agonist," J NUCL MED MEETING ABSTRACTS , vol. 52, no. 1_MeetingAbstracts, p. 516, May2011.
26. S. Mistry, M. L. Pan, E. Sevrioukov, C. Constantinescu, and J. Mukherjee, "Evaluation of 18F-FBM in triple transgenic mice," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 1572, May2011.
27. N. Pithia, N. Gulati, M. L. Pan, E. Sevrioukov, C. Constantinescu, and J. Mukherjee, "18F-Festron: A potential serotonin 5-HT3 receptor PET imaging agent," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 227, May2011.
28. W. Tang, V. Galitovskiy, E. Sevrioukov, M. L. Pan, C. Constantinescu, S. Grando, and J. Mukherjee, "18F-FAHA PET for imaging histone deacetylase in lung cancer," J NUCL MED MEETING ABSTRACTS, vol. 52, no. 1_MeetingAbstracts, p. 411, May2011.
29. T. Jerjian, C Constantinescu, E. Sevrioukov, J. Mukherjee. "Evaluation of dopamine D3 receptor binding of 18F-fallypride". J Nuclear Medicine, vol. 51, Supplement 2, p. 1762, 2010
30. K. Vu, E. Sevrioukov, C Constantinescu, M-L Pan, R. Pichika, J. Mukherjee. "Dual probe microPET imaging of rodent pancreas". J Nuclear Medicine, vol. 51, Supplement 2, p. 219, 2010
31. E. Sevrioukov, A. Garcia, C. Constantinescu, J. Mukherjee. "Inveon microCT imaging of the rat spleen". J Nuclear Medicine, vol. 51, Supplement 2, p. 219, 2010
32. A. Garcia, E. Sevrioukov, C Constantinescu, M-L Pan, K.G. Chandy, J. Mukherjee. "Dual probe microPET imaging of rodent pancreas". J Nuclear Medicine, vol. 51, Supplement 2, p. 88, 2010
33. C. Constantinescu, K. Kasabwala, S. Pandey, M. Pan, R. Coleman, R. Kant, J Mukherjee. "Comparative Rodent MicroPET Study of 18F-Fallypride and 18F-Desmethoxyfallypride". J Nuclear Medicine, vol. 50, Supplement 2, p. 1207, 2009
34. R.Kant, P. Parekh, S. Pandey, M. Pan, R. Coleman, C. Constantinescu, J Mukherjee. "Evaluation of {alpha}4{beta}2 receptor occupancy by

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CURRICULUM VITAE
Danna Jennings, MD

21 May 2013

60 Temple Street, Suite 8B
New Haven, CT 06510
(203) 401-4333

75 Spring Garden Street
Hamden, CT 06517
(203) 668-5649 cell

CURRENT EMPLOYMENT:

2001-present	Vice President and Senior Director of Research, Molecular NeuroImaging, LLC
2001-present	Senior Director of Research, Institute for Neurodegenerative Disorders
1998-2001	Assistant Professor of Neurology, Yale University
1998-2001	Attending Physician, Department of Neurology, Yale University

EDUCATION:

1996-98	Movement Disorders Fellow, Columbia Presbyterian, NY
1995-96	Neurology Chief Resident, Boston University Medical Center
1993-96	Neurology Resident, Boston University Medical Center
1992-93	Internal Medicine Internship, Boston City Hospital
1988-92	Oregon Health Sciences University, School of Medicine, MD
1985-88	Oregon Health Sciences University, School of Nursing, BSN
1981-85	Willamette University, Salem, Oregon, BSc

PROFESSIONAL ASSOCIATIONS

American Neurological Association (1994 - present)
The Movement Disorders Society (1996 - present)
Huntington Study Group (1998 - present)
Parkinson Study Group (1998 - present)

COMMITTEE ASSIGNMENTS

Michael J. Fox Foundation, PPMI Steering Committee (10/09 – present)
Michael J. Fox Foundation, Peer Grant Review (4/07 - present)
Parkinson Disease Foundations, Peer Grant Review (3/10 – present)
Parksinon Diseae Foundation, Medical Advisory Board (10/13 – present)

TEACHING EXPERIENCE

Yale Medical School, Teaching Faculty (1998-2001)
Yale Medical School, Lecturer (2001-2005)
Yale School of Public Health, Lecturer (1999-2007)

CLINICAL RESEARCH EXPERIENCE

Alzheimer Disease Study Participation

Site Investigator, A Phase 3 Multi-Center, Randomized, Double-blind, Placebo-controlled, Parallel-Group, Efficacy and Safety Trial of Bapineuzumab (AAB-001, ELN115727) in Patients with Mild to Moderate Alzheimer's Disease

Site Investigator, An open-label, non-randomized, multi-center study to optimize image assessment and evaluate the efficacy and safety of BAY 94-9172 positron emission tomography (PET) for detection/exclusion of cerebral amyloid beta in patients with probable Alzheimer's disease compared to healthy volunteers

Site Investigator, Effect of Passive Immunization on the Progression of Alzheimer's Disease: LY2062430 versus Placebo

Coordinating Investigator, An open-label, non-randomized study to evaluate the efficacy and safety of Bay 94-9172 positron emission tomography (PET) for detection of cerebral β-amyloid in individuals with Down syndrome compared to individuals without Down syndrome.

Site Investigator, Safety Assessment of Neuroptix SAPPHIRE II System to Aid in the Diagnosis of Probable Alzheimer's Disease in five normal subjects and five patients with probable Alzheimer's disease.

Site Investigator, An open label, parallel group, multi-center study, evaluating the safety and imaging characteristics of 18F-AV-45 in healthy volunteers, patients with mild cognitive impairment (MCI) and patients with Alzheimer's disease (AD)

Site Investigator, A Clinical Trial to Characterize the Performance of MK-3328 in Subjects with Alzheimer's Disease or Mild Cognitive Impairment

Principal Investigator, An Exploratory, open-label, non-randomized study to evaluate the efficacy and safety of MNI-558 positron emission tomography (PET) for detection/exclusion of cerebral amyloid beta in patients with Alzheimer's disease compared to healthy volunteers.

Site Investigator, A Phase II clinical trial to evaluate the efficacy and safety of [^{18}F]AZD4694 PEY in the detection of beta amyloid in subjects with probable Alzheimer's disease, older healthy volunteers and young healthy volunteers.

Investigator Initiated Studies

Principal Investigator, A single dose open label study of CNS distribution of [^{123}I]NAV5001 (Altropane) in healthy volunteers by Single Photon Emission Tomography (SPECT)

Principal Investigator, An open-label, non-randomized study to evaluate the feasibility of [^{18}F]Florbetapir positron emoission tomography (PET) for assessment of demyelination and subsequent remyelination in patients with relapsing remitting multiple sclerosis

Principal and Coordinating Investigator, Query-PD Study: Development of [^{123}I]β-CIT SPECT as a diagnostic tool in Parkinsonian Syndrome, NIH SBIR funded study

Coordinating Investigator, InSPECT: Investigation of the effect of levodopa and pramipexole on [^{123}I]β-CIT/SPECT, multi-site clinical and imaging study (12 sites), DOD funded

Principal Investigator, ANAM-PD Study: (Automated Neuropsychological Assessment Metric in Parkinson Disease) Development of Imaging and Neuropsychological Markers for Changes in Parkinson Disease, DOD funded

Coordinating Investigator, PARS Study: Parkinson Associated Risk Syndrome Study (An at-risk study for first degree relative of PD patients), multi-site study to evaluate olfaction and annual clinical examinations in relatives of PD patients (20 sites), DOD funded

Coordinating Investigator, VIEW-AD Study: Validation of Imaging Evaluations in Alzheimer Disease, multi-site clinical and imaging study in AD subjects (6 sites), privately funded

Principal Investigator, Development of Amyloid Imaging Agents in Alzheimer Disease, Alzheimer Association funding (J. Seibyl)

Sub-Investigator, Pfizer Speech Study: Randomized, Open-Label Methods of Validation Study of Acoustical Analyses of Speech to Detect Change in Motor Control of the Vocal Apparatus in Mild and Moderate Parkinson's Disease, and Relationship Between Speech Changes and Striatal Dopamine Transporter

NIH Exploratory Trials in Parkinson's Disease
Site Investigator, A Multicenter, Double-Blind, Futility Study of Minocycline, Creatine and CoQ10 in subjects with early untreated Parkinson's Disease (PD) (**FS-1**)

Site Investigator, A Multicenter, Double-Blind, Pilot Study of CoQ10 and GPI 1485 in subjects with early untreated Parkinson's Disease (PD) (**FS-TOO**)

Site Investigator, A Multicenter, Double-Blind Parallel Group, Placebo Controlled Study of Creatine in Subjects with Treated Parkinson's Disease (PD) (**LS-1**)

Parkinson Study Group Studies
Site Investigator, A Multicenter Randomized Double-Blind Placebo Controlled Parallel Group Dose Ranging Study to Assess the Efficacy Safety and Tolerability of Escalating Transdermal Doses of SMP 962 (Rotigotine) in Subjects with Early Stage Parkisnon's Disease (**PATCH**)

Site Investigator, A Multicenter US and Canada Double Blind Randomized Placebo Controlled Parallel Group Study for the Efficacy Tolerability and Safety of Rasagiline Mesylate in Levodopa Treated Parkinson's Disease Patients with Motor Fluctuations (**PRESTO**)

Site Investigator, A Multicenter Randomized Double Blind Dummy Parallel Group Study Comparing TV-1203/Carbidopa Dispersible Tablets with Levodopa/Carbidopa Tablets in Advanced Parkinson's Disease Patients with Motor Fluctuations (**RAPID**)

Site Investigator, Earlier Versus Later Levodopa in Parkinson's Disease: A Multi-center Investigation by the Parkinson's Study Group (**ELLDOPA**)
Site Investigator PROGENI

Site Investigator, A Multi-center Open Label Study to Evaluate the Effect of CEP-1347 on CIT Uptake in SPECT Imaging in Participants with PD (**CEPCIT**)

Site Investigator, A Randomized Double Blind Placebo Controlled Study to Evaluate the Pharmacokinetics Tolerability and Safety of CEP-1347 when Administered With and Without Levodopa in Participants with Parkinson's Disease (**CONCEPT**)

Site Investigator, Parkinson's Disease Collaborative Study of Genetic Linkage (**PROGENI**)

Site Investigator, A Randomized, Double-Blind, Placebo-Controlled, Dose-Finding Study to Assess the Efficacy and Safety of CEP-1347 in Patients With Early Parkinson's Disease Assessing the determinants of PD progression (**PRECEPT**) and Long-term dopamine transporter imaging in the PRECEPT cohort

Site Investigator, A longitudinal Observational Follow-up of the PRECEPT Study Cohort (**PostCEPT**)

Site Investigator, Blood α-Synuclein, Gene Expression, and Smell Testing as Diagnostic and Prognostic Biomarkers in Parkinson's Disease (**PROBE**)

Huntington Study Group Studies
Site Investigator, Prospective Huntington At Risk Observational Study (**PHAROS**)

Site Investigator, Minocycline Dosing and Safety in Huntington's disease (**MINO**)

Site Investigator, A Multi-Center, Double-Blind, Randomized, Parallel Group, Placebo-Controlled Trial of Ethyl-EPA (Miraxion™) in Subjects with Mild to Moderate Huntington's Disease (**TREND-HD**)

Site Investigator, Pilot Safety and Tolerability Study of Coenzyme Q10 in Huntington's Disease and in Normal Subjects (**Pre2CARE**)

Site Investigator, Coenzyme Q10 in Huntington's Disease (**2CARE**)

Other Study Participation

Steering Committee Member and Site Investigator, Parkinson Progression Marker Initiative (PPMI) (June 2010- present).

Lead Investigator, Boehringer Ingelheim Mirapex Eye Safety Study (10/05 – 6/2011)

Sub-Investigator, A Phase, 2 Multi-Center, Randomized, Double-Blind, Placebo Controlled, Parallel-Group, 2-Year Study To Evaluate The Effects of GPI 1485 (1000 Mg QID) on [^{123}I]ß CIT/SPECT Scanning And Clinical Efficacy In Symptomatic Parkinson's Disease Patients Receiving Dopamine Agonist Therapy

Sub-Investigator, Randomized Double-Blind, Placebo Controlled, Parallel Group, 6-Month Safety Efficacy and Neuroimaging Trial of AMG-474-00 in the Treatment of Patients with Parkinson's Disease

Sub-Investigator, Double Blind Placebo Controlled Dose Response Study of Tolerability, Safety and Efficacy of Sumanirole in Patients with Early Parkinson's disease

Sub-Investigator, A Phase III Multi-Center Parallel Group Placebo Controlled Study of the Effect of Riluzole 50 mg bid or 100 mg bid on the Progression of Parkinson's disease Patients

Site Investigator, A Prospective Randomized Parallel Group Double-Blind Placebo Controlled Multi-center Study to Evaluate the Short Term Safety and Efficacy of Entacapone Administered together with Levodopa in Patients with Non-Fluctuating Parkinson's Disease

Sub-Investigator, A randomized Double Blind Parallel Group Study to Compare the safety and Efficacy of Zydis Selegiline 1.25 QD with Placebo as an Adjunct in the Management of Parkinsonian Patients being Treated with Levodopa who Exhibit Deterioration in the Quality of life

Site Investigator, A multi-center, multi-national, phase III, randomized, double-blind, placebo controlled trial, of the efficacy and safety of the rotigotine CDS patch in subjects with early stage, idiopathic Parkinson's disease (Part I), and an open-label extension to assess the safety of long-term treatment of rotigotine CDS (Part II)

Site Investigator, A multi-center, multinational, phase III, randomized, double-blind, placebo controlled trial of the efficacy and safety of rotigotine CDS patch (2 target doses) in subjects with advanced stage, idiopathic Parkinson's disease who are not well controlled on levodopa (Part I) and open-label extension to assess the safety of long-term treatment of rotigotine CDS (Part II)

Site Investigator, A Multi-Center, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study of the Efficacy, Safety, and Tolerability of E2007 in Levodopa Treated Parkinson's Disease Patients with Motor Fluctuations

Site Investigator, ^{18}F-AV-133-A02: Test-retest reproducibility of ^{18}F-AV-133 for PET brain imaging of striatum in healthy volunteers and Parkinson's disease patients

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Marek K, Jennings D, Tamagnan G, Seibyl J. Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression. Ann Neurol, 2008 (Suppl 2):S111-121.

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Marek K, Jennings D, Tamagnan G, Seibyl J. Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression. Ann Neurol 2008; 64 (suppl 2):S111-21.

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Durso R, Evans BA, Handler JS, Jennings D, Browne TR. Central Levodopa Metabolism in Parkinson's Disease after Administration of Isotope Labeled Levodopa. Ann Neurol 42(3): 300-304, 1997.

Durso R, Evans JE, Josephs E, Szabo B, Handler J, Jennings D, Feldman RG. Reexamination of Peripheral and Central Levodopa Metabolism Using Stable Isotope Labeled Levodopa, Neurology 45:416S, 1995.

Curriculum Vitae and Bibliography

Name: **David Stewart Russell, M.D., Ph.D.**

Associate Director for Clinical Research
Institute for Neurodegenerative Disorders
and
Molecular NeuroImaging, LLC
New Haven, CT
drussell@indd.org

Employer:

2007-present:
Institute for Neurodegenerative Disorders (IND)
60 Temple Street, Suite 8B
New Haven, CT 06510
phone: 203-401-4346
fax: 203-401-4301
email: drussell@indd.org

2007-present:
Molecular Neuroimaging, L.L.C. (MNI)
60 Temple Street, Suite 8A
New Haven, CT 06510
phone: 203-401-4346
fax: 203-789-8037
email: drussell@mnimaging.com

Current Academic Appointment: 1995-present:
Assistant Clinical Professor
Yale University School of Medicine
New Haven, CT 06510
david.russell@yale.edu

Clinical Trial Experience:

Principal/Principal Site Investigator for 10-15 clinical trials primarily in Alzheimer disease and Parkinson disease; and also multiple sclerosis, Huntington disease, and post-traumatic stress disorder; 1995-present.

Co-Investigator/Site Co-Investigator for 30-40 clinical trials primarily in Alzheimer disease and Parkinson disease; and also multiple sclerosis, Huntington disease, Fragile X Syndrome, and Down Syndrome; 1995-present.

Principal Investigator or Co-Investigator for 10-15 Phase I trials with healthy control subjects.

Born: May 13, 1960, Cleveland, Ohio

Education:

Medical Scientist Training Program (MD-PhD)
Cornell University Medical College and
Memorial-Sloan Kettering Cancer Center,
1983-1991, New York, N.Y.
-M.D., C.U.M.C., 1991
-Ph.D., Molecular Biology, 1991
Ora M. Rosen, MD, sponsor
in the Sloan Kettering Division, C.U.M.C.
Thesis: *The human insulin receptor: Mechanisms of action*
B.A., Oberlin College
1978-1982, Oberlin, Ohio

Post-Doctoral Medical Training:

Chief Resident, Neurology, Yale-New Haven Hospital
1994-1995, New Haven, Connecticut
Residency, Neurology, Yale-New Haven Hospital
1992-1994, New Haven, Connecticut
Internship, Internal Medicine, Yale-New Haven Hospital

1991-1992, New Haven, Connecticut

Board Certification: 1997, American Board of Psychiatry and Neurology (Neurology)
2007, recertification

Movement Disorders Experience: 1995-2001, Attending, Yale Movement Disorders Clinic
2001-2007, Director, Yale Movement Disorders Consultation Clinic, responsible for the evaluation and treatment of a broad range of movement disorders, other neurodegenerative disorders and dementias, and dystonias.

Clinical Trial Experience: 1995-present: PI or co-I for 32 trials of Parkinson disease
2007-present: PI or co-I for 18 trials of Alzheimer disease
2008-present: PI or co-I for 8 trials for other brain disorders

Further Basic Research Training during Residency/Fellowship:
Eric J. Nestler, MD, PhD, sponsor
in the Laboratory of Molecular Psychiatry,
Departments of Psychiatry and Pharmacology,
Yale University School of Medicine
1993-1994, New Haven, Connecticut

Ongoing Teaching Responsibilities:
Yale School of Medicine,
Medical Students, Courses Years 1,2,3,and 4
Residents, Departments of Neurology and Psychiatry
Physician Assistants Training Program

Hospital Clinical Appointments:
Attending Neurologist, University Staff, Yale-New Haven Hospital
1995-present, New Haven, Connecticut

Professional Memberships:

American Association for the Advancement of Science	1985-present
Society for Neuroscience	1993-present
Movement Disorders Society	1997-present
American Academy of Neurology	2007-present

Other Research Experience:
Honors Research Program at Oberlin College
Research Technician, St. Luke's Hospital (Dr. Dale Cowan)
Summers: 1976, 1979-1981, Cleveland, Ohio
Research Technician, Case Western Reserve University
(Dr. Charles Miller), Summer: 1982, Cleveland, Ohio

Research Fellowships:	Rudin/Morton Fellowship	1988-90
	KSB Medical Scientist Fellowship	1983, 1986-87
	Surdna Foundation Medical Scientist Fellowship	1984-85

Honors and Awards:	Administrative Chief Resident, Neurology	1994
	Julian R. Rachele Award for an Outstanding Research Paper	1987
	C.U.M.C. representative at the New York State Association of Medical Schools Research Symposium	1988

Phi Beta Kappa	1981
Sigma Xi	1982
Graduation with Honors in Biology	1982
F.F. Jewett Award for Chemistry	1980
Bausch and Lomb Award for Scientific Achievement	1978
Ohio Academic Scholarship	1978
National Honors Society	1978
Commended Merit Scholar	1978

Publications:

Original Publications (peer reviewed)

1. Chou, C.K., Dull, T., Russell, D.S., Gherzi, R., Lebwohl, D., Ullrich, A., and Rosen, O.M. "Human insulin receptors mutated in the ATP-binding site fail to mediate post-receptor actions of insulin." J. Biol. Chem., 262:1842-1847 (1987).

2. Russell, D.S., Gherzi, R., Johnson, E.L., Chou, C.K., and Rosen, O.M. "The protein tyrosine kinase activity of the insulin receptor is necessary for insulin-mediated receptor down-regulation." J. Biol. Chem., 262:11833-11840 (1987).

3. Gherzi, R., Russell, D.S., Taylor, S.I., and Rosen, O.M. "Reevaluation of the evidence that an antibody to the insulin receptor is insulinmimetic without activating the protein tyrosine kinase activity." J. Biol. Chem., 262:16900-16905 (1987).

4. Villalba, M., Wente, S.R., Russell, D.S., Ahn, J.C., Reichelderfer, C.F., and Rosen, O.M. "Another version of the human insulin receptor kinase domain: Expression, purification and characterization." Proc. Natl. Acad. Sci U.S.A., 86:7848-7852 (1989).

5. Villalba, M., Alvarez, J.F., Russell, D.S., Mato, J.M., and Rosen, O.M. "Hydrolysis of glycosyl-phosphatidylinositol in response to insulin is reduced in cells bearing kinase-deficient insulin receptors." Growth Factors, 2:91-97 (1990).

6. Widnell, K.L., Russell, D.S., and Nestler, E.J. "Regulation of cAMP response element binding protein (CREB) expression in the locus coeruleus in vivo and in a LC-like cell line in vitro." Proc. Natl. Acad. Sci U.S.A., 91:10947-10951 (1994).

7. Berhow, M.T., Russell, D.S., Terwilliger, R.S., Beitner-Johnson, D., Self, D.W., Lindsay, R.M., and Nestler, E.J. "Influence of neurotrophic factors on morphine- and cocaine-induced biochemical changes in the mesolimbic dopamine system." Neuroscience, 68:969-979 (1995).

8. Widnell, K.W., Self, D.S., Lane, S.B., Russell, D.S., Vaidya, V.A., Miserendino, M.J.D., Rubin, C.S., Duman, R.S., and Nestler, E.J. "Regulation of CREB expression: In vivo evidence for a functional role in morphine action in the nucleus accumbens." J. Pharmacol. Exp. Ther., 276:306-315 (1996).

9. Numan, S., Lane-Ladd, S.B., Zhang, L., Lundgren, K.H., Russell, D.S., Seroogy, K.B., and Nestler, E.J. "Differential regulation of neurotrohins and their Trk receptors in catecholaminergic nuclei during chronic opiate treatment and withdrawal." J. Neurosci 18:10700-10708 (1998)

10. Oh, J.D., Russell, D.S., Vaughan, C.L., and Chase T.N. "Enhanced tyrosine phosphorylation of dopaminergic denervation and L-DOPA administration." Brain Research 813:150-159 (1998)

11. Numan, S., Lane, S.B., Zhang, L., Lundgren, K.H., Russell, D.S., Seroogy, K.B., and Nestler, E.J. "Differential regulation of neurotrophin and trk receptor mRNAs in catecholaminergic nuclei during chronic opiate treatment and withdrawal." J. Neurosci. 18:10700-10708. (1998)

12. Wolf, D.H., Numan, S., Nestler, E.J., and Russell, D.S. "Regulation of phospholipase C □ in the mesolimbic dopamine system by chronic morphine administration." J. Neurochem. 73:1520-1528 (1999)

13. Nibuya, M., Takahashi, M., Russell, D.S., and Duman, R.S. "Repeated stress increases catalytic TkrB mRNA in rat hippocampus." Neurosci. Letts. 267:81-84. (1999)

14. Fryer, H.J.L., Wolf, D.H., Knox, R., Strittmatter, S.M., Pennica, D., O'Leary, R., Russell, D.S., and Kalb R.G. "Brain-derived neurotrophic factor induces excitotoxic sensitivity in cultured embryonic rat spinal

motor neurons through activation of the phosphatidylinositol 3-kinase pathway." J. Neurochem. 74:582-595. (1999)

15. Numan, S. and Russell, D.S. "Discrete expression of insulin receptor substrate-4 mRNA in adult rat brain." Molecular Brain Research 72:97-102 (1999)

16. Messer, C.J., Eisch, A.J., Carlezon, W.A., Whisler, K., Shen, L., Wolf, D.H., Westphal, H., Collins, F., Russell, D.S., and Nestler, E.J. "Role for GDNF in biochemical and behavioral adaptations to drugs of abuse." Neuron 26:247-257 (2000).

17. Holloway, R. Shoulson, I. Kieburtz, K. McDermott, M. Tariot, P. et al. "Pramipexole vs levodopa as initial treatment for Parkinson disease - A randomized controlled trial." JAMA 284:1931-1938 (2000)

18. White, B.H., Cummins, T.R., Wolf, D.H., Waxman, S.G., Russell, D.S., and Kaczmarek, L.K. "The HSV-1 helper virus 5DL1.2 supresses sodium currents in amplicon-transduced neurons." J. Neurophysiol. 87:2149-2157 (2002)

19. Shirayama, Y., Chen, A.C.-H., Nakagawa, S., Russell, D.S., and Duman, R.S. "Brain derived neurotrophic factor produces antidepreessant effects in behavioral models of depression." J. Neurosci. 22:3251-3261 (2002)

20. Brunzell, D.H., Russell, D.S. and Picciotto, M.R. "In vivo nicotine treatment regulates mesocorticolimbic CREB and ERK signaling in C57Bl/6J mice." J. Neurochem. 84:1431-1441 (2003)

21. Bolaños, C.A., Perrotti, L.I., Edwards. S., Eisch, A.J., Barrot, M., Olson, V.G., Russell, D.S., Neve, R.L. and Nestler, E.J. "Phospholipase Cgamma in distinct regions of the ventral tegmental area differentially modulates mood-related behaviors." J. Neurosci. 23:7569-7576 (2003)

22. Sheehan, T.P., Neve, R.L., Duman, R.S., Russell, D.S. "Antidepressant effect of the calcium-activated tyrosine kinase Pyk2 in the lateral septum." Biol. Psychiatry 54:540-551 (2003)

23. Tolbert, L.M., Russell, D.S., and Duman, R.S. "Norepinephrine activates extracellular-regulated kinase in cortical neurons." Biol. Psychiatry 15:983-993 (2003)

24. Madsen, T.M., Newton, S.S., Eaton, M.E., Russell, D.S., and Duman, R.S. "Chronic electroconvulsive seizures up-regulates beta-catenin expression in rat hippocampus: Role in adult neurogenesis." Biol. Psychiatry 15:1006-1014 (2003)

25. Sheehan, T.P., Chambers, R.A., and Russell, D.S. "Regulation of affect by the lateral septum: Implications for neuropsychiatry." Brain Res. Rev. 46:71-117 (2004)

26. Newton, S.S., Collier, E.F., Bennett A.H., Russell, D.S., and Duman, R.S. "Regulation of growth factor bound 2 by electroconvulsive seizure." Mol. Brain Res. 129:185-188 (2004)

27. Parkinson Study Group. "Levodopa and the rate of progression of Parkinson's disease, the ELLDOPA study." N. Engl. J. Med. 351:2498-2508 (2004)

28. Holloway, R.G., Shoulson, I., Fahn, S., Kieburtz, K., Lang, A., et al. "Pramipexole vs levodopa as initial treatment for Parkinson disease: A 4-year randomized controlled trial." Arch Neurol. 61:1044-1053 (2004)

29. Dow, A.L., Russell, D.S., and Duman, R.S. "Regulation of activin mRNA and Smad2 phosphorylation by antidepressant treatment in the rat brain: effects in behavioral models." J Neurosci. 25:4908-4916 (2005)

30. Kodama, M., Russell, D.S., and Duman, R.S. "Electroconvulsive seizures increase the expression of MAP kinase phosphatases in limbic regions of rat brain." Neuropsychopharmacology. 30:360-371 (2005)

31. Duman, C.H., Schlesinger, L., Kodama M., Russell D.S., and Duman R.S. "A role for MAP Kinase signaling in behavioral models of depression and antidepressant treatment." Biol. Psychiatry 61:661-670 (2007)

32. Mojsilovic-Petrovic, J., Jeong, G.B., Crocker, A., Arneja, A., David, S., Russell, D.S., Kalb, R.G. "Protecting motor neurons from toxic insult by antagonism of adenosine A2a and Trk receptors." J Neurosci. 26:9250-9263 (2006).

32. Russell, D.S. "Benign tremulous parkinsonism." Arch Neurol. 63:1346 (2006).

33. Russo, S.J., Bolanos, C.A., Theobald, D.E., Decarolis, N.A., Renthal, W., Kumar, A., Winstanley, C.A., Renthal, N.E., Wiley, M.D., Self, D.W., Russell, D.S., Neve, R.L., Eisch, A.J., Nestler, E.J. "IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates." Nat Neurosci. 10:93-99 (2007)

34. Wolf, D.H., Nestler, E.J., and Russell, D.S. "Regulation of neuronal PLCgamma by chronic morphine." Brain Res. 1156:9-20 (2007).

35. Kosten, T.A., Galloway, M.P. Duman, R.S., Russell, D.S., and D'Sa, C. "Repeated Unpredictable Stress and Antidepressants Differentially Regulate Expression of the Bcl-2 Family of Apoptotic Genes in Rat Cortical, Hippocampal, and Limbic Brain Structures." Neuropsychopharmacology 33:1545-1558 (2008).

36. Duman, C.H. Schlesinger, L., Russell, D.S., and Duman, R.S. "Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice." Brain Res. 1199:148-158 (2008). [Erratum in: Brain Res. 1218:313 (2008)].

37. Hunsberger, J.G., Newton, S.S., Bennett, A.H., Duman, C.H. Russell, D.S., Salton, S.R. and Duman, R.S. "Antidepressant actions of the exercise-regulated gene VGF", Nat Med. 13:1476-1482 (2007)

38. Schifitto, G., Friedman, J.H., Oakes, D., Shulman, L., Comella, C.L., Marek, K., Fahn, S. and the Parkinson Study Group ELLDOPA Investigators. "Fatigue in levodopa-naive subjects with Parkinson disease." Neurology 71:481-485 (2008)

39. Kosten, T.A., Galloway, M.P., Duman, R.S., Russell, D.S., and D'Sa, C. "Repeated unpredictable stress and antidepressants differentially regulate expression of the bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures." Neuropsychopharmacology. 33:1545-58 (2008)

40. Duman, C.H., Schlesinger, L., Terwilliger, R., Russell, D.S., Newton, S.S., and Duman, R.S. "Peripheral insulin-like growth factor-I produces antidepressant-like behavior and contributes to the effect of exercise." Behav Brain Res. 198:366-371 (2009)

41. Parkinson Progression Marker Initiative. "The Parkinson Progression Marker Initiative (PPMI)." Prog Neurobiol. 95:629-35 (2011)

42. Siderowf, A., Jennings, D., Eberly, S., et al. "Impaired olfaction and other prodromal features in the Parkinson At-Risk Syndrome Study." Mov Disord. 27:406-12 (2012)

43. Seibyl, J., Russell, D.S., Jennings, D., and Marek, K. "Neuroimaging over the course of Parkinson's disease: from early detection of the at-risk patient to improving pharmacotherapy of later-stage disease." Semin Nucl Med. 42:406-14 (2012).

44. Elm, J.J. and the NINDS NET-PD Investigators. "Design innovations and baseline findings in a long-term Parkinson's trial: the National Institute of Neurological Disorders and Stroke Exploratory Trials in Parkinson's Disease Long-Term Study-1." Mov Disord. 27:1513-21 (2012).

Case Reports

1. Hisama, F.M., Lee, H.H., Vashlishan, A., Tekumalla, P., Russell, D.S., Auld, E., Goldstein, J.M. "Clinical and molecular studies in a family with probable X-linked dominant Charcot-Marie-Tooth disease involving the central nervous system." Arch. Neurol. 58:1891-1896 (2001)

Reviews

1. Russell, D.S. "Neurotrophins: Mechanisms of action" The Neuroscientist, 1:3-6 (1995).

2. Russell, D.S. "Neurotrophins: New players, clinical uses?" The Neuroscientist, 1:119-122 (1995).

3. Russell, D.S., Widnell, K.L., and Nestler, E.J. "Antisense oligonucleotides: New tools for the study of brain function." The Neuroscientist, 2:79-82 (1996).

4. Russell, D.S. and Duman, R.S. "Neurotrophic factors and intracellular signal transduction pathways." Neuropsychopharmacology: The Fifth Generation of Progress. David, K.L., Charney, D., Coyle, J.T. and Nemeroff, C. (eds.), Lippincott, Williams & Wilkins, New York (2002)

5. Seibyl, J., Russell, D., Jennings, D., and Marek, K. "The molecular basis of dopaminergic brain imaging in Parkinson's disease" Q J Nucl Med Mol Imaging, 56:4-16 (2012)

gnicoletti@mnimaging.com

Gina M. Nicoletti

Experience

2000 to Present
Institute of Neurodegenerative Disorders/
Molecular NeuroImaging, LLC.
New Haven, CT 06510

Nuclear Medicine Technologist
- Chief Technologist
- Staff Nuclear Medicine technologist

Major Responsibilities
Primary responsibilities to fulfill this role include:
- Inject and image subjects according to defined protocols, and investigational Nuclear Medicine procedures
- Interpret, synthesize and analyze the quality of image data on a variety of computer platforms
- Interact with other managers to assure compliance to protocol specifications & needs
- Review equipment daily to ensure it confirms to quality control standards
- Review paper trail, from point of injection, to ensure compliance
- Write papers on brain imaging for peer reviewed journals; make presentations on work being performed at MNI to the staff and at regional and national professional meetings.
- Perform image analysis for specific studies.

1998
Yale University NeuroSPECT Center
New Haven, CT 06510
Nuclear Medicine Image Analysis
- Analysis of Nuclear Medicine Images for research in Epilepsy

1986-1998
Hospital of Saint Raphael
New Haven, CT 06510
- Staff Technologist in a large teaching hospital

1985
Yale University School of Medicine
New Haven, CT 06510

1-27-14

- Nuclear Medicine Image Analysis of cardiac scans

Education

1984-1986
South Central Community College
New Haven, CT 06510

- Associate of Science Degree in Nuclear Medicine
- Graduated *cum laude*

Professional Certifications

CNMT
RT(N)

Memberships

Society of Nuclear Medicine
New England Chapter of the Society of Nuclear Medicine Technology
Brain Imaging Council

Publications

GN Morano, John P. Seibyl
Technical overview of brain SPECT imaging: improving acquisition and processing of data.
J Nucl Med Technol. 2003 Dec;31(4):191-5

Robert A. Avery, Susan Spencer, Colin Studholme, Rik Stokking, **Gina Morano**, Maria Corsi, John P. Seibyl, Dennis D. Spencer, I. George Zubal
Reproducibility of serial peri-ictal single-photon emission tomography difference images in epilepsy patients undergoing surgical resection.
Eur J Nucl Med. 2000 Jan;27(1):50-5

Regional Cerebral Blood Flow Measured with SPECT Difference Imaging During ElectroConvulsive Therapy (ECT)-Induced Seizures
Robert A. Avery, Susan Spencer, Colin Studholme, Arien, Jonathan Smith, Maria Corsi, **Gina Morano**, Robert Ostroff, John Seibly, I George Zubal, Susan S. Spencer, and Hal Blumenfeld

Effect on quantitative ROI Striatal Binding Ratios (SBR) due to external point sources used as fiducial markers for brain imaging.
Andrea Perez*, George Zubal, **Gina Nicoletti**, Stacey Ross, Danna Jennings, Kenneth Marek, John Seibyl. Division of Imaging Sciences, Institute for Neurodegenerative Disorders, New Haven, Connecticut, 06510, USA

Presentations

Dopaminergic Function in Movement Disorders: A look at Pre, Intra, and Post Synaptic Imaging. **Gina N. Morano**, A. Perez, S. Ross, J. Bartosik Molecular NeuroImaging, LLC. New Haven, CT.

Review of Clinical Brain Imaging. **Gina N. Morano**, CNMT, RT(N)
Research Technologist. Molecular NeuroImaging, LLC. New Haven, CT 06510

Multi-Center Brain Imaging, Clinical Research Trials
Gina Morano, CNMT., J. Seibyl MD.Andrea Perez BS., CNMT.,
Molecular NeuroImaging L.L.C.

Jennifer R. Madonia, MS, PA-C

Madonia 2024
21 May

SUMMARY

Recognized as an energetic, articulate leader, effective communicator and team player. Participation in professional organizations on the state and national level and with hospital leadership and administration has allowed for the development of strong organizational and interpersonal skills and the opportunity to act as an effective advocate, forward thinker and communicator. I am currently seeking a position that will provide challenging opportunities and allow for professional development.

PROFESSIONAL EXPERIENCE

More than twelve years experience working with challenging patient populations. My compassion and work ethic has instilled confidence in my patients and the community of referring physicians.

2012-present	**Associate Director of Clinical Research Affairs** Clinical Research Physician Assistant (Oct 2012- May 2014) Molecular NeuroImaging/ Institute for Neurodegenerative Disorders, New Haven, CT
2013- present (per diem)	Neurosurgical Associates of Southwestern Connecticut, Danbury
2012-2014 (per-diem)	Ortho/Neuro Unit Physician Assistant, Danbury Hospital
2005- 2012	Physician Assistant in Orthopaedic Spine Surgery Connecticut Neck & Back Specialists, LLC, Danbury, CT
2002 -2004	Neurosurgical Physician Assistant Perry Shear, M.D., PC, Bridgeport, CT
1999 -2002	Emergency Department Physician Assistant Danbury Hospital, Danbury, CT Saint Francis Hospital and Medical Center, Hartford, CT
1995-1997	Chemist, Quality Assurance Bayer Corporation, West Haven, CT

EDUCATION

1999 Master of Science in Physician Assistant Studies, Beaver College, Glenside, PA
1995 Bachelor of Science in Chemistry, Sacred Heart University, Fairfield, CT

AWARDS RECEIVED

Distinguished Fellow of the American Academy of Physician Assistants 2011
Leadership Award, Connecticut Academy of Physician Assistants 2004
Connecticut Physician Assistant Foundation 1997 Scholarship
President Clinton's Volunteer Youth Service Award 1994

LANGUAGES Fluent in Spanish

Jennifer R. Madonia, MS, PA-C

Jmadonia 27 May 2014

PUBLICATIONS

Madonia-Barr J., Lumbar Artificial Disc Replacement: A Triumph of Technology Over Reason? *Journal of the American Academy of Physician Assistants* 2007 Aug (20)8 30-33.

Madonia-Barr J., Kramer DL. Interspinous Process Decompression with the X-Stop Device for Lumbar Spinal Stenosis: A Retrospective Review.

- Submitted for publication to the *Journal of the American Academy of Orthopaedic Surgeons*, 2008.
- Presented at the CTACS Annual Chapter Meeting 2008

PROFESSIONAL ACHIEVEMENTS

National Commission on Certification of Physician Assistants (NCCPA)

- Member, Board of Directors 2010-2014

Connecticut Academy of Physician Assistants

- Responsible for transition to new Association Management Firm; including request for proposal process, interviewing and selection of new Executive Director.
- Worked with ConnAPA Lobbyist to achieve passage of HB5477 "An Act Concerning the Supervision of Physician Assistants"eliminating confusing statute language regarding on site supervision.
- Reestablished ties with the Connecticut State Medical Society (CSMS). Created an Associate Membership category for PA's thereby allowing them to have representation and an opportunity for dialogue. Was appointed as the first PA associate member in the history of the Society.
- Developed strong alliances with the Connecticut Chapter of the American College of Surgeons (CTACS) and the Connecticut Orthopaedic Society (COS).
  - PA Liaison to the CTACS 2005-2012
- Conducted a formal strategic planning session with the ConnAPA Leadership team to evaluate the status of the organization and to determine and prioritize plans for chapter development and advancement.

Danbury Hospital

- Chairman of the Associate Medical Staff Committee.
- First Physician Assistant appointed to the Danbury Hospital Medical Executive Committee. Represented the mid-level practitioners (PA's, APRN's & CRNA's), acting as an advocate and critical voice for that constituency.
- Undertook the initiative to have a formally designated category of privileges within the hospital medical staff as "Associate" members.
- Participated on the Physician's Health Link Advisory Committee, a committee charged with integrating the multiple community electronic medical record (EMR) systems with the hospital's open platform EMR.
- Member of the Pharmacy and Therapeutics Committee and the Spine Center Peer Review Committee.

21 MAY 2014

- Assisted with grant development and garnering support (15K) for a Spine Center sponsored Category I CME event in conjunction with the Miami Project to Cure Paralysis (Current Concepts in Spinal Cord Injury Management).

Community Based

- Took the lead role in planning the 2007 DAPA 5K: Sprint for Sales, a road race event for a local 13 year old boy with leukemia raising over $9,000.
- Organized the second annual 2008 DAPA 5K: Fans of Than, raising over $18,000 for a local young man diagnosed with a suprasellar germinoma.
- Captain of a 2011 St. Vincent's Medical Center Swim Across the Sound team; lead the team to become the highest fundraising team in the event raising over $22,000 for their cancer foundation.

VOLUNTEER ACTIVITIES

National Commission on Certification of Physician Assistants (NCCPA)
Member, Board of Directors 2010-2014

Consultant, Practitioner Licensing Investigations, State of Connecticut, Department of Public Health 2012-present

American Academy of Physician Assistants (AAPA) Leadership
State Advocates for Reimbursement Network 2010-present
Government Affairs and Reimbursement Committee 2007-2009

Involvement in the Connecticut Academy of Physician Assistants (ConnAPA)
ConnAPA Immediate Past President, Reimbursement Chair 2007-2010
Nominating Committee Chair 2007-08
ConnAPA President 2006-2007
ConnAPA President-Elect 2005-2006
PA Liaison to the Connecticut Chapter of the American College of Surgeons (CTACS) 2005-present
ConnAPA Regional Director 2002- 2005

Danbury Hospital Committee Service (2005-2012)
Chairman, Associate Medical Staff Committee
Member, Pharmacy and Therapeutics (P&T) Committee
Member, Medical Executive Committee (Associate Medical Staff Representative)
Member, Spine Center Peer Review Committee
Member, Healthlink Physician Advisory Committee

J P Seibyl 11 JAN 2013

CURRICULUM VITAE

Name: John Peter Seibyl, MD
60 Temple Street, 8A
New Haven, CT 06510 USA

Education: BA Yale College, 1981
MD Case-Western Reserve University, 1986

Career/Academic Appointments:

1986-1987	Medical Intern, Greenwich Hospital Association, Greenwich, CT
1987-1990	Postdoctoral Fellow, Specialty Track in Neuroscience, Yale Psychiatry
1990- 1992	Associate Unit Chief, Neuropsychiatric Studies Unit, VA Medical Center, West Haven, CT
1992-1994	Resident/Fellow in Nuclear Medicine, Section of Nuclear Medicine, Department of Diagnostic Radiology, Yale University School of Medicine
1994 - 2001	Director, NeuroSPECT Center, Section of Nuclear Medicine, Department of Diagnostic Radiology, Yale University Assistant Professor, Department of Psychiatry, Yale University
1994 – 1996	Assistant Professor of Diagnostic Radiology and Psychiatry, Yale University School of Medicine
1996 – 2001	Associate Professor of Diagnostic Radiology and Psychiatry, Yale University School of Medicine
1998 – 2001	Director, Yale Positron Emission Tomography Center
1996 – 2001	Chief, Section of Nuclear Medicine, Yale University
2001- present	Exec. Director and Senior Scientist, Institute for Neurodegenerative Disorders

Administrative Positions:

2001- present	President, Molecular NeuroImaging, LLC

Board Certifications: American Board of Psychiatry and Neurology
American Board of Nuclear Medicine

Current Grants:

W23RYX-8263-N602 Seibyl (PI) 10/15/08-7/1/12
DOD/USMRMC
SPECT Imaging to Evaluate Post Traumatic Stress Disorder
This project will evaluate neuroinflammatory imaging biomarkers in subjects with post traumatic stress disorder.
Role: Principal Investigator

W81XWH-06-1-0679 Marek (PI) 9/1/06-8/31/12
DOD/USMRMC
Assessing the Determinants of PD Progression – Long Term Dopamine Transporter Imaging in the PRECEPT Cohort
This study will investigate the utility of dopamine transporter imaging in monitoring and predicting the progression of PD

Role: Investigator

W81XWH-06-1-0678 Marek (PI) 9/1/06-8/31/12
DOD/USMRMC
Establishing an At-Risk Cohort for Parkingon's Disease Neuroprevention Using Olfactory Testing and
DAT Imaging
This study will develop a strategy to detect pre-symptomatic parkinsonism in a large population of individuals at increased risk for PD.
Role: Investigator

R42-NS055475-01 Zubal (PI) 8/01/07 – 6/30/12
NIH
Develop an automated software processing package which will objectively yield quantitative striatal uptake values for evaluating Alzheimers and Parkinsons diagnosis and progression.
Role: Investigator

PPMI Parkinsons Progression Marker Initiative (Seibyl PI) 11/1/09-11/1/12
Core Imaging Lab
Michael J. Fox Foundation
Develop multicenter imaging techniques and qualify international imaging sites for 5 year PD progression study evaluating imaging and non-imaging biomarkers
Role: Principal Investigator

Lectures, Courses, and Web-based Education:

2011-2012 only

Yale School of Medicine:
Nuclear Medicine Fellows: Four lectures on epilepsy, Alzheimer's, Parkinson's imaging, and clinical cases in neurodegenerative disorders

Yale PET Center invited lecture, 14 May 2012 "Histopathological validation of β-amyloid - targeting PET tracers: Lessons from the Phase III clinical diagnostic trials"

Scientific meetings

1. Society of Nuclear Medicine San Antonio, Texas 6/7/2011 *Clinical Imaging with Ioflupane in Parkinson's Disease*

2. Alzheimer's Association Annual Meeting Paris, France 7/20/2011 *Amyloid Imaging Biomarkers in Alzheimer's Disease*

3. European Assoc of Nuclear Medicine Birmingham, UK 10/19/11 *Imaging Biomarkers in Therapeutic Trials of Neurodegenerative Disorders*

4. Northshore Hospital Grand Rounds Long Island, NY 2/27/2012 *Brain Imaging with Ioflupane (DaTscan) in Neurodegenerative Diseases*

5. International Conference on Research and Standardization in Alzheimer's Disease

Melbourne, Australia, 3/28/12 *PET Imaging Biomarkers in AD Therapeutic Trials: Core Imaging Lab Perspectives*

6. Parkinson Progression Marker Initiative Annual Meeting New York, NY 5/2/12 PPMI Study Imaging Core Lab Update

Courses

1. Movement Disorder Society *Dopamine Transporter Imaging in Clinical Practice* 7/23/11, Course Co-Director, New Haven, CT

2. Movement Disorder Society *Dopamine Transporter Imaging in Clinical Practice* 9/10/11 Chicago, Ill

Professional Societies:
Society of Nuclear Medicine

PROFESSIONAL SERVICE

Peer Review Groups/Grant Study Sections:

2005- current Referee, NIMH Special Emphasis Panel, Imaging in Mood Disorders
2008- current Referee, INSERM (Institut national de la santé de la recherché médicale), France

Journal Service:

Referee: Psychiatry Research, Archives of General Psychiatry, Journal of Nuclear Medicine, Journal of Neuropsychiatry and Clinical Neurosciences, Brain Research, Neurology, Journal of Neuroimaging, Psychiatry Research, Biological Psychiatry, Neuroimaging, Neuroscience Letters, Movement Disorders, Neuropsychopharmacology

Professional Organizations:

Past President, Greater New York Chapter, Society of Nuclear Medicine,(2008-2010)
President, Brain Imaging Council, Society of Nuclear Medicine (2012-2013)
Past President, Brain Imaging Council, Society of Nuclear Medicine (2004-2005)
Member, Parkinson's Study Group PRECEPT Steering Committee
Member, Steering Committee, Parkinsons Progression Marker Initiative (PPMI)

Yale University Service:

Member, Yale- New Haven Hospital Radioactive Drug Research and Radiation Safety Committees

Other:
Columbia University PET Center consultant

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Peer-reviewed manuscripts

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143. Seegal, R.F., K.L. Marek, J.P. Seibyl, D.L. Jennings, E.S. Molho, D.S. Higgins, S.A. Factor, E.F. Fitzgerald, E.A. Hills, S.A. Korrick, M.S. Wolff, R.F. Haase, A.C. Todd, P. Parsons, and R.J. McCaffrey, *Occupational exposure to PCBs reduces striatal dopamine transporter densities only in women: a beta-CIT imaging study.* Neurobiol Dis. 2010 **38**(2): p. 219-25.
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150. Seegal, R. F., E. F. Fitzgerald, et al. *Estimating the half-lives of PCB congeners in former capacitor workers measured over a 28-year interval.* J Expo Sci Environ Epidemiol 2011 **21**(3): 234-246.
151. Cosgrove, K. P., J. K. Staley, et al. *SPECT imaging with the serotonin transporter radiotracer [123I]p ZIENT in nonhuman primate brain.* Nucl Med Biol 2010 **37**(5): 587-591.
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Semin Nucl Med 2010 **40**(5): 338-346.
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158. Schwarzschild, M. A., K. Marek, et al. *Serum urate and probability of dopaminergic deficit in early "Parkinson's disease* Mov Disord 2011 26(10): 1864-1868..
159. Seibyl, J., I. G. Zubal, et al. *Molecular PET imaging in multicenter Alzheimer's therapeutic trials: current trends and implementation strategies.* Expert Rev Neurother 2011 11(12): 1783-1793.
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Case Reports, Technical Notes, Letters

1. **Seibyl**, J.P., J.H. Krystal, L.H. Price, and D.S. Charney, *Use of yohimbine to counteract nortriptyline-induced orthostatic hypotension.* J Clin Psychopharmacol, 1989. **9**(1): p. 67-8.
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3. **Seibyl**, J.P., J.H. Krystal, and D.S. Charney, *Marijuana (cannabis) use is anecdotally said to precipitate anxiety symptoms in patients with panic disorder. Is there any research evidence to support this? Also, can marijuana use precipitate or expose paranoia in patients with an underlying bipolar disorder?* J Clin Psychopharmacol, 1990. **10**(1): p. 78.
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5. Karper, L.P., S.P. Salloway, J.P. **Seibyl**, and J.H. Krystal, *Prolonged postictal encephalopathy in two patients with clozapine-induced seizures.* J Neuropsychiatry Clin Neurosci, 1992. **4**(4): p. 454-7.
6. Karper, L.P., J.P. **Seibyl**, and J.H. Krystal, *Valproate management of psychosis in a patient with carbamazepine-induced hyponatremia.* J Clin Psychopharmacol, 1992. **12**(2): p. 137-9.
7. **Seibyl**, J.P., L. Brenner, J.H. Krystal, R. Johnson, and D.S. Charney, *Mazindol and cocaine addiction in schizophrenia.* Biol Psychiatry, 1992. **31**(11): p. 1179-81.
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Editorials, Reviews, Chapters, Books

1. **Seibyl**, J., D. Jennings, R. Tabamo, and K. Marek, *Neuroimaging trials of Parkinson's disease progression.* J Neurol, 2004. **251 Suppl 7**: p. vII9-13.
2. Marek, K., D. Jennings, and J. **Seibyl**, *Dopamine agonists and Parkinson's disease progression: what can we learn from neuroimaging studies.* Ann Neurol, 2003. **53 Suppl 3**: p. S160-6; discussion S166-9.
3. **Seibyl** J., Scanley BE., Krystal J., Innis, R. *Neuroimaging Methodologies: Utilizing Radiotracers or Nuclear Magnetic Resonance*, in Neurobiology of Mental Illness, 2nd Edition, Charney D and Nestler E, ed. New York: Oxford University Press. 2003, 190-209.
4. Marek, K, Jennings, D, **Seibyl**, J. *Neuroimaging in Parkinson's Disease* in Handbook of Parkinson's Disease, Pahwa R. ed., New York: Marcel Decker, Inc. 2003, 179-202.
5. Marek, K, Jennings, D, **Seibyl**, J. *Single photon Emission Tomography and Dopamine Transporter Imaging in Parkinson's Disease* in Advances in Neurology: Parkinson's Disease, Gordin A. ed., New York: Lippincott Williams, and Wilkins. 2003, 183-191.
6. **Seibyl**, J., D. Jennings, R. Tabamo and K. Marek. *Unique roles of SPET brain imaging in clinical and research studies. Lessons from Parkinson's disease research.* Q J Nucl Med Mol Imaging 49(2): 215-21.2005.
7. **Seibyl**, J., D. Jennings, R. Tabamo and K. Marek. *The role of neuroimaging in the early diagnosis and evaluation of Parkinson's disease*. Minerva Med 96(5): 353-64. 2005
8. **Seibyl, J.** Movement Disorders in *Functional Cerebral SPECT and PET*, 2008, in press.
9. Marek, K., D. Jennings, G. Tamagnan, and J. Seibyl, *Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression.* Ann Neurol, 2008. **64 Suppl 2**: p. S111-21.

Principal Investigator/Program Director
(Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kenneth L. Marek, M.D.	President and Senior Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Princeton University, Princeton, NJ	BA	1974	Biochemistry
Yale University, New Haven, CT	MD	1978	Medicine

A. Positions and Honors.

Positions and Employment

1978-1979 Intern in internal medicine on Osler Medical Service Johns Hopkins Hospital.
1978-1980 Post-doctoral fellow in neurochemistry Institute of Neurology, Queens Square, London.
1981-1982 Resident in preventive medicine Yale University School of Medicine
1982-1983 Resident in internal medicine on Osler Medicine Service Johns Hopkins Hospital.
1983-1985 Resident in neurology Johns Hopkins Hospital.
1985-1986 Chief resident in neurology Johns Hopkins Hospital.
1986-1988 Instructor, Department of Neurology Johns Hopkins University.
1988-1989 Assistant Professor, Department of Neurology Johns Hopkins University.
1989-1995 Assistant Professor, Department of Neurology Yale University.
1990-2001 Director Movement Disorders Center, Department of Neurology Yale University.
1995-2001 Associate Professor, Department of Neurology Yale University.
2004-2009 Clinical Professor, Department of Neurology, Yale University
1989-present Attending Neurologist Yale New Haven Hospital, New Haven, Connecticut
2001-present President and Senior Scientist Institute for Neurodegenerative Disorders, New Haven, Connecticut
2001-present Chief Executive Officer Molecular NeuroImaging, New Haven, Connecticut

Other Professional Experience

1998 Parkinson's disease Advisory Group Veterans Administration
1998-2001 Parkinson Study Group Executive Committee
1998-2002 Huntington Study Group Chair Publications committee
2001-present Scientific Advisory Board Michael J Fox Foundation
2003-present Founder Amadeus international imaging Consortium
2006-present Principal Investigator: PARS
2010-present Principal Investigator: Parkinson Progression Marker Initiative

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B. Selected peer-reviewed publications (in chronological order)

1. Marek KL, Roth RH. Ergot alkaloids: Interaction with presynaptic dopamine receptors in the neostriatum and olefactory tubercles. Eur J Pharm 62:137-146, 1980.

2. Marek KL, Bowen DM, Sims NR, Davison AN. Stimulation of acetycholine synthesis by blockade of presynaptic muscarinic autoreceptors. Life Sciences 30:1517-1524, 1982.

3. Marek KL and Mains RE. Biosynthesis, development and regulation of Neuropeptide Y in superior cervical ganglion culture. J. Neurochem 52:1807-1816, 1989.

4. Marek, KL and Mains, RE. Differential regulation of Neuropeptide Y and catecholamine production in superior cervical ganglion cultures. J Molec and Cell Neurosci 1:262-269, 1991.

5. Spencer D, Robbins RH, Naftolin F, Marek KL, Vollmer T, Leranth C, Roth RH, Price LH, Gjedde A, Bunney BS, Sass KJ, Elsworth JD, Kier EL, Makuch R, Hoffer PB, Redmond DE. Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of Parkinsonian Patients: Functional effect for 18 months. NEJM 327:1541-1548, 1992.

6. Innis RB, Seibyl JP, Scanley BE, Laruelle M, Abi-Dargham A, Wallace E, Baldwin RM, Zea-Ponce Y, Zoghbi S, Wang S, Gao Y, Neumeyer JL, Charney DS, Hoffer PB, Marek KL . SPECT imaging demonstrates loss of striatal monoamine transporters in Parkinson's disease. Proc. Natl. Acad. Sci. USA. 90: 11965-11969, 1993.

7. Gracco LC, Gracco VL, Lofqvist A, Marek KL. An aerodynamic evaluation of parkinsonian dysarthria: Laryngeal and supralaryngeal manifestations. In Motor Speech Disorders: Applied speech science. Eds. Till J, Yorkston K, and Benkleman K. Brookes Press, Baltimore MD. p65-78, 1994.

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9. Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B, Charney DS, van Dyck C, Hoffer PB, Innis RP [123I] beta-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. Neurology 46(1):231-237, 1996.

10. The Parkinson's Study Group. Dose-ranging study of the safety and efficacy of the dopamine agonist pramipexole in early Parkinson's disease. JAMA, 278:125-130, 1997. (Role: Site investigator)

11. Seibyl JP, Marek KL, Sheff K, Baldwin RM, Zoghbi S, Charney DS, van Dyck C, Hoffer P, Innis RB. Test/Retest reproducibility of [123I]CIT brain measurement of dopamine transporters in Parkinson's Disease. J. Nucl Med. 38: 1453-1461, 1997.

12. Seibyl JP, Marek KL, Sheff K, Baldwin RM, Zoghbi S, Charney DS, van Dyck C, Hoffer P, Innis RB. Within subject comparison of [123I]ß-CIT and [123I]FP-CIT SPECT brain measurement of dopamine transporters in healthy subjects and Parkinson's patients. J. Nucl Med. 39:1500-1507, 1998.

13. Innis RB, Marek KL, Sheff K, Zoghbi S, Castronuovo J, Feigin A, Seibyl JP Effect of treatment with L-dopa/carbidopa or L-selegiline on striatal dopamine transporter SPECT imaging with [123I]beta-CIT. Mov Disord 14(3):436-442, 1999.

14. Marek KL, Seibyl JP. Imaging: A molecular map for neurodegeneration. Science 289:409-411, 2000

15. Parkinson Study Group. A randomized controlled trial comparing the agonist pramipexole with levodopa as initial dopaminergic treatment in Parkinson's disease. JAMA 284:231-238, 2000 Role: Imaging Study- Principal investigator).

16. Parkinson Study Group. A Multicenter Assessment Of Dopamine Transporter Imaging With Dopascan/SPECT In Parkinsonism, Neurology 55:1540-1547, 2000. (Role Study-Principal investigator).

17. Marek KL, Innis RB, van Dyck C; Fussell B, Early M, Eberly S, Oakes D, Seibyl JP.[123I]ß-CIT SPECT imaging assessment of the rate of Parkinson's disease progression Neurology, 57:2089-2094, 2001

18. Varrone A, Marek KL, Jennings D, Innis RB, Seibyl JP [(123)I]beta-CIT SPECT imaging demonstrates reduced density of striatal dopamine transporters in Parkinson's disease and multiple system atrophy. Mov Disord 16(6):1023-1032, 2001.

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20. Parkinson Study Group. Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on Parkinson's disease progression, JAMA 287:1653-1661, 2002. (role- Principal Investigator).

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21. DeKosky S, Marek KL. Looking backward to move forward: Early detection of neurodegenerative disorders, Science 302:830-834, 2003.
22. Parkinson Study Group. Pramipexole vs levodopa as initial therapy for Parkinson's disease: A 4-year randomized controlled study. Arch Neurol 61:1044-1053, 2004;. *(role- Steering Committee).*
23. Jennings DL, Seibyl JP, Oakes D, Eberly S, Murphy J, Marek KL[^{123}I]b-CIT and SPECT Imaging Versus Clinical Evaluation in Parkinsonian Syndrome: Unmasking an Early Diagnosis. Arch Neurol 61:1219-1228, 2004.
24. Parkinson Study Group. Levodopa and the progression of Parkinson's disease. NEMJ 351:18-28, 2004;. *(role- PI-imaging/Steering Committee/).*
25. Ravina, B., et al., *The role of radiotracer imaging in Parkinson disease.* Neurology, 2005. **64**(2): p. 208-15.
26. Seibyl, J., et al., Unique roles of SPET brain imaging in clinical and research studies. Lessons from Parkinson's disease research. Q J Nucl Med Mol Imaging, 2005. **49**(2): p. 215-21.
27. Parkinson Study Group, *Mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson disease.* Neurology, 2007. **69**(15): p. 1480-90.
28. Scherfler, C., et al., *Role of DAT-SPECT in the diagnostic work up of parkinsonism.* Mov Disord, 2007. **22**(9): p. 1229-38.
29. Shang, Y., et al., *Displacement of serotonin and dopamine transporters by venlafaxine extended release capsule at steady state: a [123I]2beta-carbomethoxy-3beta-(4-iodophenyl)-tropane single photon emission computed tomography imaging study.* J Clin Psychopharmacol, 2007. **27**(1): p. 71-5.
30. Siderowf, A., et al., *Risk factors for Parkinson's disease and impaired olfaction in relatives of patients with Parkinson's disease.* Mov Disord, 2007. **22**(15): p. 2249-55.
31. van Dyck, C.H., et al., *Striatal dopamine transporters correlate with simple reaction time in elderly subjects.* Neurobiol Aging, 2007.
32. Zubal, I.G., et al., *Optimized, automated striatal uptake analysis applied to SPECT brain scans of Parkinson's disease patients.* J Nucl Med, 2007. **48**(6): p. 857-64.
33. Schwarzschild, M.A., et al., *Serum Urate as a Predictor of Clinical and Radiographic Progression in Parkinson Disease.* Arch Neurol, 2008 65, p716-723.
34. Gaenslen, A., et al., *The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study.* Lancet Neurol, 2008. **7**(5): p. 417-24.
35. Marek, K., et al., Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression. Ann Neurol, 2008. 64 Suppl 2: p. S111-21.
36. Ravina B, Tanner C, DiEuliis D, Eberly S, Flagg E, Galpern WR, Fahn S, Goetz CG, Grate S, Kurlan R, Lang AE, Marek K, Kieburtz K, Oakes D, Elliott R, Shoulson I and the Parkinson Study Group LABS-PD Investigators. A longitudinal program for biomarker development in Parkinson disease: a feasibility study. *Movement Disord* 2009;24(14): 2081-2090.
37. Poewe, W., et al., *Supplement neuroimaging movement disorders.* Movement disorders : official journal of the Movement Disorder Society, 2009. **24 Suppl 2**: p. S655.
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39. Ravina, B., et al., *A longitudinal program for biomarker development in Parkinson's disease: a feasibility study.* Movement disorders : official journal of the Movement Disorder Society, 2009. **24**(14): p. 2081-90.
40. Marek, K. and D. Jennings, Can we image premotor Parkinson disease? *Neurology* 2009;72(7 Suppl):S21-
41. Schapira, A.H., et al., *Rationale for delayed-start study of pramipexole in Parkinson's disease: the PROUD study.* Mov Disord, 2010. **25**(11): p. 1627-32.
42. Seegal, R.F., et al., *Occupational exposure to PCBs reduces striatal dopamine transporter densities only in women: a beta-CIT imaging study.* Neurobiology of disease, 2010. **38**(2): p. 219-25.

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44. Seibyl, J., et al., *Molecular PET imaging in multicenter Alzheimer's therapeutic trials: current trends and implementation strategies.* Expert review of neurotherapeutics, 2011. **11**(12): p. 1783-93.
45. Schwarzschild, M.A., et al., *Serum urate and probability of dopaminergic deficit in early "Parkinson's disease".* Movement disorders : official journal of the Movement Disorder Society, 2011.
46. Marras, C., et al., *Predictors of time to requiring dopaminergic treatment in 2 Parkinson's disease cohorts.* Movement disorders : official journal of the Movement Disorder Society, 2011. **26**(4): p. 608-13.
47. Hall, D.A., et al., *FMR1 gene expansion and scans without evidence of dopaminergic deficits in parkinsonism patients.* Parkinsonism & related disorders, 2010. **16**(9): p. 608-11.
48. Seegal, R.F., et al., *Estimating the half-lives of PCB congeners in former capacitor workers measured over a 28-year interval.* Journal of exposure science & environmental epidemiology, 2011. **21**(3): p. 234-
49. Parkinson Progression Marker Initiative, *Parkinson Progression Marker Initiative* Movement Progress in Neurobiology 95 (2011), pp. 629-635
50. Siderowf, A., et al., *Impaired olfaction and other prodromal features in the Parkinson At-Risk Syndrome Study.* Movement disorders : official journal of the Movement Disorder Society, 2012. **27**(3): p. 406-12.
51. Seibyl, J., et al., *The molecular basis of dopaminergic brain imaging in Parkinson's disease.* The quarterly journal of nuclear medicine and molecular imaging : official publication of the Italian Association of Nuclear Medicine, 2012. **56**(1): p. 4-16.
52. Berg, D., et al., *Defining at-risk populations for Parkinson's disease: lessons from ongoing studies.* Movement disorders : official journal of the Movement Disorder Society, 2012. **27**(5): p. 656-65.

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Meghan J. Pajonas 11-Jul-2013

Work Experience

Molecular NeuroImaging, New Haven, CT. July 8, 2013- Present
Clinical Research Nurse Full-Time

- Responsible for the day-to-day activities associated with the implementation and conduct of clinical studies in compliance with all company SOPs.
- Closely interacts with subjects, physicians, and other staff members. Collaborates with other study coordinators and sites.
- Major Responsibilities include:
  - Possessing a detailed understanding of the study protocol and study consent forms
  - Developing and maintaining source documents and tracking logs relevant to the imaging study
  - Participating in study initiation and close-out processes
  - Completion of Case Report Forms
  - Responsible for recruiting and scheduling of study participants, tracking the participant's visits and projected dates of return as well as overseeing all data management within the study
  - Determining eligibility of study participants and the consent process
  - Completion of study visit activities including: EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs
  - Responsible for study medication accounting, dispensing, and destruction as required by study protocol and procedures
  - Communicates all protocol violations, deviations, or study results outside of the normal range to PI

Hartford Hospital, Hartford, CT. September 2011- July 2013
Registered Nurse – Chest Pain Unit/Angioplasty Suite Full-Time

- Completed core cardiology training. Proficient in cardiac telemetry monitor reading and 12-Lead EKG reading. Certified in Basic Life Support and Advanced Cardiac Life support.
- Provides comprehensive nursing care to patients with chest pain, patients with pulmonary embolism/deep vein thrombosis, palliative care, and general medical surgical patients.
- Works closely with the cardiac catheterization lab staff to manage patients pre- and post- cardiac angiogram/angioplasty.
- Skilled in the education of patients and families on disease processes, health promotion, medication, preventative care, and overall health management.
- Certified as a Resource Nurse. Responsibilities include overseeing the nursing operations of the unit, coordination of staffing for the designated shift, and patient assignments for nursing.
- Completed the Graduate RN Residency Program with a research project component.
  Project: "Minimizing Distractions While Performing Patient Care."
  Worked in a three-person team. The aim of the study was focused on improving patient satisfaction while decreasing distractions that the nurse experiences while performing care. Research has shown that limiting the amount of distractions the nurse experiences during patient care activities can decrease the amount of medical errors. Through data collection and implementation of new methods, the study results were shown to be successful in decreasing the amount of distractions experienced by the nurse while performing direct patient by decreasing from 35% to 23%, thus decreasing the possibility of medical errors. These methods were then presented to the unit and implemented by the nursing staff.

Perceptive Informatics, Billerica, MA. June 2007- January 2010
Imaging Research Associate I

- Developed and coordinated study related activities in compliance with FDA and Good Clinical Practices. Experience includes Phase II, III, and IV trials including areas of Cardiology and Oncology.
- Utilized computer systems to convert medical image data to digital form and performed preliminary image analysis on medical images. Possesses medical imaging experience with CT, MR, Bone Scan, PET, and X-Ray.
- Duties included qualifying sites for study participation, data collection, processing, and quality checks of medical imaging to prepare for independent radiology review, query issuing and resolution.
- Maintained a database of medical images and measurement results.
- Acted as a Lead Imaging Research Associate with duties that included communication of project details with the Project Manager, client, and third party vendors. Responsible for developing image processing procedures and operation manuals, along with training team members.
- Interacted with investigator sites and attended meetings globally (China, Spain) to educate on imaging guidelines set forth by our facility and to resolve issues that arose throughout the clinical trial.
- Assisted as a Requirements Analyst working with the software development team and project managers to document the needs and specifications necessary to develop computer applications for projects.

Meghan J. Pajonas

Meghan J. Pajonas 11-Jul-2013

Education

Quinnipiac University, Hamden, CT. May 2010- May 2011
Accelerated Bachelor of Science in Nursing for Second Degree Students
Endorsed by the American Holistic Nurses' Certification Corporation
- Received "**The Alumni Award for Holistic Nursing Practice**"

Worcester Polytechnic Institute, Worcester, MA. August 2003- May 2007
Bachelor of Science, Biomedical Engineering

Projects:

- **Major Qualifying Project (MQP):** Worcester, MA.

  Worked on a two-person team. "Nanostructured Surface Engineering for Biomedical Implants." This project was designed to investigate the effects of applying different surface coatings to medical implants in order to increase their effectiveness when implanted into the body. Lab work included creating nanostructured aluminum oxide templates, developing a reliable method for producing desirable surface coatings, capturing TEM and SEM images, contact angle measurements, and studying cell and protein activity and adhesion. (3-course equivalent).
  - Received "**Honorable Mention Award**" from the Mechanical Engineering Department
  - Received "**First Place Prize Award**" from The Materials Information Society (ASM)
- **Interdisciplinary Qualifying Project (IQP):** Worcester, MA.

  Worked on a two-person team. "Injuries in Sports and the Workplace." The goal of this report was to characterize different injuries that occur in sports and the workplace. Topics included exploration into the mechanics of the human body, factors that change the dynamics of an injury environment, and a look at medicine's input to these injuries, revealed by the types of rehabilitation and prevention techniques that exist today. (3-course equivalent).

Skills and Certification

Certification:	Registered Nurse, State of Connecticut (expires 07/2014) Advanced Cardiac Life Support, American Heart Association (expires 01/2014) CPR/AED for the Professional Rescuer, American Heart Association (expires 09/2013)
Software:	Microsoft Word, Microsoft Excel, Microsoft Power Point, Pro-Engineer, MATLAB, Sunrise Clinical Manager, Pyxis, Micromedex, and Citrix Applications
Language:	Basic abilities in Spanish
Cultural:	Lived overseas in Stockholm, Sweden

Leadership Activities

The New England Society of Clinical Engineering, Member	September 2012- Present
The Connecticut League for Nursing, Member	September 2010- June 2011
National Student Nurses' Association, Member	June 2010- June 2011
Quinnipiac Student Nurses' Association, Member	June 2010- June 2011
National Biomedical Engineering Society, Member	2005-2008
Mu Sigma Delta- Pre-health Society, Member	2005-2007
Women in Industry, Member	2006-2007
Member of the WPI Women's Basketball Team	2003-2007

Team Captain (2006-2007), First Team All-Conference (2006-2007), New England Senior All-Star (2006-2007), Eastern College Athletic Conference MVP (2006-2007), and Second Team All- Conference (2005-2006).

Community Service

Volunteer at The First Congregational Church Food Pantry, Old Lyme, CT	2011
Mock Disaster, The Hospital of St. Raphael, New Haven, CT	December 2010
2010 Making Strides Against Breast Cancer, New Haven, CT	October 2010
Family Health Fair- Connecticut Beardsley Zoo, Bridgeport, CT	September 2010
Easter Seals Charity Softball Game, Boston, MA	2009
Volunteer at Salem Animal Rescue League, Salem, NH	2008-2010
Special Olympics of Massachusetts: Bioball, Boston, MA	2008
Relay for Life, Worcester, MA	2007
Member of Big Brothers, Big Sisters of America, Worcester, MA	2004-2007
Volunteer at Community Animal Hospital, Shrewsbury, MA	Summer 2005 & July-October 2006

Nicholas Sandella

Imaging Technical Quality Control & Processing Specialist at Molecular NeuroImaging

nicholas.sandella@me.com

Summary

Radiology professional with experience in the clinical, academic, and pharmaceutical Setting

Specialties

In-Vivo & In-Vitro Nuclear Medicine, Nuclear Cardiology, PET, PET/CT Imaging, Diagnostic CT Imaging, Bone Densitometry, MRI/MRA, DCE-MRI, Neuroimaging, 3D Post Processing, Multimodality Fusion, Radiation Health & Safety, ACR & ICANL Accreditation

Healthcare Administration, Revenue Cycle Management, Union & Non-Union Staff Management/Supervision, PACS Administration, Performance Improvement Process & Implementation, Technical Training & Development, Coding & Reimbursement, Patient & Customer Advocacy

FDA Regulations Relating to Good Clinical Practices and Clinical Trials, Preambles to GCP Regulations, Operational Management of Clinical trials, Monitoring & Management of CRO's, Maintaining / Achieving Clinical Trial Timelines & Milestones, The Hallmarks of Cancer, RECIST 1.0 & 1.1, PERCIST, Cheson, McDonald & RANO Criteria's

Certifications

Certified to practice the specialty of Nuclear Medicine Technology (CNMT)
By the Nuclear Medicine Technology Certification Board (NMTCB) License 019553 October 1996

Certified to practice the specialty of Positron Emitted Tomography (PET)
By the Nuclear Medicine Technology Certification Board (NMTCB) License S-80448 September 2004 to September 2011

Certified to practice the specialty of Computed Tomography (CT)
By the American Registry of Radiologic Technologists (ARRT) License 417652 April 2007

Licensed Nuclear Medicine Technologist
By the State of New York Department of Health (NYDOH) License 320943 January 2012 to February 2016

Licensed Nuclear Medicine Technologist
By the South Carolina Radiation Quality Standards Association (SQRQSA) License 01-1609 July 2012 to July 2014

Licensed Computed Tomography Technologist
By the South Carolina Radiation Quality Standards Association (SQRQSA) License 01-1609 July 2012 to

30-JUN-2014

Experience

Imaging Technical Quality Control & Processing Specialist at Molecular NeuroImaging
October 2013 - Present (8 months)

Responsible for conducting a variety of Molecular Imaging QC/analysis procedures, reviewing Molecular Imaging data to confirm specific protocol requirements have been met as well as ensuring technical adequacy and data integrity. In addition serves as technical support for internal and external imaging centers and staff.

Emergency 1st Responder (Contract Position) at Recovery Logistics Inc
June 2012 - Present (1 year 9 months)

Emergency first responder in areas declared a natural disaster from tropical storms, hurricanes, snow/ice storms, etc.. Most notable deployments have been to the Gulf Coast for Hurricane Isaac & to the Northeast for Hurricane Sandy.

Oncology Imaging Specialist / Manager (Contract Position) at Eisai Pharmaceuticals
December 2011 - June 2012 (7 months)

Responsible for executing imaging in clinical trials for the development of targeted oncology therapies by providing imaging strategy input to clinical protocols, manage imaging CRO's, perform data review, and overall support to clinical teams. Implement RECIST 1.0 & 1.1, Cheson, and RANO Criteria in clinical trials.

Oncology Imaging Study Manager at Novartis Pharmaceuticals
April 2008 - September 2010 (2 years 6 months)

Responsible for executing imaging in pre-clinical, translational, and early phase clinical trials (I, II) for the development of targeted oncology therapies by providing imaging strategy input to clinical protocols, manage imaging CRO's, perform data review, and overall support to clinical teams. Maintain operational efficiency & budgetary management of all imaging components across global oncology clinical trials.

Oncology Imaging Manager at Saint Vincent's Catholic Medical Center (Aptium Oncology)
November 2003 - April 2008 (4 years 6 months)

Responsible for overseeing the operations of the Saint Vincent's Comprehensive Cancer Centers Diagnostic Radiology Department by providing clinical, strategic staff, and budgetary management. Additionally, held a secondary role of providing preceptorship functional imaging training to the parent organization personnel (Astrazeneca Pharmaceuticals) quarterly.

Nuclear Medicine Instructor (Part-Time Position) at Saint Vincent Catholic Medical Centers
November 2003 - April 2008 (4 years 6 months)

Part-Time Didactic & Clinical instructor of advanced Nuclear Medicine Technology, which included PET, CT, PET/CT, and fusion imaging.

Regional Nuclear Medicine Manager (Contract Position) at American Diagnostic Medicine
August 2002 - November 2003 (1 year 4 months)

Responsible for overseeing over fifteen turn-key imaging centers in the eastern region of the United States that included General Nuclear Medicine, Nuclear Cardiology, PET, and Computed Tomography; with involvement in every aspect of a turn-key business model from the initial sales agreement to the daily management of imaging centers.

Nuclear Medicine Technologist at Yale New Haven Hospital
January 1994 - August 2002 (8 years 8 months)

Responsible for performing In-Vivo & In-Vitro Nuclear Medicine procedures, Nuclear Cardiology procedures, and assisting Physician's with Radiotherapies; additionally provided system support to mini-PACS across multiple locations.

Skills & Expertise

Nuclear Medicine
Nuclear Cardiology
PET/CT
Computed Tomography
Molecular Imaging
Fusion Imaging
Pre-Clinical Imaging
Imaging Science
Medical Imaging
Diagnostic Imaging Managment
Healthcare Management
Revenue Cycle Management
Clinical Trials
Pre-clinical Studies
Clinical Research
Oncology
Cardiology
Clinical Development
Radiology
GCP
FDA
CRO
Digital Imaging

Publications

PET/CT image fusion error due to urinary bladder filling changes: consequence and correction
Ann Nucl Med () (2009) PMID 19787311 2009
Authors: Nicholas Sandella

Authors:
Sherif Heiba, Barbara Raphael, Ivan Castellon, Erkan Altinyay, Nick Sandella, Gerald Rosen and Hussein Abdel-Dayem

Impact of PET/CT in comparison with same day contrast enhanced CT in breast cancer management.
Clin Nucl Med 32(6): 429-34 (2007) PMID 17515747 2007

Authors: Nicholas Sandella

Authors:

Elena Piperkova, Barbara Raphael, Ivan Castellon, Richard Libes, Nick Sandella, Sherif Heiba, and Hussein Abdel-Dayem.

The distinctive role of positron emission tomography/computed tomography in breast carcinoma with brown adipose tissue 2-fluoro-2-deoxy-d-glucose uptake.
Breast J 11(6): 457-61 (2005) PMID 16297092 2005

Authors: Nicholas Sandella

Authors:

Sherif I Heiba, Stephanie Bernik, Barbara Raphael, Nick Sandella, Witold Cholewinski and Paula Klein.

Education

Charter Oak State College
Bachelor of Science, Health Studies, 2012 - 2014

Gateway Community Technical College
Associates of Applied Science, Nuclear Medicine Technology, 1994 - 1996

Yale New Haven Hospital
Certificate of Clinical Accomplishment, Nuclear Medicine Technology, 1994 - 1996

Nicholas Sandella

Imaging Technical Quality Control & Processing Specialist at Molecular NeuroImaging

nicholas.sandella@me.com

Linked

Contact Nicholas on LinkedIn

RICARDO HIDALGO, CNMT, NCT, R.T.(N)(CT)

[redacted]

PROFESSIONAL EXPERIENCE

VA Medical Center — West Haven, CT
- ***Nuclear Medicine Technologist*** *(02/2013 - current)*

Molecular NeuroImaging and The Instituted for Neurodegenerative Disorders(MNI-IND) — New Haven, CT
- ***Nuclear Medicine Research Technologist*** *(07/2010 - current)*

Bristol Hospital — Bristol, CT
- ***Nuclear Medicine Technologist*** *(06/2008- current)*

Griffin Hospital — Derby, CT
- ***Nuclear Medicine Technologist*** *(12/2007-06/2008)*

EDUCATION, CERTIFICATIONS & ASSOCIATIONS

The Nuclear Medicine Certification Board *(NMTCB)* — Tucker, GA
- ***Nuclear Cardiology Technologist Specialty Certification*** – **NCT** *(2011-current)*
- ***Nuclear Medicine Technologist Certification*** – **CNMT** *(2008-current)*

The American Registry of Radiology Technologist (ARRT) — St. Paul, MN
- ***Computer Tomography Technologist Specialty Certification*** – **CT** *(2009-current)*
- ***Nuclear Medicine Technologist Certification*** – **N** *(2008-current)*

Charter Oak State College — New Britain, CT
- ***Bachelors in Health Science/Administration*** *(In progress)*

St. Vincent's College — Bridgeport, CT
- ***Certification Program for Computed Tomography*** *(2009-2010)*

Gateway Community College — North Haven, CT
- ***Associate in Science, Nuclear Medicine Technology*** *(2005-2008)*

Certified BLS Healthcare Provider American Heart Association – ***CPR*** *(2005-current)*

Bilingual ***(Spanish & English)***

CLINICAL EXPERIENCE

St. Vincent's Medical Center (CT only)	Bridgeport, CT
Yale New Haven Hospital (General & PET/CT & Cardiology)	New Haven, CT
Middlesex Hospital (General & PET/CT)	Middletown, CT
UConn Health Center (General & Cardiology)	Farmington, CT
Saint Francis Hospital (General & Cardiology)	Hartford, CT

CAMERAS, SOFTWARES & INFORMATION SYSTEMS

GE ▫ Discovery ST PET/CT , Millennium VG SPECT/CT, Myosight, LightSpeed VCT.	Discovery Dimension/Xeleris
Picker ▫ Prism 2000, Prism 1500, Axis, XP2.	Odyssey LX/FX
Philips ▫ Genesys EPIC, Solus, Vertex PLUS, Argus, Forte, Skylight, Cardio MD, BrightView	Pegasys/JET Stream
Siemens ▫ Multispect 2, Orbiter 7500, ECAT-EXACT HR+ PET	Apple/JET Stream/ECAT

Information Systems ▫ Philips Isuite/Isite-PACs enterprise, Series 2000, CareLINK, NMIS, Pandora

PRESENTATIONS & PUBLICATIONS

Spring 2009 Yale New Haven Grass Roots Meeting, Technologist Section. Society of Nuclear Medicine: presenter, "Advantage of Simultaneous PET-MR image co-registration"

Spring 2008 Symposium of the New England Chapter, Technologist Section, Society of Nuclear Medicine: presenter, "Introduction to hybrid PET-MR"

The New England Journal of Nuclear Medicine Technology, "Overview of hybrid PET-MR" abstract *(published)*

02/20/13

19DEC2013

Scott M. Vogel RN, BSN

Professional Profile

Registered nurse with 7 years of clinical experience working in medical/surgical and critical care and cardiac clinical areas, as well as 10 years of experience in all facets of clinical research management via the medical device industry

- Surgical/Trauma ICU
- Advanced telemetry
- Inpatient dialysis
- Advanced hemodynamic and intracranial pressure monitoring
- Experience with EMR
- Study Director for many field-based research efforts
- Familiarization with GCP/ICH; IRB research study submission
- Head of clinical education of all internal and external customers
- Development of education/marketing materials

Professional Accomplishments

EDUCATIONAL/NURSING

- Graduated with honors (Cum Laude) and 4th out of a graduating class of 50 students
- Was one of 10 students chosen out of my entire nursing class to participate in one of Yale-New Haven Hospital's early capstone programs during the summer before my senior year in 1996
- Was placed in a critical care environment for the duration of the program; Only a select few of the 50 students in this program were deemed qualified to serve their preceptorship in a critical care clinical setting
- Continued to work in this unit during my senior year of nursing school and was offered a full-time nursing position after I graduated
- Recently completed an RN Refresher course at Charter Oak State College

FIELD-BASED CLINICAL RESEARCH

- Led research efforts as Study Director; successful IRB submission for many field-based, multi-centered research studies (TSL/TEI Biosciences)
- Collected/analyzed data (utilizing SAS/STAT software)and co-authored/ghost-wrote 3 published research projects (TSL/TEI Biosciences)
- Successful development of Key Opinion Leaders (KOLs) and luminary accounts (TSL/TEI Biosciences)

FIELD-BASED CLINICAL EDUCATION

- Clinical Applications specialist – Rookie of the Year (CardioDynamics)
- Chosen by Medical Director to serve on the National Speaker's Bureau (CardioDynamics)
- Educated all employees on the clinical/disease state aspects of our medical devices (TSL, TEI)

Work History

June 1996 – December 2000
Clinical Nurse II, Yale-New Haven Hospital, New Haven, CT

March 2001 – January 2002
Registered Nurse, Level II, Midstate Medical Center, Meriden, CT

January 2002 – June 2004
Clinical Account Manager, Deltex Medical, Chichester, Sussex, United Kingdom

- Worked with current accounts to educate them on the function of Deltex's minimally invasive hemodynamic monitoring device in ICU and OR settings
- Spent much time in the ICU & OR settings providing bedside teaching
- I left Deltex when the company down-sized and laid off all US-based employees

June 2004 – December 2006
Senior Clinical Applications Specialist, CardioDynamics, San Diego, CA

- Worked with current accounts to educate them on the function of CardioDynamics' non-invasive hemodynamic monitoring device in outpatient settings
- Awarded CAS Rookie of the Year in 2004
- I left CardioDynamics for a more lucrative opportunity with a company that was in a more stable financial state. This company ultimately ended up going out of business after I left

December 2006 – April 2008
Senior Clinical Specialist, Tissue Science Labs (TSL), Aldershot, Hampshire, United Kingdom

- TSL manufactured a biologically-derived dermal tissue surgical implant used in various reconstructive surgical procedures
- I provided all clinical education pertaining to the product and associated disease states to internal employees and surgeon end-users
- Spent copious time providing education to surgeons in the OR theatre setting
- Cultivated all field-based research data projects
- I left TSL when the company was wholly purchased by Covidien, as the new regime did not employ field-based clinical personnel

April 2008 – May 2009
Senior Clinical Consultant, TEI Biosciences, Boston, MA

- TEI is a direct competitor of TSL. My position at TEI was identical in every way to my position at TSL in terms of responsibilities
- I left TEI immediately following the conclusion of a legal struggle where I was awarded full custody of my children. I became a stay-at-home parent and raise my kids
- After resigning, I searched for about 18 months for a similar clinical position in the medical device industry that did not require extensive overnight travel but was not able to identify a position like this. At this point that I decided to refocus my efforts back to clinical nursing

May 2009 – March 2013
Stay-at-home Father, Voluntarily resigned to be home to raise my children

- Did much volunteer work at my kids' school and for the local soccer club as a coach
- During this time I completed an RN Refresher course and re-took NCLEX-RN, as required by the CT DPH for all nurses that have been away from the profession for more than 5 years

March 2013 - Present
Assistant Manager of Operations, Advanced SportsPlex, Middletown, CT

- A friend of mine owns this indoor sports facility and brought me on as a full-time manager
- Manage membership and food/beverage bar operations, as well as new business development and facility maintenance.

December 2013 – Present
Clinical Research Nurse, Molecular Neuroimaging, New Haven, CT

Education

Bachelors of Science in Nursing, Cum Laude
Southern Connecticut State University
New Haven, CT
May 1, 1997

References

[References are available upon request.]

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Nuclear Medicine Research Technologist

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Injects and images subjects to generate data using basic and investigational Nuclear Medicine procedures..

Scope & Impact:

The Nuclear Medicine Research Technologist carries out the proper processes necessary for radiopharmaceutical injection and imaging as utilized in clinical research.

Major Responsibilities:

Daily Responsibilities

- Expertise in the use of nuclear medicine imaging equipment (calibration, QC monitoring, utilization, and calibrations).
- Instructs and serves as a resource of information on testing techniques, procedures, and equipment operation.
- Provides imaging data to Principal Investigators and Imaging Processing personnel for further analysis.
- Performs appropriate quality controls on imaging equipment and maintains active quality control information to assure the safe and appropriate use of imaging equipment and radiopharmaceuticals for research and studies.
- Ensures correct calculation and appropriateness of radiopharmaceuticals and doses administered.
- Performs venipuncture or catheterization to provide access for the administration of radiopharmaceuticals, and fluids or the removal of blood for metabolite, survival, or recovery (of the radiopharmaceutical) analysis.

Job description approved (date) 2 JUL 14 by (initials) JM

- Provides image reconstruction and data conversion for projects and publications.
- Maintains patient/ subject flow Imaging division for maximum efficiency.
- Communicates with principal investigators about issues and problems pertaining to any imaging studies completed on site.

Weekly Responsibilities:

- Monitors radiation work areas (wipe testing, weekly surveys) and other areas as requested.
- Provides data quality assurance (data flow, and storage).
- Attends scheduling meetings to promote appropriate subject flow through the company and Imaging division.
- Assists with coordination of studies from the VA or other facilities for subjects who are imaged at MNI/IND

Monthly and Other Responsibilities:

- Assists with monthly camera quality control.
- Maintains quarterly equipment (dose calibrator) linearity and constancy checks.
- Maintains division supplies inventory.
- Orders materials and division technical supplies.
- Drafts papers or portions of papers for publications and/or presentation.
- May present abstracts at annual professional meetings.
- Maintains professional credentialing obligations through continuing education opportunities.
- Additional responsibilities as deemed necessary by supervisor
- Participates in company committees as needs, e.g. Safety Committee
- Provides, collects and monitors radiation safety badges

Qualifications:

- **Required:**
    - Nuclear Medicine Technologist Board Certification

Other Requirements (as applicable):

Job description approved (date) 2JUL14 by (initials) JMM

- **On-going Required Training:**
  - Required to maintain Nuclear Medicine Technologist's Certification (CNMT)

Employee Signature: _____ Date: 7/7/14

Supervisor Signature: _____ Date: 07 JUL 2014

Job description approved (date) 7JUL14 by (initials) JMM

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Clinical Research Nurse

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Responsible for the day to day activities associated with the implementation and conduct of clinical studies.

Scope & Impact:

This position requires frequent close interactions with subjects, physicians and other staff members. Complete, accurate and timely reporting of data is essential.

Major Responsibilities:

- Must possess a detailed understanding of the study protocol and study consent forms
- Develops and maintains source documents and tracking logs relevant to the imaging study
- Participates in study initiation and close-out process
- Completes the study Case Report Form (CRF)
- Responsible for recruiting and scheduling of study participants, tracking the participant's visits and projected dates of return as well as overseeing all data management within the study
- Completes the consenting process and inclusion/exclusion criteria to determine eligibility of study participants
- Completes study visit activities including EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs

Job description approved (date) 7JUL14 by (initials) JPR

- Completes follow up activities with both the site and the participant after the participant has departed from the facility
- Familiar with Good Clinical Practices (GCP) complies with all company Standard Operating Procedures (SOP)
- Communicates all protocol violations, deviations and any study results that are not within normal ranges to the PI and/or the Associate Director of Clinical Research Affairs
- Responsible for study medication accounting, dispensing and destruction as required by studies and procedures
- Works collaboratively with other study coordinators and sites
- Additional responsibilities to be delegated as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Current registered nurse license
  - Bachelor's Degree &/or equivalent experience in a related field
  - Knowledge of Good Clinical Practices (GCP) and other Regulations
  - Able to multi task in fast paced environment
- **Preferred:**

Microsoft Office, WORD, EXCEL, Filemaker PRO, and Outlook

Employee Signature: Candace L Catto RN Date: 7 July 2014

Supervisor Signature: [signature] Date: 07 JUL 2014

Job description approved (date) 7 JUL 14 by (initials) [initials]

MNI Molecular Neuroimaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Clinical Research Nurse

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Responsible for the day to day activities associated with the implementation and conduct of clinical studies.

Scope & Impact:

This position requires frequent close interactions with subjects, physicians and other staff members. Complete, accurate and timely reporting of data is essential.

Major Responsibilities:

- Must possess a detailed understanding of the study protocol and study consent forms
- Develops and maintains source documents and tracking logs relevant to the imaging study
- Participates in study initiation and close-out process
- Completes the study Case Report Form (CRF)
- Responsible for recruiting and scheduling of study participants, tracking the participant's visits and projected dates of return as well as overseeing all data management within the study
- Completes the consenting process and inclusion/exclusion criteria to determine eligibility of study participants
- Completes study visit activities including EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs

Job description approved (date) 7JUL14 by (initials) _____

- Completes follow up activities with both the site and the participant after the participant has departed from the facility
- Familiar with Good Clinical Practices (GCP) complies with all company Standard Operating Procedures (SOP)
- Communicates all protocol violations, deviations and any study results that are not within normal ranges to the PI and/or the Associate Director of Clinical Research Affairs
- Responsible for study medication accounting, dispensing and destruction as required by studies and procedures
- Works collaboratively with other study coordinators and sites
- Additional responsibilities to be delegated as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Current registered nurse license
  - Bachelor's Degree &/or equivalent experience in a related field
  - Knowledge of Good Clinical Practices (GCP) and other Regulations
  - Able to multi task in fast paced environment
- **Preferred:**

Microsoft Office, WORD, EXCEL, Filemaker PRO, and Outlook

Employee Signature: Cheryle Rickard for Date: 07 JUL 2014

Supervisor Signature: [signature] Date: 07 JUL 2014

Job description approved (date) 7 JUL 14 by (initials) [initials]

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: **Associate Director Imaging Translational Research**

Department: **Chemistry and Translational Research**

Reports To: **Director of Imaging Translational Research**

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

The purpose of this position is to conduct research dealing with the understanding of human diseases by engaging in clinical investigations and developing compounds to be evaluated in animal and human studies.

Scope & Impact:

This role is critical in establishing MNI as a leader in the development and application of novel radiopharmaceuticals in support of drug development, contributing to organizational growth through development, validation, and use of novel imaging tools. To be successful in this role, the individual must have significant experience in the field of image analysis for novel CNS radiotracers and associated radioligand evaluation.

Major Responsibilities:

- Manage PET and SPECT acquisition protocols for pre-clinical and early clinical studies
- Conduct image-processing, analysis and modeling for existing or novel radiotracers
- Provide guidance on selection of radiotracers for further development and clinical study
- Assess the acquisition and processing of image data in support of validation of quantitative outcome measures
- Install and evaluate available software packages for image processing
- Research and assess new development in image processing or data modeling
- Develop needed software, methods and modeling when not already available
- Lead studies to investigate human and animal disease, preventive methods, and treatments
- Writing publications and reports

Job description approved (date) 1/23/14 by (initials) OB

- In addition to the above duties, will be instrumental in the completion of additional ad-hoc projects
- Additional responsibilities as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Ph.D. in nuclear medicine, physics, biophysics, or biomedical engineering with a minimum of 4 years of experience in both academic and/or industrial instrumentation/image-processing environments
  - Must be aware of the status of current and relevant science
  - Extremely attentive to detail able to work well independently, and relevant experience should exhibit organizational capabilities
  - Must possess strong written and verbal communication skills
  - Must possess ability to work within a team
  - Must possess experience in modeling of PET or SPECT data
- **Preferred:**
  - Familiarity with image processing software
  - Algorithmic and computational software design and development skills

Employee Signature: Cristian Cantarineanu Date: 1/23/2014

Supervisor Signature: O.B Date: 1/23/14

Job description approved (date) 1/23/14 by (initials) O.B.

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Vice President and Senior Director of Clinical Research

Department: Clinical Research

Reports To: General Partners

Personnel reporting To: Senior Clinical Research Nurse Manager, Recruiting Director, Research Program Specialist, Associate Director of Clinical Research

Purpose of the position/Job Summary: The purpose of this position is to oversee all aspects of clinical research at MNI and IND. As Senior Director of Clinical Research, this position is responsible for the development of collaborations and new clinical research projects with sponsors. As Vice President, this position plays a key role in senior management and providing guidance regarding directions for the company.

Scope & Impact: The Vice President and Senior Director of Clinical Research is responsible for defining the clinical trial strategy and management of all clinical studies being conducted by MNI/IND. The Vice President and Senior Director of Clinical Research will manage operational and logistical tasks of clinical development to ensure efficient execution of trials within established budgets and timelines, ensuring all activities occur in compliance with the appropriate regulations.

Major Responsibilities:

- Set strategic direction and leads large clinical trials.
- Manage clinical research program including design and initiation of clinical trials to optimize tactical and clinical value through global site selection and data portability.
- Coordinate and oversee clinical trials to support regulatory submissions.
- Author and/or work with others to develop publications based on the results of the clinical trials.
- Manage the budget for clinical trials. Ensure overall operation is within the approved budget and timeline.
- Create and/or approve study documents for IRB and IND submissions as it pertains to the clinical research activities.
- Develop and implement SOPs for clinical trials and related activities.

Job description approved (date) 7/11/2012 by (initials) KM

- Ensure MNI/IND compliance with all applicable regulatory standards related to global clinical trials and interactions with physicians.
- Develop and maintain professional relationships with academic and community-based physicians, clinicians and investigators to assure good clinical input to MNI/IND research product development process and marketing.
- Interface with departments within and outside of MNI/IND including, Regulatory Affairs and Quality Assurance, Operations, Chemistry, and Finance.
- Manage staff and outside partners/service providers.
- Represent the company at major annual conferences.

Qualifications:

Required:

- M.D. degree required
- 10+ years of experience in biotech or pharmaceutical industry with at least five years of hands-on managerial experience running clinical trials and managing teams.
- Experience with all aspects of management of large clinical trials from inception to completion.
- Strong knowledge of FDA regulations.
- Previous clinical trial site management experience
- Knowledge of and experience in experimental design.
- Basic understanding of statistics and statistical methods.
- Working knowledge of Good Clinical Practice (CGP).

Preferred:

- Strong project planning, leadership, negotiation and presentation skills as well an ability to contribute creative yet practical solutions to problems.
- Extraordinary researching skills and expertise in searching medical literature and databases for clinical and technical information.

Job description approved (date) 7/11/12 by (initials) JCM

- Ability to multi-task and manage several projects in parallel .
- Ability to forge cross-functional working relationships with internal teams and external project partners.
- Ability to be proactive in identifying issues and hurdles that may handicap the effective implementation of the trial and resolve the issues in a timely fashion.
- Superior writing skills and ability to effectively communicate with technical and non-technical people.

Employee Signature: _____ Date: 7/18/2012

Supervisor Signature: _____ Date: 7/11/12

Job description approved (date) 7/11/2012 by (initials) KM

MNI Molecular Neuroimaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Associate Director of Clinical Research

Department: Clinical Research

Reports To: Vice President and Senior Director of Clinical Research

Personnel reporting To: N/A

Purpose of the position/Job Summary: The purpose of this position is to oversee and manage all aspects of clinical research programs at MNI and IND. As Associate Director of Clinical Research, this position also assists in the development of collaborations and new clinical research projects with sponsors.

Scope & Impact: The Associate Director of Clinical Research is responsible for implementing the clinical trial strategy and management of selected clinical studies being conducted by MNI/IND. The Associate Director of Clinical Research will manage operational and logistical tasks of clinical participant visits to ensure efficient execution of trials within established budgets and timelines, ensuring all activities occur in compliance with the appropriate regulations.

Major Responsibilities:

- Leads selected clinical trials.
- Perform clinical evaluations and clinical procedures with study participants per protocol requirements.
- Coordinate and oversee clinical trials to support regulatory submissions.
- Author and/or work with others to develop publications and presentations based on the results of the clinical trials.
- Create and/or approve study documents and IRB submissions.
- Ensure MNI/IND compliance with all applicable regulatory standards related to clinical trials and interactions with physicians.
- Develop and maintain professional relationships with academic and community-based physicians, clinicians and investigators to assure good clinical input to MNI/IND research product development process and marketing.
- Interface with departments within and outside of MNI/IND including, Regulatory Affairs and Quality Assurance, Operations, Chemistry, and Finance.

Job description approved (date) 6/7/17 by (initials) [signature]

- Represent the company at major annual conferences.
- Develop or promote new potential scientific, research, or business avenues

Qualifications:

Required:

- M.D. degree required
- 5+ years of experience in biotech or pharmaceutical industry with at least five years of hands-on experience running clinical trials and overseeing the conduct of studies..
- Experience with all aspects of management of clinical trials Phase I - IV from inception to completion.
- Strong knowledge of FDA regulations.
- Previous clinical trial site management experience
- Knowledge of and experience in experimental design.
- Basic understanding of statistics and statistical methods.
- Strong Knowledge of Good Clinical Practice (CGP).

Preferred:

- Strong clinical evaluation skills and participant interaction skills
- Strong project planning, leadership, negotiation and presentation skills as well an ability to contribute creative yet practical solutions to problems.
- Strong researching skills and expertise in searching medical literature and databases for clinical and technical information.
- Ability to multi-task and manage several projects in parallel, paying attention to detail.
- Ability to forge cross-functional working relationships with internal teams and external project partners.
- Ability to be proactive in identifying issues and hurdles that may handicap the effective implementation of the trial and resolve the issues in a timely fashion.

Job description approved (date) 7/27/2012 by (initials) [signature]

- Strong understanding of current scientific information regarding diseases under study
- Superior writing skills and ability to effectively communicate with technical and non-technical people.
- Board Certification in Neurology (Adult)

Employee Signature: _____ Date: 7/13/12

Supervisor Signature: _____ Date: 7/17/12

Job description approved (date) 7/17/12 by (initials) _____

MNI Molecular Neuroimaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Nuclear Medicine Research Technologist

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Injects and images subjects to generate data using basic and investigational Nuclear Medicine procedures..

Scope & Impact:

The Nuclear Medicine Research Technologist carries out the proper processes necessary for radiopharmaceutical injection and imaging as utilized in clinical research.

Major Responsibilities:

Daily Responsibilities

- Expertise in the use of nuclear medicine imaging equipment (calibration, QC monitoring, utilization, and calibrations).
- Instructs and serves as a resource of information on testing techniques, procedures, and equipment operation.
- Provides imaging data to Principal Investigators and Imaging Processing personnel for further analysis.
- Performs appropriate quality controls on imaging equipment and maintains active quality control information to assure the safe and appropriate use of imaging equipment and radiopharmaceuticals for research and studies.
- Ensures correct calculation and appropriateness of radiopharmaceuticals and doses administered.
- Performs veinipuncture or catheterization to provide access for the administration of radiopharmaceuticals, and fluids or the removal of blood for metabolite, survival, or recovery (of the radiopharmaceutical) analysis.

Job description approved (date) 7 JUL 14 by (initials) JLM

- Provides image reconstruction and data conversion for projects and publications.
- Maintains patient/ subject flow Imaging division for maximum efficiency.
- Communicates with principal investigators about issues and problems pertaining to any imaging studies completed on site.

Weekly Responsibilities:

- Monitors radiation work areas (wipe testing, weekly surveys) and other areas as requested.
- Provides data quality assurance (data flow, and storage).
- Attends scheduling meetings to promote appropriate subject flow through the company and Imaging division.
- Assists with coordination of studies from the VA or other facilities for subjects who are imaged at MNI/IND

Monthly and Other Responsibilities:

- Assists with monthly camera quality control.
- Maintains quarterly equipment (dose calibrator) linearity and constancy checks.
- Maintains division supplies inventory.
- Orders materials and division technical supplies.
- Drafts papers or portions of papers for publications and/or presentation.
- May present abstracts at annual professional meetings.
- Maintains professional credentialing obligations through continuing education opportunities.
- Additional responsibilities as deemed necessary by supervisor
- Participates in company committees as needs, e.g. Safety Committee
- Provides, collects and monitors radiation safety badges

Qualifications:

- **Required:**
    - Nuclear Medicine Technologist Board Certification

Other Requirements (as applicable):

Job description approved (date) 2JUL14 by (initials) JMM

- **On-going Required Training:**
  - Required to maintain Nuclear Medicine Technologist's Certification (CNMT)

Employee Signature: _____ Date: 08 JUL 2014

Supervisor Signature: _____ Date: 08 JUL 2014

Job description approved (date) 8 JUL 14 by (initials) _____

MNI Molecular Neuroimaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: **Associate Director of Clinical Research Affairs**

Department: Clinical Research

Reports To: Vice President and Senior Director of Clinical Research

Personnel reporting to: Nurse coordinators, non-nurse coordinators, nuclear medicine technologists, other clinic administrative personnel.

The Associate Director of Clinical Research Affairs is a licensed medical practitioner responsible for the management of all clinical studies conducted at MNI/IND. This position manages the operational and logistical responsibilities of the clinic to ensure efficient execution of trials within established budgets and timelines. Ensures that all activities occur in compliance with appropriate regulations. Coordinates the efforts of internal and external resources to ensure timely patient recruitment, trial plan optimization and execution of trials with a focus on quality. Responsible for medical practices in the clinic being consistent and that MNI/IND is in compliance with all applicable regulatory standards related to clinical trials and interactions with physicians.

Major Responsibilities:

- In conjunction with VP and Senior Director of Clinical Research Affairs, sets objectives for Clinic staff consistent with MNI/IND goals; links these to individual performance throughout the department.
- Coordinates cross departmental meetings to ensure flow of essential information.
- Monitors and upgrades diagnostic and laboratory equipment as needed in order to keep the clinic in optimal functional form.
- Coordinates new programs and provides training for the clinical staff and companywide as appropriate.
- Provides solutions to issues at hand, unforeseen events and medical emergencies.
- Assigns research study responsibilities to the clinical staff.

Job description approved (date) 7/Jun/12 by (initials) WO

- Supervises the work flow within the clinic including patient screening, assessment and imaging.
- Evaluates work performance of clinical research staff and personnel; measures performance against individual and departmental objectives.
- Develops, implements and updates relevant SOP's and WID's for the clinic, clinical trials and related activities. Ensures that all SOP's are followed by the clinic staff and personnel.
- Participates in internal committees as requested; takes a leadership role in areas directly related to areas of expertise and scope of responsibility.
- Serves as Clinic organizational contact for external sponsors.
- Takes on clinical trial/study assignments as required. Performs clinical evaluations and clinical procedures with study participants per protocol requirements.
- Performs additional duties as delegated by the VP and Senior Director of Clinical Research.

Skills/Certifications:

- Clinical degree, training and acumen e.g. PA or RN.
- Strong project planning, leadership, and organizational skills; ability to contribute creative yet practical solutions to problems.
- Strong written, verbal and presentation skills; able to effectively communicate with both clinical and non-clinical people. Manage and multi-task several projects in parallel while attending to detail.
- Works and manages effectively within, a fast-paced team environment.
- Holds state licensure, BLS and ACLS certification.

Job description approved (date) 7/JUL/14 by (initials) AO

Requirements:

- Bachelor's degree in the natural or health sciences.
- 10+ years of experience in health care or pharmaceutical industry with hands-on patient care experience.
- Experience in supervision, running clinical trials and managing teams.
- Experience with all aspects of Phase I – Phase IV trials from inception to completion.
- Basic understanding of statistics and statistical methods.
- Knowledge of Good Clinical Practice (CGP).

Preferred:

- Masters Degree in related field preferred.
- Strong knowledge of FDA regulations a plus.

Employee Signature: _____ Date: 07 JUL 2014

Supervisor Signature: _____ Date: 07 JUL 2014

Job description approved (date) 7/JUL/14 by (initials) _____

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Clinical Research Nurse

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Responsible for the day to day activities associated with the implementation and conduct of clinical studies.

Scope & Impact:

This position requires frequent close interactions with subjects, physicians and other staff members. Complete, accurate and timely reporting of data is essential.

Major Responsibilities:

- Must possess a detailed understanding of the study protocol and study consent forms
- Develops and maintains source documents and tracking logs relevant to the imaging study
- Participates in study initiation and close-out process
- Completes the study Case Report Form (CRF)
- Responsible for recruiting and scheduling of study participants, tracking the participant's visits and projected dates of return as well as overseeing all data management within the study
- Completes the consenting process and inclusion/exclusion criteria to determine eligibility of study participants
- Completes study visit activities including EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs

Job description approved (date) 7JUL14 by (initials) _____

- Completes follow up activities with both the site and the participant after the participant has departed from the facility
- Familiar with Good Clinical Practices (GCP) complies with all company Standard Operating Procedures (SOP)
- Communicates all protocol violations, deviations and any study results that are not within normal ranges to the PI and/or the Associate Director of Clinical Research Affairs
- Responsible for study medication accounting, dispensing and destruction as required by studies and procedures
- Works collaboratively with other study coordinators and sites
- Additional responsibilities to be delegated as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Current registered nurse license
  - Bachelor's Degree &/or equivalent experience in a related field
  - Knowledge of Good Clinical Practices (GCP) and other Regulations
  - Able to multi task in fast paced environment
- **Preferred:**

Microsoft Office, WORD, EXCEL, Filemaker PRO, and Outlook

Employee Signature: [signature] Date: 7-Jul-2014

Supervisor Signature: [signature] Date: 09 JUL 2014

Job description approved (date) 7 JUL 14 by (initials) [initials]

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Imaging Quality Control & Processing Specialist

Department: Imaging Services

Reports To: Manager Quality Control & Processing

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

The position of the **Imaging Quality Control & Processing Specialist** is responsible for conducting a variety of QC/analysis procedures. This person is responsible for reviewing the imaging data to confirm specific protocol requirements have been met as well as ensuring technical adequacy and data integrity. In addition this person serves as technical support for internal and external imaging centers and staff.

Scope & Impact:

The **Imaging Quality Control & Processing Specialist** plays in integral role in the day to day conductance of imaging QC and processing procedures.

Major Responsibilities:

Specific duties include:

- Technical quality review of images
- Imaging data reconstruction
- Development and completion of quality control and processing documentation
- Generation of Technical Issue (data) clarification forms for outstanding issues and tracking of these technical issues to ensure timely resolution.
- Imaging center support and trouble-shooting
- Technical imaging center training and set up, on-site and technical t-cons
- Quantitative image analysis

Job description approved (date) 24-Jul-2014 by (initials) SA

- Develop additional QC processes and standard operating procedures.
- In addition to the above duties, the person will be instrumental in the completion of additional ad-hoc projects
- Additional responsibilities as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Nuclear Medicine Technologist with a minimum of 2 years experience or or equivalent formal qualification for handling radiation
  - Proficiency with MS Word and Excel
  - Must possess strong written and verbal skills for effectively communicating with departmental staff and research centers
  - Relevant experience should exhibit organizational capabilities
  - Should be process focused for ensuring efficient follow-through of internal procedures
  - Demonstrated ability to work well independently and in project teams
- **Preferred:**
  - Familiarity with image processing software such as Medex, ANALYZE, IDL, Matlab or SPM, PMOD
  - Understanding of image file formats, DICOM knowledge a plus

Other Requirements (as applicable):

- **On-going Required Training:**
  - Maintenance of Nuclear Medicine Technologist's certification (CNMT) or equivalent formal qualification for handling radiation

Employee Signature: _____ Date: 28-July-2014

Supervisor Signature: _____ Date: 28-Jul-2014

Job description approved (date) 24-Jul-2014 by (initials) JA

MNI Molecular NeuroImaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Nuclear Medicine Research Technologist

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Injects and images subjects to generate data using basic and investigational Nuclear Medicine procedures..

Scope & Impact:

The Nuclear Medicine Research Technologist carries out the proper processes necessary for radiopharmaceutical injection and imaging as utilized in clinical research.

Major Responsibilities:

Daily Responsibilities

- Expertise in the use of nuclear medicine imaging equipment (calibration, QC monitoring, utilization, and calibrations).
- Instructs and serves as a resource of information on testing techniques, procedures, and equipment operation.
- Provides imaging data to Principal Investigators and Imaging Processing personnel for further analysis.
- Performs appropriate quality controls on imaging equipment and maintains active quality control information to assure the safe and appropriate use of imaging equipment and radiopharmaceuticals for research and studies.
- Ensures correct calculation and appropriateness of radiopharmaceuticals and doses administered.
- Performs venipuncture or catheterization to provide access for the administration of radiopharmaceuticals, and fluids or the removal of blood for metabolite, survival, or recovery (of the radiopharmaceutical) analysis.

Job description approved (date) JUL 14 by (initials) JLM

- Provides image reconstruction and data conversion for projects and publications.
- Maintains patient/ subject flow Imaging division for maximum efficiency.
- Communicates with principal investigators about issues and problems pertaining to any imaging studies completed on site.

Weekly Responsibilities:

- Monitors radiation work areas (wipe testing, weekly surveys) and other areas as requested.
- Provides data quality assurance (data flow, and storage).
- Attends scheduling meetings to promote appropriate subject flow through the company and Imaging division.
- Assists with coordination of studies from the VA or other facilities for subjects who are imaged at MNI/IND

Monthly and Other Responsibilities:

- Assists with monthly camera quality control.
- Maintains quarterly equipment (dose calibrator) linearity and constancy checks.
- Maintains division supplies inventory.
- Orders materials and division technical supplies.
- Drafts papers or portions of papers for publications and/or presentation.
- May present abstracts at annual professional meetings.
- Maintains professional credentialing obligations through continuing education opportunities.
- Additional responsibilities as deemed necessary by supervisor
- Participates in company committees as needs, e.g. Safety Committee
- Provides, collects and monitors radiation safety badges

Qualifications:

- **Required:**
    - Nuclear Medicine Technologist Board Certification

Other Requirements (as applicable):

Job description approved (date) 2JUL14 by (initials) JMM

- **On-going Required Training:**
  - Required to maintain Nuclear Medicine Technologist's Certification (CNMT)

Employee Signature: _____ Date: 03/19/14

Supervisor Signature: _____ Date: 07 JUL 2014

Job description approved (date) JUN14 by (initials) JRM

MNI Molecular Neuroimaging

IND Institute for Neurodegenerative Disorders

Job Description

Job Title: Clinical Research Nurse

Department: Clinical Research

Reports To: Associate Director of Clinical Research Affairs

Personnel Reporting To: N/A

Purpose of the position/Job Summary:

Responsible for the day to day activities associated with the implementation and conduct of clinical studies.

Scope & Impact:

This position requires frequent close interactions with subjects, physicians and other staff members. Complete, accurate and timely reporting of data is essential.

Major Responsibilities:

- Must possess a detailed understanding of the study protocol and study consent forms
- Develops and maintains source documents and tracking logs relevant to the imaging study
- Participates in study initiation and close-out process
- Completes the study Case Report Form (CRF)
- Responsible for recruiting and scheduling of study participants, tracking the participant's visits and projected dates of return as well as overseeing all data management within the study
- Completes the consenting process and inclusion/exclusion criteria to determine eligibility of study participants
- Completes study visit activities including EKGs, laboratory samples, adverse event assessments, dispensing of thyroid blockade, concomitant medication review, neuropsychological assessments, and vital signs

Job description approved (date) 2JUL14 by (initials) JPL

- Completes follow up activities with both the site and the participant after the participant has departed from the facility
- Familiar with Good Clinical Practices (GCP) complies with all company Standard Operating Procedures (SOP)
- Communicates all protocol violations, deviations and any study results that are not within normal ranges to the PI and/or the Associate Director of Clinical Research Affairs
- Responsible for study medication accounting, dispensing and destruction as required by studies and procedures
- Works collaboratively with other study coordinators and sites
- Additional responsibilities to be delegated as deemed necessary by supervisor

Qualifications:

- **Required:**
  - Current registered nurse license
  - Bachelor's Degree &/or equivalent experience in a related field
  - Knowledge of Good Clinical Practices (GCP) and other Regulations
  - Able to multi task in fast paced environment
- **Preferred:**

Microsoft Office, WORD, EXCEL, Filemaker PRO, and Outlook

Employee Signature: _____ Date: 08 JUN 2014

Supervisor Signature: _____ Date: 08 JUNE 2014

Job description approved (date) 7 JUN 14 by (initials) _____

Appendix C: DPH Licenses issued to applicants

John Seibyl, MD: Nuclear Medicine Physician and General Partner
Kenneth Marek, MD: General Partner

EMPLOYER'S COPY

STATE OF CONNECTICUT
DEPARTMENT OF PUBLIC HEALTH

NAME
JOHN P. SEIBYL, MD

VALIDATION NO.	LICENSE NO.	CURRENT THROUGH
03-510420	029212	12/31/13

PROFESSION
PHYSICIAN/SURGEON

SIGNATURE

COMMISSIONER

State of Connecticut

Lookup Detail View

Name

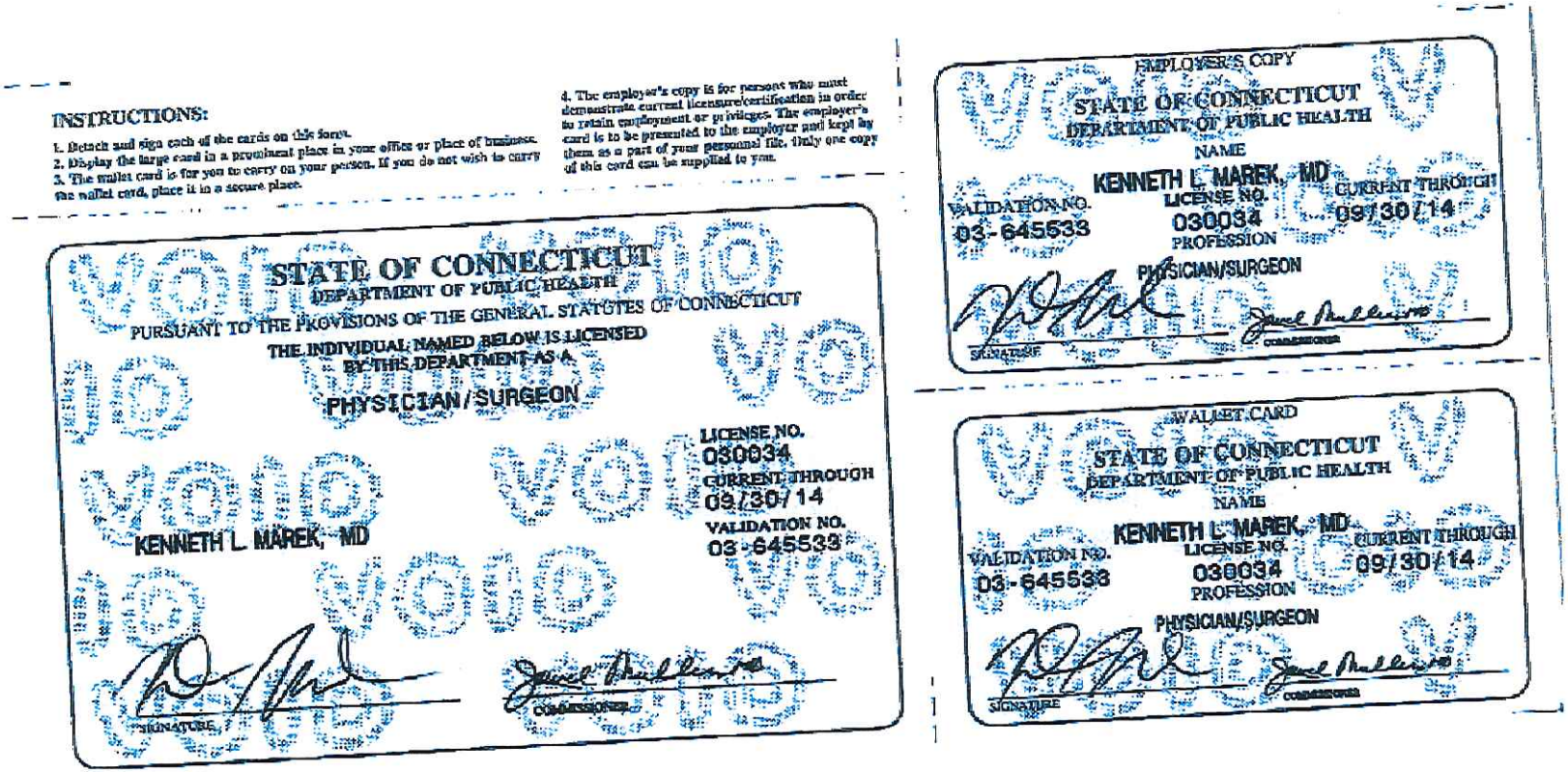
Name
KENNETH L MAREK

License Information

License Information

License Type	License Number	Expiration Date	Granted Date	License Name	License Status	Licensure Act Pending Char
Physician/Surgeon	30034	09/30/2015	06/23/1989	KENNETH L. MAREK	ACTIVE	None

Generated on: 9/26/2014 3:25:27 PM



INSTRUCTIONS:

1. Detach and sign each of the cards on this form.
2. Display the large card in a prominent place in your office or place of business.
3. The wallet card is for you to carry on your person. If you do not wish to carry the wallet card, place it in a secure place.
4. The employer's copy is for persons who must demonstrate current licensure/certification in order to retain employment or privileges. The employer's card is to be presented to the employer and kept by them as a part of your personnel file. Only one copy of this card can be supplied to you.

STATE OF CONNECTICUT
DEPARTMENT OF PUBLIC HEALTH
PURSUANT TO THE PROVISIONS OF THE GENERAL STATUTES OF CONNECTICUT
THE INDIVIDUAL NAMED BELOW IS LICENSED BY THIS DEPARTMENT AS A
PHYSICIAN/SURGEON
KENNETH L. MAREK, MD
LICENSE NO. 030034
CURRENT THROUGH 09/30/14
VALIDATION NO. 03-645533
SIGNATURE
COMMISSIONER

EMPLOYER'S COPY
STATE OF CONNECTICUT
DEPARTMENT OF PUBLIC HEALTH
NAME
KENNETH L. MAREK, MD
VALIDATION NO. 03-645533
LICENSE NO. 030034
CURRENT THROUGH 09/30/14
PROFESSION
PHYSICIAN/SURGEON
SIGNATURE
COMMISSIONER

WALLET CARD
STATE OF CONNECTICUT
DEPARTMENT OF PUBLIC HEALTH
NAME
KENNETH L. MAREK, MD
VALIDATION NO. 03-645533
LICENSE NO. 030034
CURRENT THROUGH 09/30/14
PROFESSION
PHYSICIAN/SURGEON
SIGNATURE
COMMISSIONER

Appendix D: 2011, 2012 and 2013Audited Financial Statements

2011 Molecular NeuroImaging, LLC Audited Financials
2012 Molecular NeuroImaging, LLC Audited Financials
2013 Molecular NeuroImaging, LLC Audited Financials

MOLECULAR NEUROIMAGING, LLC

AUDITED FINANCIAL STATEMENTS

DECEMBER 31, 2011

MOLECULAR NEUROIMAGING, LLC

TABLE OF CONTENTS

MICHAEL A. OLENSKI, CPA, P.C. Certified Public Accountant

Independent Auditor's Report

To the Members
Molecular Neuroimaging, LLC
New Haven, Connecticut

We have audited the accompanying balance sheet of Molecular Neuroimaging, LLC as of December 31, 2011, and the related statements of income and members' equity and cash flows for the year then ended. These financial statements are the responsibility of Molecular Neuroimaging, LLC's management. Our responsibility is to express an opinion on these financial statements based on our audit.

We conducted our audit in accordance with auditing standards generally accepted in the United States of America. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

In our opinion, the financial statements referred to above present fairly, in all material respects, the financial position of Molecular Neuroimaging, LLC as of December 31, 2011, and the changes in its members' equity and its cash flows for the year then ended in conformity with accounting principles generally accepted in the United States of America.

Michael A. Olenski, CPA, PC
March 2, 2012

110 Washington Avenue, 3rd Floor, North Haven, CT 06473 • Phone: (203) 234-6370 • Fax: (203) 234-6367

MOLECULAR NEUROIMAGING, LLC
BALANCE SHEET
DECEMBER 31, 2011

ASSETS		
CURRENT ASSETS		
Cash and Cash Equivalents	$ 172,513	
Accounts Receivable	3,081,204	
Other Current Assets	40,845	
TOTAL CURRENT ASSETS		$3,294,562
PROPERTY AND EQUIPMENT		
Furniture and Fixtures	2,581,562	
Leasehold Improvements	2,056,197	
	4,637,759	
Less: Accumulated Depreciation	(2,704,642)	
TOTAL PROPERTY AND PROPERTY		1,933,117
OTHER ASSETS		
Intangible Assets, net of amortization of $192,442	1	
Security Deposits	73,776	
TOTAL OTHER ASSETS		73,777
TOTAL ASSETS		$5,301,456
LIABILITIES AND PARTNERS' CAPITAL		
CURRENT LIABILITIES		
Accounts Payable	$ 686,080	
Accrued Expenses	298,255	
Current Notes Payable	152,346	
TOTAL CURRENT LIABILITIES		$1,136,681
LONG-TERM LIABILITIES		
Notes Payable	1,606,663	
Lease Payable	-	
Other Liabilities	13,662	
TOTAL LONG TERM LIABILITIES		1,620,325
TOTAL LIABILITIES		2,757,006
Members' Equity		2,544,450
TOTAL LIABILITIES AND MEMBERS' EQUITY		$5,301,456

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF INCOME AND MEMBERS' EQUITY
FOR THE YEAR ENDED DECEMBER 31, 2011

Revenue	$13,722,420
Expenses	
Salaries	5,219,948
Employee Benefits	1,204,850
Insurance	23,339
Payroll Taxes	369,569
Depreciation Expense	482,212
Amortization	68,994
Office Rent	619,749
Equipment Rent	15,725
Partner Consulting Fees	194,557
Dues and Subscriptions	32,807
Licenses and Permits	8,712
Repairs and Maintenance	214,088
Utilities	54,420
Telephone	47,679
Professional Fees	416,047
Study Costs	3,066,714
Property Tax	25,334
Printing and Postage	95,822
Office Expense	324,187
Travel and Entertainment	429,144
Interest Expense	55,591
Miscellaneous	31,198
Total Expense	13,000,686
Income From Operations	721,734
Other Income - Interest	17
Other Expense - Taxes	3,646
Net Income	718,105
Members' Equity, Beginning of Year	1,833,025
Less Members' Draws	(6,680)
Members' Equity, End of Year	$2,544,450

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED DECEMBER 31, 2011

Cash Flows from Operating Activities	
Net Income	$ 718,105
Adjustments to Reconcile Net Income to	
Net Cash Provided by Operating Activities	
Depreciation	482,212
Amortization	68,994
Changes in Operating Assets And Liabilities	
Increase in Accounts Receivable	(847,134)
Increase in Other Assets and Security Deposit	(2,536)
Decrease in Accounts Payable, Accrued Expenses and Other Liabilities	(1,558)
Net Cash Flow Provide by Operating Activities	418,083
Cash Flows from Investing Activities	
Purchase of Equipment and Leasehold Improve	(1,247,854)
Net Cash Used in Investing Activities	(1,247,854)
Cash Flows from Financing Activities	
Distributions to Members	12,621
Principal Payments on Long Term Debt	786,958
Net Cash Flows from Financing Activities	799,579
Net Decrease in Cash	(30,315)
Cash and Cash Equivalents at Beginning of Period	202,828
Cash and Cash Equivalents at End of Period	$ 172,513
Supplemental Disclosure:	
Interest paid	$ 55,591

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
NOTES TO FINANCIAL STATEMENTS
FOR THE YEAR ENDED DECEMBER 31, 2011

NOTE 1 - Summary of Significant Accounting Policies

Organization and Nature of Operations

Molecular Neuroimaging, LLC is a partnership that performs clinical research into improvements and diagnostic tools for Parkinson's disease, Huntington's disease, dystonia, and Alzheimer's disease. Research is focused on three related programs: functional brain imaging studies for diagnosis and monitoring of disease, clinical trials of medications and genetic and environmental studies. The Company works with hundreds of participants each year in performing this research.

Cash and Cash Equivalents

The company considers its investments with maturity of three months or less to be cash equivalents, which are reflected at their approximate fair value.

Basis of Accounting

The financial statements of the Company have been prepared on the accrual basis of accounting and accordingly reflect all significant receivables, payables, and other liabilities.

Estimates

The preparation of financial statements in conformity with generally accepted accounting principles requires management to make estimates and assumptions that affect certain reported amounts. Accordingly, actual results could differ from those estimates.

Property and Equipment

Acquisitions of property and equipment in excess of $1,000 are capitalized. Property and equipment are carried at cost.

Depreciation

Depreciation is computed for financial statement purposes using the tax method over the estimated useful lives of the assets. The equipments' estimated useful life is five and seven years for furniture, fixtures and equipment and thirty-nine years for leasehold improvements.

Income Tax Status

The Company is taxed as a partnership.

NOTE 2 - Note Payable

At December 31, 2011, the Company had two notes payable to Bank of America totaling $1,759,009.

The note payable to Bank of America is for $1,154,900 and is payable in monthly installments of $13,188 and is secured by the assets of the Company. The interest rate is fixed at 5.3%. The note is due 3/9/2021.	$1,133,926
The equipment line of credit from Bank of America is payable in monthly installments of $7,194 and is secured by the assets of the Company. The interest rate is fixed at 5.53%. The loan is due 3/9/2021.	625,083
	1,759,009
Less current portion	(152,346)
	$1,606,663

Maturities for the following five years of the note payable are as follows:

Fiscal year December 31,	2012	$ 152,346
	2013	162,137
	2014	171,078
	2015	180,512
	2016	190,466
	Thereafter	902,470
		$1,759,009

NOTE 3 - Related Party

The Company has related party transactions with a not-for-profit corporation, Institute for Neurodegenerative Disorders, Inc. (IND), where two of the officers of the not-for-profit are also principals of the Company. IND provided space at an MNI rented facility and various services to the Institute. Rent and other costs for utilities, phone, office supplies and administrative services provided by MNI staff, such as receptionist, computer support and accounting services, are allocated to the Institute based on the ratio of direct costs for research at the Institute vs. the Company. In addition, brain-imaging services were provided by MNI to the Institute. Amounts charged by MNI for imaging services were based on industry standard rates.

MOLECULAR NEUROIMAGING, LLC

AUDITED FINANCIAL STATEMENTS

DECEMBER 31, 2012

MOLECULAR NEUROIMAGING, LLC

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Independent Auditor's Report

To the Members
Molecular Neuroimaging, LLC
New Haven, Connecticut

We have audited the accompanying balance sheet of Molecular Neuroimaging, LLC as of December 31, 2012, and the related statements of income and members' equity and cash flows for the year then ended. These financial statements are the responsibility of Molecular Neuroimaging, LLC's management. Our responsibility is to express an opinion on these financial statements based on our audit.

We conducted our audit in accordance with auditing standards generally accepted in the United States of America. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

In our opinion, the financial statements referred to above present fairly, in all material respects, the financial position of Molecular Neuroimaging, LLC as of December 31, 2012, and the changes in its members' equity and its cash flows for the year then ended in conformity with accounting principles generally accepted in the United States of America.

Michael A. Olenski, CPA, PC
March 22, 2013

Michael A. Olenski
CPA, P.C.
9 Research Drive - Milford - CT - 06460 (203)693-3617 Phone (203) 693 -3619 Fax

MOLECULAR NEUROIMAGING, LLC
BALANCE SHEET
DECEMBER 31, 2012

ASSETS		
CURRENT ASSETS		
Cash and Cash Equivalents	$ 810,420	
Accounts Receivable, net of reserves of $225,000	2,976,496	
Other Current Assets	278,674	
TOTAL CURRENT ASSETS		$4,065,590
PROPERTY AND EQUIPMENT		
Furniture and Fixtures	2,961,271	
Leasehold Improvements	2,082,614	
	5,043,885	
Less: Accumulated Depreciation	(3,178,066)	
TOTAL PROPERTY AND PROPERTY		1,865,819
OTHER ASSETS		
Intangible Assets, net of amortization of $199,571	346	
Security Deposits	400	
TOTAL OTHER ASSETS		746
TOTAL ASSETS		$5,932,155
LIABILITIES AND PARTNERS' CAPITAL		
CURRENT LIABILITIES		
Accounts Payable	$ 171,102	
Accrued Expenses	438,853	
Current Notes Payable	181,307	
TOTAL CURRENT LIABILITIES		$ 791,262
LONG-TERM LIABILITIES		
Notes Payable	1,532,187	
Other Liabilities	-	
TOTAL LONG TERM LIABILITIES		1,532,187
TOTAL LIABILITIES		2,323,449
Members' Equity		3,608,706
TOTAL LIABILITIES AND MEMBERS' EQUITY		$5,932,155

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF INCOME AND MEMBERS' EQUITY
FOR THE YEAR ENDED DECEMBER 31, 2012

Revenue	$15,871,521
Expenses	
Salaries	6,120,488
Employee Benefits	1,242,488
Insurance	35,066
Payroll Taxes	484,914
Depreciation Expense	496,089
Amortization	30,943
Office Rent	932,415
Equipment Rent	19,499
Partner Consulting Fees	373,655
Dues and Subscriptions	39,295
Licenses and Permits	73,948
Repairs and Maintenance	285,455
Utilities	42,215
Telephone	47,814
Professional Fees	374,565
Study Costs	3,113,442
Property Tax	50,658
Printing and Postage	93,078
Office Expense	157,184
Travel and Entertainment	455,539
Interest Expense	94,891
Miscellaneous	7,637
Total Expense	14,571,278
Income From Operations	1,300,243
Other Income - Interest	13
Net Income	1,300,256
Members' Equity, Beginning of Year	2,544,450
Less Members' Draws	(236,000)
Members' Equity, End of Year	$3,608,706

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED DECEMBER 31, 2012

Cash Flows from Operating Activities	
Net Income	$1,300,256
Adjustments to Reconcile Net Income to Net Cash Provided by Operating Activities	
Depreciation	496,089
Amortization	30,943
Changes in Operating Assets And Liabilities	
Decrease in Accounts Receivable	104,708
Increase in Other Assets and Security Deposit	(164,453)
Decrease in Accounts Payable, Accrued Expenses and Other Liabilities	(388,042)
Net Cash Flow Provide by Operating Activities	1,379,501
Cash Flows from Investing Activities	
Purchase of Equipment and Leasehold Improve	(460,079)
Net Cash Used in Investing Activities	(460,079)
Cash Flows from Financing Activities	
Distributions to Members	(236,000)
Principal Payments on Long Term Debt	(45,515)
Net Cash Flows from Financing Activities	(281,515)
Net Decrease in Cash	637,907
Cash and Cash Equivalents at Beginning of Period	172,513
Cash and Cash Equivalents at End of Period	$ 810,420
Supplemental Disclosure:	
Interest paid	$ 94,891

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
NOTES TO FINANCIAL STATEMENTS
FOR THE YEAR ENDED DECEMBER 31, 2012

NOTE 1 - Summary of Significant Accounting Policies

Organization and Nature of Operations

Molecular Neuroimaging, LLC is a partnership that performs clinical research into improvements and diagnostic tools for Parkinson's disease, Huntington's disease, dystonia, and Alzheimer's disease. Research is focused on three related programs: functional brain imaging studies for diagnosis and monitoring of disease, clinical trials of medications and genetic and environmental studies. The Company works with hundreds of participants each year in performing this research.

Cash and Cash Equivalents

The company considers its investments with maturity of three months or less to be cash equivalents, which are reflected at their approximate fair value.

Basis of Accounting

The financial statements of the Company have been prepared on the accrual basis of accounting and accordingly reflect all significant receivables, payables, and other liabilities.

Estimates

The preparation of financial statements in conformity with generally accepted accounting principles requires management to make estimates and assumptions that affect certain reported amounts. Accordingly, actual results could differ from those estimates.

Property and Equipment

Acquisitions of property and equipment in excess of $1,000 are capitalized. Property and equipment are carried at cost.

Depreciation

Depreciation is computed for financial statement purposes using the tax method over the estimated useful lives of the assets. The equipments' estimated useful life is five and seven years for furniture, fixtures and equipment and thirty-nine years for leasehold improvements.

Income Tax Status

The Company is treated as a partnership for federal income tax purposes. Consequently, federal income taxes are not payable by, or provided for, the Company. Members are taxed individually on their shares of the Company's earnings. The Company's net income or loss is allocated among the members in accordance with the regulations of the Company.

NOTE 2 - Note Payable

At December 31, 2012, the Company had four notes payable to Bank of America totaling $1,713,494.

The note payable to Bank of America is for $1,154,900 and is payable in monthly installments of $13,188 and is secured by the assets of the Company. The interest rate is fixed at 5.3%. The note is due 3/9/2021.	$1,044,222
The note payable to Bank of America is for $24,235 and is payable in monthly installments of $452 and is secured by the assets of the Company. The interest rate is fixed at 4.5%. The note is due 9/10/2017.	22,713
The note payable to Bank of America is for $79,467 and is payable in monthly installments of $1,484 and is secured by the assets of the Company. The interest rate is fixed at 4.5%. The note is due 9/10/2017.	74,477
The equipment line of credit from Bank of America is payable in monthly installments of $7,194 and is secured by the assets of the Company. The interest rate is fixed at 5.53%. The loan is due 3/9/2021.	572,081
	1,713,494
Less current portion	(181,307)
	$1,532,187

Maturities for the following five years of the note payable are as follows:

Fiscal year December 31,	2013	$ 181,307
	2014	191,129
	2015	201,484
	2016	212,401
	2017	216,032
	Thereafter	711,141
		$1,713,494

NOTE 3 - Related Party

The Company has related party transactions with a not-for-profit corporation, Institute for Neurodegenerative Disorders, Inc. (IND), where two of the officers of the not-for-profit are also principals of the Company. MNI has provided IND space at an MNI rented facility and various services to the Institute. Rent and other costs for utilities, phone, office supplies and administrative services provided by MNI staff, such as receptionist, computer support and accounting services, are allocated to the Institute based on the ratio of direct costs for research at the Institute vs. the Company. In addition, brain-imaging services were provided by MNI to the Institute. Amounts charged by MNI for imaging services were based on industry standard rates.

NOTE 4 - Significant Concentrations of Credit Risk

The Company has concentrated its risk for cash by maintaining deposits in a one bank. The excess of the deposit liabilities reported by the bank over the amounts covered by federal insurance totalled $560,420 at December 31, 2012, and $0 at December 31, 2011.

NOTE 5 - Subsequent Events

Management has evaluated subsequent events through March 22, 2013, the date which the financial statements were available to be issued.

MOLECULAR NEUROIMAGING, LLC

AUDITED FINANCIAL STATEMENTS

DECEMBER 31, 2013

MOLECULAR NEUROIMAGING, LLC

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Independent Auditor's Report

To the Members
Molecular Neuroimaging, LLC
New Haven, Connecticut

We have audited the accompanying balance sheet of Molecular Neuroimaging, LLC as of December 31, 2013, and the related statements of income and members' equity and cash flows for the year then ended. These financial statements are the responsibility of Molecular Neuroimaging, LLC's management. Our responsibility is to express an opinion on these financial statements based on our audit.

We conducted our audit in accordance with auditing standards generally accepted in the United States of America. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

In our opinion, the financial statements referred to above present fairly, in all material respects, the financial position of Molecular Neuroimaging, LLC as of December 31, 2013, and the changes in its members' equity and its cash flows for the year then ended in conformity with accounting principles generally accepted in the United States of America.

Michael A. Olenski, CPA, PC
April 2, 2014

Michael A. Olenski
CPA, P.C.
9 Research Drive - Milford - CT - 06460 (203)693-3617 Phone (203) 693-3619 Fax

MOLECULAR NEUROIMAGING, LLC
BALANCE SHEET
DECEMBER 31, 2013

ASSETS		
CURRENT ASSETS		
Cash and Cash Equivalents	$ 306,286	
Accounts Receivable	4,522,756	
Other Current Assets	154,231	
TOTAL CURRENT ASSETS		$4,983,273
PROPERTY AND EQUIPMENT		
Furniture and Fixtures	3,068,528	
Leasehold Improvements	2,231,502	
	5,300,030	
Less: Accumulated Depreciation	(3,381,739)	
TOTAL PROPERTY AND PROPERTY		1,918,291
OTHER ASSETS		
Intangible Assets, net of amortization of $363,256	4,297	
TOTAL OTHER ASSETS		4,297
TOTAL ASSETS		$6,905,861
LIABILITIES AND PARTNERS' CAPITAL		
CURRENT LIABILITIES		
Accounts Payable	$ 276,908	
Accrued Expenses	169,194	
Current Notes Payable	191,126	
TOTAL CURRENT LIABILITIES		$ 637,228
LONG-TERM LIABILITIES		
Notes Payable	1,341,607	
Other Liabilities	-	
TOTAL LONG TERM LIABILITIES		1,341,607
TOTAL LIABILITIES		1,978,835
Members' Equity		4,927,026
TOTAL LIABILITIES AND MEMBERS' EQUITY		$6,905,861

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF INCOME AND MEMBERS' EQUITY
FOR THE YEAR ENDED DECEMBER 31, 2013

Revenue	$17,844,114
Expenses	
Salaries	6,709,813
Employee Benefits	1,357,781
Insurance	46,581
Payroll Taxes	514,385
Depreciation Expense	194,159
Amortization	158,940
Office Rent	963,867
Equipment Rent	24,664
Partner Consulting Fees	371,961
Dues and Subscriptions	30,388
Licenses and Permits	124,672
Repairs and Maintenance	333,996
Utilities	38,625
Telephone	48,920
Professional Fees	1,176,175
Study Costs	2,636,001
Property Tax	79,460
Printing and Postage	133,235
Office Expense	290,737
Travel and Entertainment	782,290
Interest Expense	87,061
Miscellaneous	23,402
Total Expense	16,127,113
Income From Operations	1,717,001
Other Income - Interest	13
Net Income	1,717,014
Members' Equity, Beginning of Year	3,608,706
Less Members' Draws	(398,694)
Members' Equity, End of Year	$4,927,026

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED DECEMBER 31, 2013

Cash Flows from Operating Activities	
Net Income	$1,717,014
Adjustments to Reconcile Net Income to Net Cash Provided by Operating Activities	
Depreciation	194,159
Amortization	158,940
Changes in Operating Assets And Liabilities	
Decrease in Accounts Receivable	(1,546,260)
Increase in Other Assets and Security Deposit	124,843
Decrease in Accounts Payable, Accrued Expenses and Other Liabilities	(163,854)
Net Cash Flow Provide by Operating Activities	484,842
Cash Flows from Investing Activities	
Purchase of Equipment and Leasehold Improve	(409,521)
Net Cash Used in Investing Activities	(409,521)
Cash Flows from Financing Activities	
Distributions to Members	(398,694)
Principal Payments on Long Term Debt	(180,761)
Net Cash Used in Financing Activities	(579,455)
Net Decrease in Cash	(504,134)
Cash and Cash Equivalents at Beginning of Period	810,420
Cash and Cash Equivalents at End of Period	$ 306,286
Supplemental Disclosure:	
Interest paid	$ 87,061

See accompanying notes to financial statements.

MOLECULAR NEUROIMAGING, LLC
NOTES TO FINANCIAL STATEMENTS
FOR THE YEAR ENDED DECEMBER 31, 2013

NOTE 1 - Summary of Significant Accounting Policies

Organization and Nature of Operations

Molecular Neuroimaging, LLC is a partnership that performs clinical research into improvements and diagnostic tools for Parkinson's disease, Huntington's disease, dystonia, and Alzheimer's disease. Research is focused on three related programs: functional brain imaging studies for diagnosis and monitoring of disease, clinical trials of medications and genetic and environmental studies. The Company works with hundreds of participants each year in performing this research.

Cash and Cash Equivalents

The company considers its investments with maturity of three months or less to be cash equivalents, which are reflected at their approximate fair value.

Basis of Accounting

The financial statements of the Company have been prepared on the accrual basis of accounting and accordingly reflect all significant receivables, payables, and other liabilities.

Estimates

The preparation of financial statements in conformity with generally accepted accounting principles requires management to make estimates and assumptions that affect certain reported amounts. Accordingly, actual results could differ from those estimates.

Property and Equipment

Acquisitions of property and equipment in excess of $1,000 are capitalized. Property and equipment are carried at cost.

Depreciation

Depreciation is computed for financial statement purposes using the tax method over the estimated useful lives of the assets. The equipments' estimated useful life is five and seven years for furniture, fixtures and equipment and thirty-nine years for leasehold improvements.

Income Tax Status

The Company is treated as a partnership for federal income tax purposes. Consequently, federal income taxes are not payable by, or provided for, the Company. Members are taxed individually on their shares of the Company's earnings. The Company's net income or loss is allocated among the members in accordance with the regulations of the Company.

NOTE 2 - Note Payable

At December 31, 2013, the Company had four notes payable to Bank of America totaling $1,532,733.

The note payable to Bank of America is for $1,154,900 and is payable in monthly installments of $13,188 and is secured by the assets of the Company. The interest rate is fixed at 5.3%. The note is due 3/9/2021.	$ 938,768
The note payable to Bank of America is for $24,235 and is payable in monthly installments of $452 and is secured by the assets of the Company. The interest rate is fixed at 4.5%. The note is due 9/10/2017.	18,226
The note payable to Bank of America is for $79,467 and is payable in monthly installments of $1,484 and is secured by the assets of the Company. The interest rate is fixed at 4.5%. The note is due 9/10/2017.	59,764
The equipment line of credit from Bank of America is payable in monthly installments of $7,194 and is secured by the assets of the Company. The interest rate is fixed at 5.53%. The loan is due 3/9/2021.	515,975
	1,532,733
Less current portion	(191,126)
	$1,341,607

Maturities for the following five years of the note payable are as follows:

Fiscal year December 31,	2014	$ 191,126
	2015	201,484
	2016	212,401
	2017	216,032
	2018	212,053
	Thereafter	449,667
		$1,532,733

NOTE 3 - Related Party

The Company has related party transactions with a not-for-profit corporation, Institute for Neurodegenerative Disorders, Inc. (IND), where two of the officers of the not-for-profit are also principals of the Company. MNI has provided IND space at an MNI rented facility and various services to the Institute. Rent and other costs for utilities, phone, office supplies and administrative services provided by MNI staff, such as receptionist, computer support and accounting services, are allocated to the Institute based on the ratio of direct costs for research at the Institute vs. the Company. In addition, brain-imaging services were provided by MNI to the Institute. Amounts charged by MNI for imaging services were based on industry standard rates.

NOTE 4 - Significant Concentrations of Credit Risk

The Company has concentrated its risk for cash by maintaining deposits in a one bank. The excess of the deposit liabilities reported by the bank over the amounts covered by federal insurance totaled $44,704 at December 31, 2013, and $560,420 at December 31, 2012.

NOTE 5 - Subsequent Events

Management has evaluated subsequent events through April 2, 2014, the date which the financial statements were available to be issued.

Appendix E: Financial Attachments I-A and II

Attachments I-A
Attachments II
Vender Quote on proposed PET CT Camera

13. B i. Please provide one year of actual results and three years of projections of **Total Facility** revenue, expense and volume statistics without, incremental to and with the CON proposal in the following reporting format:

Total Facility: Description	FY 2013 Actual Results	FY 2014 Projected W/out CON	FY 2014 Projected Incremental	FY 2014 Projected With CON	FY 2015 Projected W/out CON	FY 2015 Projected Incremental	FY 2015 Projected With CON	FY 2016 Projected W/out CON	FY 2016 Projected Incremental	FY 2016 Projected With CON
NET PATIENT REVENUE										
Non-Government				$0			$0			$0
Medicare				$0			$0			$0
Medicaid and Other Medical Assistance				$0			$0			$0
Other Government				$0			$0			$0
Total Net Patient Patient Revenue	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Other Operating Revenue	$17,844,114	$19,399,905		$19,399,905	$20,951,897	$ 2,279,060	$23,230,957	$22,418,530	$ 4,375,796	$26,794,326
Revenue from Operations	$17,844,114	$19,399,905	$0	$19,399,905	$20,951,897	$2,279,060	$23,230,957	$22,418,530	$4,375,796	$26,794,326
OPERATING EXPENSES										
Salaries and Fringe Benefits	$8,581,979	$9,962,605	$0	$9,962,605	$11,029,270	$ 408,800	$11,438,070	$11,819,502	$1,584,660	$13,404,162
Professional / Contracted Services	$1,176,175	$1,246,577	$0	$1,246,577	$1,259,043	$ 48,000	$1,307,043	$1,284,224	$ 115,200	$1,399,424
Supplies and Drugs	$2,636,001	$2,619,338	$0	$2,619,338	$2,750,305	$ 96,000	$2,846,305	$2,942,826	$ 230,400	$3,173,226
Bad Debts			$0	$0	$0	$0	$0	$0	$0	$0
Other Operating Expense	$3,292,798	$3,190,002	$0	$3,190,002	$3,349,502	$ 240,000	$3,589,502	$3,583,967	$ 576,000	$4,159,967
Subtotal	$15,686,953	$17,018,522	$0	$17,018,522	$18,388,120	$792,800	$19,180,920	$19,630,519	$2,506,260	$22,136,779
Depreciation/Amortization	$353,099	$350,734		$350,734	$350,000	$ 58,985	$408,985	$350,000	$ 58,985	$408,985
Interest Expense	$87,061	$79,000		$79,000	$79,000	$17,000	$96,000	$79,000	$14,000	$93,000
Lease Expense				$0			$0			$0
Total Operating Expenses	$16,127,113	$17,448,256	$0	$17,448,256	$18,817,120	$868,785	$19,685,905	$20,059,519	$2,579,245	$22,638,764
Income (Loss) from Operations	$1,717,001	$1,951,649	$0	$1,951,649	$2,134,778	$1,410,275	$3,545,053	$2,359,012	$1,796,551	$4,155,563
Non-Operating Income	$13			$0			$0			$0
Income before provision for income taxes	$1,717,014	$1,951,649	$0	$1,951,649	$2,134,778	$1,410,275	$3,545,053	$2,359,012	$1,796,551	$4,155,563
Provision for income taxes										
Net Income	$1,717,014	$1,951,649	$0	$1,951,649	$2,134,778	$1,410,275	$3,545,053	$2,359,012	$1,796,551	$4,155,563
Retained earnings, beginning of year	$3,608,706	$4,927,026	$0	$4,927,026	$6,878,675	$0	$6,878,675	$9,013,453	$1,410,275	$10,423,728
Less: Member Draws	($398,694)									
Retained earnings, end of year	$4,927,026	$6,878,675	$0	$6,878,675	$9,013,453	$1,410,275	$10,423,728	$11,372,465	$3,206,826	$14,579,291
FTEs	77.5	87		87	95	4	99	100	11	111

*Volume Statistics:
Provide projected inpatient and/or outpatient statistics for any new services and provide actual and projected inpatient and/or outpatient statistics for any existing services which will change due to the proposal.

FINANCIAL ATTACHMENT DESCRIPTIONS

Financial Attachment A – Long Form Total Facility Not-for-Profit

Financial Attachment B – Long Form Total Facility For-Profit

Financial Attachment C – Long Form Total Hospital Health System Not-for-Profit

Financial Attachment D – Long Form Total Hospital Health System For-Profit

12. C (i). Please provide one year of actual results and three years of projections of **Total Facility** revenue, expense and volume statistics without, incremental to and with the CON proposal in the following reporting format:

Total Facility: Description	FY Actual Results	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON
NET PATIENT REVENUE										
Non-Government				$0			$0			$0
Medicare				$0			$0			$0
Medicaid and Other Medical Assistance				$0			$0			$0
Other Government				$0			$0			$0
Total Net Patient Patient Revenue	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Other Operating Revenue										
Revenue from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
OPERATING EXPENSES										
Salaries and Fringe Benefits				$0			$0			$0
Professional / Contracted Services				$0			$0			$0
Supplies and Drugs				$0			$0			$0
Bad Debts				$0			$0			$0
Other Operating Expense				$0			$0			$0
Subtotal	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Depreciation/Amortization				$0			$0			$0
Interest Expense				$0			$0			$0
Lease Expense				$0			$0			$0
Total Operating Expense	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Gain/(Loss) from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Plus: Non-Operating Revenue				$0			$0			$0
Revenue Over/(Under) Expense	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
FTEs				0			0			0

*Volume Statistics:
Provide projected inpatient and/or outpatient statistics for any new services and provide actual and projected inpatient and/or outpatient statistics for any existing services which will change due to the proposal.

12. C (i). Please provide one year of actual results and three years of ***Total Hospital Health System*** projections of revenue, expense and volume statistics without, incremental to and with the CON proposal in the following reporting format:

Total Hospital Health System: Description	FY Actual Results	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON
NET PATIENT REVENUE										
Non-Government				$0			$0			$0
Medicare				$0			$0			$0
Medicaid and Other Medical Assistance				$0			$0			$0
Other Government				$0			$0			$0
Total Net Patient Patient Revenue	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Other Operating Revenue										
Revenue from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
OPERATING EXPENSES										
Salaries and Fringe Benefits				$0			$0			$0
Professional / Contracted Services				$0			$0			$0
Supplies and Drugs				$0			$0			$0
Bad Debts				$0			$0			$0
Other Operating Expense				$0			$0			$0
Subtotal	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Depreciation/Amortization				$0			$0			$0
Interest Expense				$0			$0			$0
Lease Expense				$0			$0			$0
Total Operating Expense	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Gain/(Loss) from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Plus: Non-Operating Revenue				$0			$0			$0
Revenue Over/(Under) Expense	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
FTEs				0			0			0

*Volume Statistics:
Provide projected inpatient and/or outpatient statistics for any new services and provide actual and projected inpatient and/or outpatient statistics for any existing services which will change due to the proposal.

13. B (i). Please provide one year of actual results and three years of ***Total Hospital Health System*** projections of revenue, expense and volume statistics without, incremental to and with the CON proposal in the following reporting format:

Total Hospital Health System: Description	FY Actual Results	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON	FY Projected W/out CON	FY Projected Incremental	FY Projected With CON
NET PATIENT REVENUE										
Non-Government				$0			$0			$0
Medicare				$0			$0			$0
Medicaid and Other Medical Assistance				$0			$0			$0
Other Government				$0			$0			$0
Total Net Patient Patient Revenue	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Other Operating Revenue										
Revenue from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
OPERATING EXPENSES										
Salaries and Fringe Benefits				$0			$0			$0
Professional / Contracted Services				$0			$0			$0
Supplies and Drugs				$0			$0			$0
Bad Debts				$0			$0			$0
Other Operating Expense				$0			$0			$0
Subtotal	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Depreciation/Amortization				$0			$0			$0
Interest Expense				$0			$0			$0
Lease Expense				$0			$0			$0
Total Operating Expenses	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Income (Loss) from Operations	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Non-Operating Income				$0			$0			$0
Income before provision for income taxes	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Provision for income taxes										
Net Income	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
Retained earnings, beginning of year		$0	$0	$0	$0	$0	$0	$0	$0	$0
Retained earnings, end of year	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0
FTEs				0			0			0

*Volume Statistics:
Provide projected inpatient and/or outpatient statistics for any new services and provide actual and projected inpatient and/or outpatient statistics for any existing services which will change due to the proposal.

12.C(ii). Please provide **three** years of projections of incremental revenue, expense and volume statistics **attributable to the proposal** in the following reporting format:

Type of Service Description _____
Type of Unit Description: _____
# of Months in Operation _____

FY _____ **FY Projected Incremental** Total Incremental Expenses:	(1)	(2) Rate	(3) Units	(4) Gross Revenue Col. 2 * Col. 3	(5) Allowances/ Deductions	(6) Charity Care	(7) Bad Debt	(8) Net Revenue Col.4 - Col.5 -Col.6 - Col.7	(9) Operating Expenses Col. 1 Total * Col. 4 / Col. 4 Total	(10) Gain/(Loss) from Operations Col. 8 - Col. 9
Total Facility by Payer Category:										
Medicare				$0				$0	$0	$0
Medicaid		$0		$0				$0	$0	$0
CHAMPUS/TriCare		$0		$0				$0	$0	$0
Total Governmental			0	$0	$0	$0	$0	$0	$0	$0
Commericial Insurers		$0	5	$0				$0	$0	$0
Uninsured		$0	2	$0				$0	$0	$0
Total NonGovernment		$0	7	$0	$0	$0	$0	$0	$0	$0
Total All Payers		$0	7	$0	$0	$0	$0	$0	$0	$0

Appendix F: Copies of Public Notice

New Haven Register Public Notice

NEW HAVEN REGISTER THE REGISTER CITIZEN The Middletown Press Farmington Valley Times CONNECTICUT
SHORELINE TIMES The Foothills Trader WEST HARTFORD NEWS MINUTEMAN Connecticut Bride
Milford-Orange Bulletin THE GRANBY NEWS the Dolphin THE LITCHFIELD COUNTY TIMES Post-Chronicle

Proof of Ad **09/02/14**

Account:	**704036**
Name:	
Company:	**MNI/IND**
Address:	**60 TEMPLE ST** **8B** **NEW HAVEN, CT 06510**
Telephone:	**(203) 401-4368**
Ad ID:	**366514**
Description:	**Public Notice: Molecular NeuroImagin**
Run Dates:	**08/28/14 to 08/30/14**
Class:	**1201**
Orig User:	**CRCGILSON**
Words:	**102**
Lines:	**26**
Agate Lines:	**28**
Column width:	**1**
Depth:	**3.083**
Blind Box:	

Public Notice:
Molecular NeuroImaging, LLC FILING APPLICATION FOR A CERTIFICATE OF NEED – OFFICE OF HEALTHCARE ACCESS
John Seibyl, MD and Kenneth Marek, MD, General Partners, at 60 Temple Street, New Haven, CT 06510 are filing an application for a Certificate of Need with the Office of Health Care Access in the State of Connecticut for the acquisition of a Siemens Biograph 6 PET/CT scanner for human research. The fair market value of the equipment to be procured is projected to be $600,000 to $800,000. The proposed location for the camera is the 8th floor of 60 Temple Street, New Haven, CT 06510.

We Appreciate Your Business!
Thank You !

21ST CENTURY media

Appendix G: List MNI Human Volunteer Research Studies from Past 3 Years

Appendix G

List of MNI Research Studies Conducted from 2011- August 2014

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
20130260: An Open-label, Non-randomized Positron Emission Tomography Study With 18F-AMG 580 to Determine PDE10A Target Occupancy of AMG 581 After Single-dose Oral Administration in Healthy Subjects	PDE10A	Schizophrenia , Huntinghton	0
An open label, multicenter study, evaluating the safety and efficacy of 18F-AV-133 PET imaging to identify subjects with dopaminergic degeneration among subjects presenting to a movement disorders specialty clinic with an uncertain diagnosis	VMAT2	Parkinson's Disease	29
Test-Retest Reproducibility of 18F-AV-1451 Injection for Brain Imaging of Tau in Healthy Volunteers and Cognitively Impaired Subjects	Tau	Alzheimer's Disease, Parkinson's Disease	20
An open label, multicenter study, evaluating the safety and imaging characteristics of 18F-AV-1451 in cognitively healthy volunteers, subjects with Mild Cognitive Impairment, and subjects with Alzheimer's's disease	Tau	Alzheimer's Disease, Parkinson's Disease	32
18F-AV-1451 Injection for Brain Imaging of Tau in Subjects with Progressive Supranuclear Palsy (PSP), Subjects with Corticobasal Degeneration (CBD) and Healthy Volunteers	Tau	Alzheimer's Disease, Parkinson's Disease	2
An open-label, non-randomized study to evaluate the feasibility of [18F]Florbetapir positron emission tomography (PET) for assessment of demyelination in patients with relapsing remitting multiple sclerosis	Demyelination/brain lesions	Multiple Sclerosis	28

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
A Placebo-controlled, Double-blind, Parallel-group, Bayesian Adaptive Randomization Design and Dose Regimen-finding Study to Evaluate Safety, Tolerability, and Efficacy of BAN2401 in Subjects With Early Alzheimer's's Disease	β-amyloid	Alzheimer's Disease	4
Protocol H8A-MC-LZAO© Continued Efficacy and Safey Monitoring of Solanezumab, an Anti-Amyloid β Antibody in Patients with Alzheimer's's Disease	β-amyloid	Alzheimer's Disease	4
Protocol H8A-MC-LZAZ Effect of Passive Immunization on the Progression of Mild Alzheimer's's Disease: Solanezumab (LY20620430) Versus Placebo	β-amyloid	Alzheimer's Disease	14
Assessing diagnostic accuracy of multiple analysis methods for 18F-FDG PET imaging in subjects with dementia or parkinsonism and healthy controls	Brain glucose metabolism	Alzheimer's Disease, Parkinson's Disease, Cancer	36
Evaluation of [^{18}F]PEB and PET as a marker of metabotropic glutamate receptor type 5 in subjects with Neuropsychiatric Conditions	mGluR5	Parkinon's Disease, Fragile X	32
Evaluation of [18F]MNI-659 as a brain PET tracer of phosphodiesterase receptor in subjects with neurodegenerative conditions in comparison to healthy subjects	PDE10A	Schizophrenia , Huntinghton	13
Evaluation of [18F]MNI-777 PET as a marker of tau pathology in subjects with clinically diagnosed tauopathies in comparison to healthy subjects	Tau	Alzheimer's Disease, Parkinson's Disease	5
A single dose open label study of CNS distribution of [18F]MNI-690 in healthy volunteers using positron emission tomography (PET)	5HT-3	Schizophrenia	2

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
A single dose open label study of CNS distribution of [18F]MNI-774 in healthy volunteers using positron emission tomography (PET)	5HT-3	Schizophrenia	2
Safety Evaluation of 18F-AV-133 in subjects participating in the The Parkinson's Progression Markers Initiative (PPMI) Protocol & Evaluation of [18F] florbetaben in subjects participating in The Parkinson's Progression Markers Initiative (PPMI) Protocol	VMAT2 & β-amyloid	Parkinson's Disease	20
Phase 1 Dose Cohort Study to Evaluate Target Occupancy as Measured by the PDE10A Ligand [^{18}F]MNI-659 and Positron Emission Tomography Following Multiple Dose Administration of OMS643762 in Healthy Male Subjects	PDE10A	Schizophrenia , Huntinghton	33
Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, 18-Month Safety and Efficacy Study of Leuco-methylthioninium bis(hydromethanesulfonate) in Subjects with Mild Alzheimer's's Disease	Reduction in decline in glucose uptake in the temporal lobe	Alzheimer's Disease	3
A Positron Emission Tomography Study to Evaluate Brain Glycine Transporter Type 1 (GlyT1) Occupancy Following Single Doses of ABT-419 in Healthy Male Subjects	GlyT1	Schizophrenia	27
An Open-Label, Non-Randomized Positron Emission Tomography Study With ^{18}F-AMG 580 to Determine PDE10A Target Occupancy of AMG 579 After Single-Dose Oral Administration in Healthy Subjects	PDE10A	Schizophrenia	11
A Preliminary Comparison and Exploratory Evaluation of the Amyloid Binding Properties and Safety of 18F-AV-657 in Healthy Volunteers and Subjects with Alzheimer's's Disease, Using Florbetapir F 18 and PET Whole Body Biodistribution	β-amyloid	Alzheimer's Disease	6

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
A Preliminary Comparison and Exploratory Evaluation of the Amyloid Binding Properties and Safety of 18F-AV-739 in Healthy Volunteers and Subjects with Alzheimer's's Disease, Using Florbetapir F 18 and PET Whole Body Biodistribution	β-amyloid	Alzheimer's Disease	2
Receptor Occupancy Determination of Single and Multiple Doses of CTP-354 in Healthy Volunteers via Positron Emission Tomography	GABA	Schizophrenia	36
A Phase 1, Open-Label, Multiple Ascending Dose, [18F]MNI-659 PET-Imaging Study to Evaluate PDE10A Occupancy After Dosing with EVP-6308 in Healthy Subjects	PDE10A	Schizophrenia , Huntinghton	29
Evaluation of [18F] CFPyPB PET as a marker of Glycine Transporter-1 (GlyT1) receptor in subjects with Parkinson disease, Alzheimer's disease and healthy subjects	GlyT1	Schizophrenia	6
Evaluation of [18F] MK-9470 and PET as a marker of cannabinoid-1 receptor activity in subjects with Parkinson disease compared with healthy controls	Cannabinoid-1 Receptor (CB-1)	Depression, Schizophrenia	16
Evaluation of [^{18}F] MNI-654 and [^{18}F] MNI-659 as brain PET tracers of phosphodiesterase receptor in subjects with neurodegenerative conditions in comparison to healthy subjects	PDE10A	Schizophrenia , Huntinghton	34
Assessment of the Biodistribution and Safety of [18F]MNI-654 and [18F]MNI-659 in Healthy Subjects	PDE10A	Schizophrenia , Huntinghton	1
Phase I evaluation of [18F]MPPF as a brain tracer of serotonin receptor 5HT1a in subjects with Parkinson disease and healthy subjects	Serotonin Receptor 5HT1a	Depression	20

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
A Phase 1 Evaluation of the Kinetics, Clearance and Cerebral Distribution of One Novel PBR PET Imaging Agent, 18F-PBR-111 Following Intravenous Administration in Healthy Subjects and Alzheimer's Disease Patients	Biomarker for Inflammatory Changes in Brain	Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, Cancer	4
Evaluation of [18F]MNI-444 as brain PET tracer of adenosine Type 2 receptor (A2aR) in subjects with neurodegenerative conditions in comparison to healthy subjects	Adenosine Type 2 Receptor (A2aR)	Parkinson's Disease	11
Assessment of the Biodistribution and Safety of [18F]MNI-444 in Healthy Subjects	Adenosine Type 2 Receptor (A2aR)	Parkinson's Disease	4
A Preliminary Comparison and Exploratory Evaluation of the Amyloid Binding Properties and Safety of [18F]MNI-764 in Healthy Volunteers and Subjects with Alzheimer's's Disease, using Florbetapir F 18 and PET Whole Body Biodistribution.	β-amyloid	Alzheimer's Disease	13
A Preliminary Comparison and Exploratory Evaluation of the Amyloid Binding Properties and Safety of [18F]MNI-762 in Healthy Volunteers and Subjects with Alzheimer's's Disease, using Florbetapir F 18 and PET Whole Body Biodistribution.	β-amyloid	Alzheimer's Disease	11
Evaluation of [18F]MNI-718 PET as a marker of tau pathology in subjects with Alzheimer's disease in comparison to healthy subjects	Tau	Alzheimer's Disease, Parkinson's Disease	4
Evaluation of [18F]MNI-720 PET as a marker of tau pathology in subjects with Alzheimer's disease in comparison to healthy subjects	Tau	Alzheimer's Disease, Parkinson's Disease	6

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
Evaluation of [18F]MNI-721 PET as a marker of tau pathology in subjects with Alzheimer's disease in comparison to healthy subjects	Tau	Alzheimer's Disease, Parkinson's Disease	2
Evaluation of [18F]MNI-723 PET as a marker of tau pathology in subjects with Alzheimer's disease in comparison to healthy subjects	Tau	Alzheimer's Disease, Parkinson's Disease	5
A Single Dose Study to Qualify [18F]MK-8040 Positron Emission Tomography (PET) for use as a Biomarker for Regional A2a Receptor Density in Human Brain – Part I	Biomarker for Regional A2a Receptor Density	Parkinson's Disease	9
Clinical Trial Protocol: NAV4-01 A Phase 2 Clinical Trial to Evaluate the Efficacy and Safety of [18F]AZD4694 PET in the Detection of Beta Amyloid in Subjects with Probable Alzheimer's's Disease, Older Healthy Volunteers, and Young Healthy Volunteers	β-amyloid	Alzheimer's Disease	14

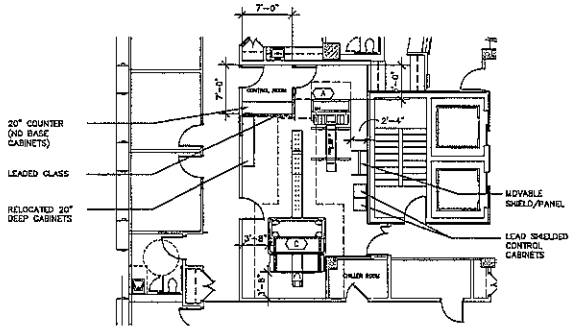
Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
Positron Emission Tomography (PET) Imaging of 18F-AMG 580 in Healthy Adult Subjects: Safety, Tolerability, Radiation Dosimetry, Biodistribution, and Test-Retest Studies	PDE10A	Schizophrenia , Huntinghton	18
An open-label, non-randomized, multi-center study to optimize image assessment and evaluate the efficacy and safety of BAY 94-9172 (ZK 6013443) positron emission tomography (PET) for detection/exclusion of cerebral amyloid beta in patients with probably Alzheimer's's disease compared to healthy volunteers	β-amyloid	Alzheimer's Disease	8
An open-label, non-randomized study to evaluate the efficacy and safety of BAY 94-9172 (ZK 6013443) positron emission tomography (PET) for detection of cerebral β-amyloid in individuals with Down Syndrome compared to individuals without Down Syndrome	β-amyloid	Alzheimer's Disease	86
Effect of Passive Immunization on the Progression of Alzheimer's Disease: LY2062430 versus Placebo	β-amyloid	Alzheimer's Disease	6
A Phase 1 evaluation of the kinetics, clearance and cerebral distribution of one novel PBR PET imaging agent, 18F-PBR-111 following intravenous administration in healthy subjects and Alzheimer's disease patients	Microglial Activation	Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, Cancer	14
Evaluation of [18F] FMH3 and PET as a marker of histamine-3 receptor activity in subjects with Alzheimer's disease compared with healthy controls	Histamine H3 Receptor	Parkinson's Disease, Schizophrenia	6
Assessment of the Biodistribution and Safety of [18F]FPEB in Healthy Subjects	Glutamate Receptor Type 5 (mGluR5)	Parkinon's Disease, Fragile X	3

Full Study Title	Research Indication	Target Potential Disease/ Patient Population	Number of PET Scans
An exploratory, open-label, non-randomized Phase 0 study to evaluate the efficacy and safety of MNI-558 positron emission tomography (PET) for detection/exclusion of cerebral amyloid beta in patients with Alzheimer disease compared to healthy volunteers	β-amyloid	Alzheimer's Disease	7
A Three Part Study to Evaluate the Safety, Radiation Dosimetry, Biokinetics, and Effectiveness of [18F]MK-3328, a Radiotracer for Use in Positron Emission Tomography	β-amyloid	Alzheimer's Disease	14
Study of the Performance of MK-3328 in Subjects with Alzheimer's's Disease or Mild Cognitive Impairment, and Healthy Young and Healthy Elderly Subjects	Quantifying Amyloid Plaque Burden in Alzheimer's's Disease Brain	Alzheimer's Disease	10
Parkinson Associated Risk Factor Study (PARS): Evaluating Potential Screening Tools for Parkinson Disease	β-amyloid	Parkinson's Disease	19
An exploratory, open-label, non-randomized Phase 1 study to evaluate the efficacy and safety of MNI-672 single photon emission tomography (SPECT) for detection/exclusion of cerebral β amyloid in patients with Alzheimer's disease compared to healthy volunteers	β-amyloid	Alzheimer's Disease	5

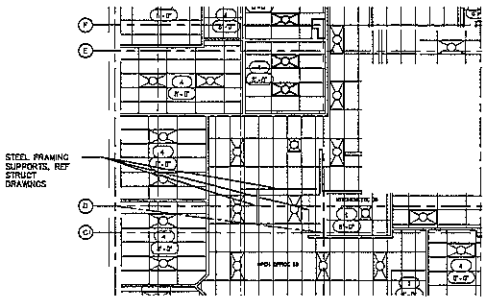
Appendix H: Initial Architectural Plans

Please note these are preliminary plans, current to date.

8th Floor Architecture Proposed Plans for the existing and new cameras
Plan Option A Proposed Plan of Camera Room

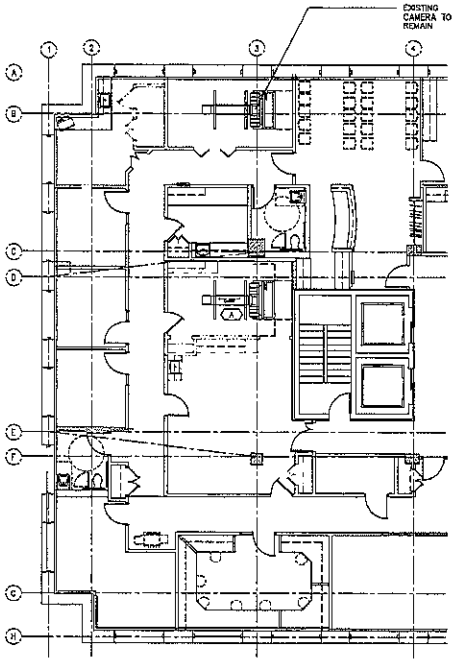


2A PLAN OPTION A
A1.02 1/8"=1'-0"

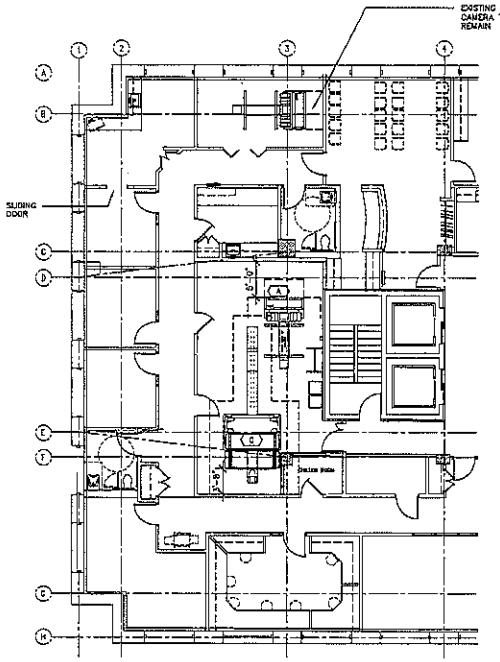


3A PART EXISTING 7TH FLOOR RCP
A1.02 1/8"=1'-0"

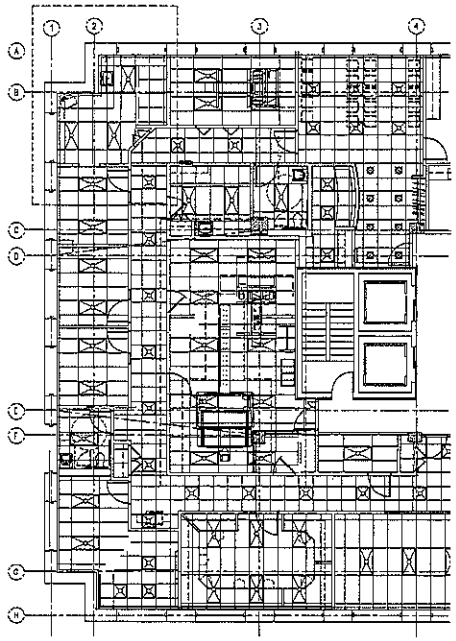
CAMERA INFORMATION		
A		C
EDAT HR+ 74x72x31.9 3400 LBS		BIOGRAPH 6 79x60x66 6500 LBS



1 DEMO PLAN 8TH FLOOR
A1.02 1/8"=1'-0"



2 PLAN 8TH FLOOR
A1.02 1/8"=1'-0"



3 RCP 8TH FLOOR
A1.02 1/8"=1'-0"

SVIGALS + PARTNERS

CONSULTANT 1:

REVISION LOG:

No.	Description	Date

PROJECT NAME:

MNI 8TH FLOOR RENOVATION

PHASE:

9-30-2014 REVIEW

DRAWING TITLE:

EXISTING & PROPOSED CAMERA PLANS

SCALE: AS NOTED

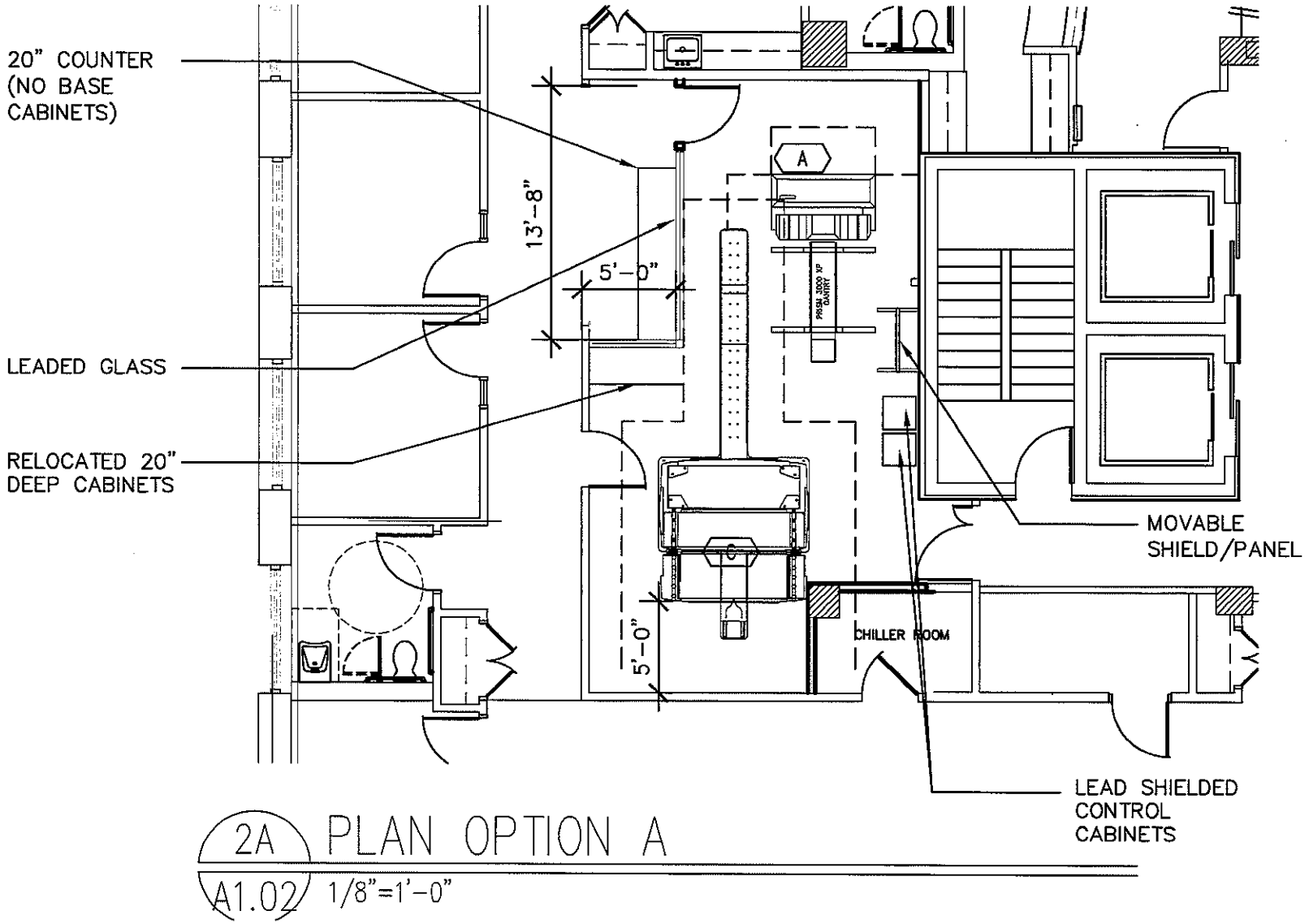
DATE: 6/25/2014

JOB NO: 1423.09

SHEET NO:

A1.02

180



2A PLAN OPTION A

A1.02 1/8"=1'-0"

Appendix I: Supporting Research Papers Published by MNI/ MNI Staff

Appendix I

Publications Contributed By MNI Staff and Data

Note: All publications are available upon request

Barret, Olivier, David Tomae, Adrianna Tavares, David Alagille, Caroline Papin, Rikki Waterhouse, Timothy McCarthy, Danna Jennings, Ken Marek, David Russell, John Seibyl, and Gilles Tamagnan. "In Vivo Assessment and Dosimetry of 2 Novel PDE10A PET Radiotracers in Humans: ^{18}F-MNI-659 and ^{18}F-MNI-654." *The Journal of Nuclear Medicine* Jnumed.113.122895 (2014). Print.
Barthel H, Gertz HJ, Dresel S, Peters O, Bartenstein P, Buerger K, Hiemeyer F, Wittemer-Rump SM, Seibyl J, Reininger C, Sabri O; Florbetaben Study Group. Cerebral amyloid PET (18F) in patients with Alzheimer's disease and healthy controls: a multicetre phase 2 diagnostic study. Lancet Neurol. 2011 May;10(5):424-35.
Batis J, Barret O, Alagille D, Koren AO, Stehouwer JS, Cosgrove K, Goodman M, Seibyl J, Tamagnan G. In vivo evaluation of [123I]mZIENT as a SPECT radioligand for the serotonin transporter. Nucl Med Biol. 2012 Nov;39(8):1137-41.
Berg D, Marek K, Ross GW, Poewe W. Defining at-risk populations for Parkinson's disease: lessons from ongoing studies. Mov Disord. 2012 Apr 15;27(5):656-65. doi: 10.1002/mds.24985. Review.
Hall DA, Jennings D, Seibyl J, Tassone F, Marek K. FMR1 gene expansion and scans without evidence of dopaminergic deficits in parkinsonism patients. Parkinsonism Relat Disord. 2010 Nov;16(9):608-11.
Hannestad JO, Cosgrove KP, DellaGioia NF, Perkins E, Bois F, Bhagwagar Z, Seibyl JP, McClure-Begley TD, Picciotto MR, Esterlis I. Changes in the cholinergic system between bipolar depression and euthymia as measured with [^{123}I]5IA single photon emission computed tomography. Biol Psychiatry. 2013 Nov 15;74(10):768-76.
Jennings D, Eberly S, Oakes D, Seibyl J, Marek K, Shoulson I. Impact of disclosure of individual imaging results in a multi-center Parksinson clinical trial. J Parkinsons Dis. 2014 Jul 25.

Joshi AD, Pontecorvo MJ, Clark CM, Carpenter AP, Jennings DL, Sadowsky CH, Adler LP, Kovnat KD, Seibyl JP, Arora A, Saha K, Burns JD, Lowrey MJ, Mintun MA, Skovronsky DM; Florbetapir F 18 Study Investigators. Performance characteristics of amyloid PERT with florbetapir F 18 in patients with Alzheimer's disease and cognitively normal subjects. J Nucl Med. 2012 Mar;53(3):378-84.
Kessler RM, Seibyl J, Cowan RL, Zald D, Young JS, Ansari MS, Stabin MG. Radiation dosimetry of 18F-FPEB in humans. J Nucl Med. 2014 May 5;55(7):1119-1121.
Marek K, Jennings D, Tamagnan G, Seibyl J. Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression. Ann Neurol. 2008 Dec;64 Suppl 2:S111-21.
Marek K, Jennings D. Can we image premotor Parkinson disease? Neurology. 2009 Feb 17;72(7 Suppl):S21-6.
Marek K, Seibyl J, Eberly S, Oakes D, Shoulson I, Lang AE, Hyson C, Jennings D; Parkinson Study Group PRECEPT Investigators. Longitudinal follow-up of SWEDD subjects in the PRECEPT study. Neurology. 2014 May 20;82(20):1791-7.
Mitsis EM, Riggio S, Kostakoglu L, Dickstein DL, Machac J, Delman B, Goldstein M, Jennings D, D'Antonio E, Martin J, Naidich TP, Aloysi A, Fernandez C, Seibyl J, DeKosky ST, Elder GA, Marek K, Gordon W, Hof PR, Sano M, Gandy S. Tauopathy PET and amyloid PET in the diagnosis of chronic traumatic encephalopathies: studies of a retired NFL player and of a man with FTD and a severe head injury. Transl Psychiatry. 2014 Sep 16;4:e441.
Ong KT, Villemagne VL, Bahar-Fuchs A, Lamb F, Langdon N, Catafau AM, Stephens AW, Seibyl J, Dinkelborg LM, Reininger CB, Putz B, Rohde B, Masters CL, Rowe CC. Ab imaging with 18F-florbetan in prodromal Alzheimer's disease: a prospective outcome study. J Neurol Neurosurg Psychiatry. 2014 Jun 26.
Poewe W, Scherfler C, Tolosa E, Marek K. Supplement neuroimaging movement disorders. Mov Disord. 2009;24 Suppl 2:S655.

Ravina B, Marek K, Eberly S, Oakes D, Kurlan R, Ascherio A, Beal F, Beck J, Flagg E, Galpern WR, Harman J, Lang AE, Schwarzschild M, Tanner C, Shoulson I. Dopamine transporter imaging is associated with long-term outcomes in Parkinson's disease. Mov Disord. 2012 Sep 15;27(11):1392-7. doi: 10.1002/mds.25157.
Russell, David, MD, PhD; Olivier Barrett, PhD; Danna Jennings, MD; Joseph H. Friedman, MD; David Tomae, PhD; David Alagille, PhD; Thomas J. Morley, PhD; Caroline Papin, PhD; Spyridon Papapetropoulos, MD, PhD; Rikki N. Waterhouse, PhD; John P. Seibyl, MD; Kenneth L. Marek, MD. "The Phosphodieterase 10 (PDE10) PET tracer, [^{18}F] MNI-659, as a Novel Biomarker for Early Huntington's Disease." Manuscript number: NEU14-0340R (2014).
Russell, David, MD, PhD; Olivier Barrett, PhD; Danna Jennings, MD; Joseph H. Friedman, MD; Gilles Tamagnan, PhD; David Tomae, PhD; David Alagille, PhD; Spyridon Papapetropoulos, MD, PhD; Rikki N. Waterhouse, PhD; John P. Seibyl, MD; Kenneth L. Marek, MD. "[^{18}F]MNI-659 and PET as an imaging biomarker of PDE10A for longitudinal studies of Huntington disease (HD)". Abstract 583.
Schapira AH, McDermott MP, Barone P, Comella CL, Albrecht S, Hsu HH, Massey DH, Mizuno Y, Poewe W, Rascol O, Marek K. Pramipexole in patients with early Parkinson's disease (PROUD): a randomized delayed-start trial. Lancet Neurol. 2013 Aug;12(8):747-55. doi: 10.1016/S1474-4422(13)70117-0.
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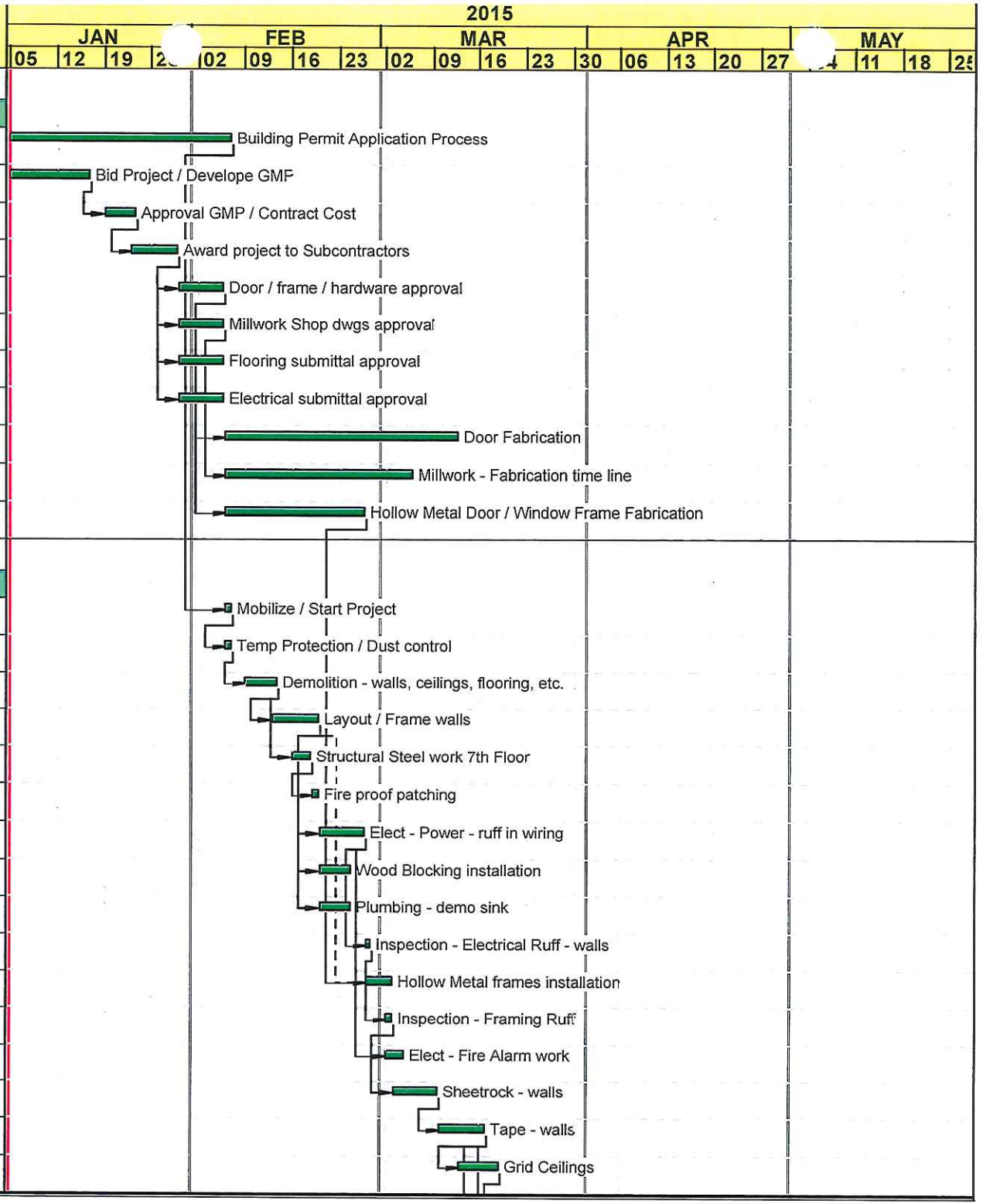
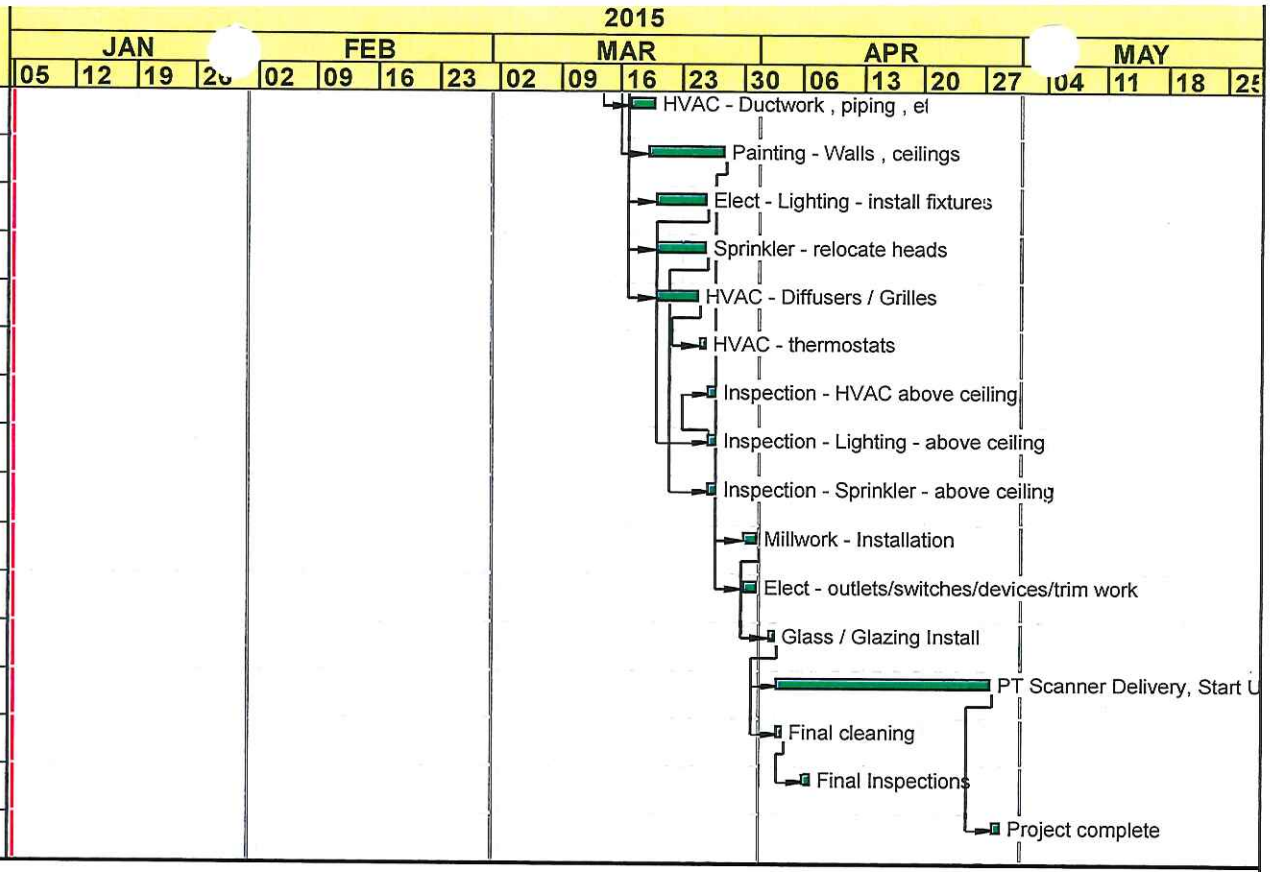
Appendix J: Projected Timelines

Projected Timelines for both Pre and Post Construction Phases

Description	Orig Dur	START	FINISH
PRE CONSTRUCTION PHASE			
Building Permit Application Process	25	05JAN15	06FEB15
Bid Project / Develope GMP	10	05JAN15	16JAN15
Approval GMP / Contract Cost	5	19JAN15	23JAN15
Award project to Subcontractors	5	23JAN15	29JAN15
Door / frame / hardware approval	5	30JAN15	05FEB15
Millwork Shop dwgs approval	5	30JAN15	05FEB15
Flooring submittal approval	5	30JAN15	05FEB15
Electrical submittal approval	5	30JAN15	05FEB15
Door Fabrication	25	06FEB15	12MAR15
Millwork- Fabrication time line	20	06FEB15	05MAR15
Hollow Metal Door / Window Frame Fabrication	15	06FEB15	26FEB15
CONSTRUCTION PHASE			
Mobilize / Start Project	1	06FEB15	06FEB15
Temp Protection / Dust control	1	06FEB15	06FEB15
Demolition - walls, ceilings, flooring, etc.	5	09FEB15	13FEB15
Layout / Frame walls	5	13FEB15	19FEB15
Structural Steel work 7th Floor	3	16FEB15	18FEB15
Fire proof patching	1	19FEB15	19FEB15
Elect - Power - ruff in wiring	5	20FEB15	26FEB15
Wood Blocking installation	3	20FEB15	24FEB15
Plumbing - demo sink	3	20FEB15	24FEB15
Inspection - Electrical Ruff - walls	1	27FEB15	27FEB15
Hollow Metal frames installation	2	27FEB15	02MAR15
Inspection - Framing Ruff	1	02MAR15	02MAR15
Elect - Fire Alarm work	3	02MAR15	04MAR15
Sheetrock - walls	5	03MAR15	09MAR15
Tape - walls	5	10MAR15	16MAR15
Grid Ceilings	4	13MAR15 *	18MAR15



Description	Orig Dur	START	FINISH
HVAC - Ductwork , piping , etc	3	17MAR15	19MAR15
Painting - Walls , ceilings	7	19MAR15	27MAR15
Elect - Lighting - install fixtures	4	20MAR15	25MAR15
Sprinkler - relocate heads	4	20MAR15	25MAR15
HVAC - Diffusers / Grilles	3	20MAR15	24MAR15
HVAC - thermostats	1	25MAR15	25MAR15
Inspection - HVAC above ceiling	1	26MAR15	26MAR15
Inspection - Lighting - above ceiling	1	26MAR15	26MAR15
Inspection - Sprinkler - above ceiling	1	26MAR15	26MAR15
Millwork - Installation	2	30MAR15	31MAR15
Elect - outlets/switches/devices/trim work	2	30MAR15	31MAR15
Glass / Glazing Install	1	02APR15	02APR15
PT Scanner Delivery, Start Up, Testing, etc.	17	03APR15	27APR15
Final cleaning	1	03APR15	03APR15
Final Inspections	1	06APR15	06APR15
Project complete	1	28APR15	28APR15



Start date	05JAN15
Finish date	28APR15
Data date	05JAN15
Run date	07NOV14
Page number	2A

Temple Medical Associates

MNI PT Camera
8th Floor
60 Temple Rd
New Haven, CT

Paniccia Construction Corp.

- Early bar
- Progress bar
- Critical bar
- Summary bar
- Start milestone point
- Finish milestone point

Appendix K: Quote for Equipment (PET CT Camera)

PROJECT: **MNI 8th Floor PET Camera Room**

LOCATION: **60 Temple Street, New Haven, CT**

Estimate Date: **October 15, 2014** **PCC Bid Estimate**

Bid PKG#		ITEM	QTY	UNIT			Labor & Material
02000	1.	**Demolition:**					
	-	demo walls	35	lf	@	=	
	-	demo doors	4	un	@	=	
	-	demo millwork	25	lf	@	=	
	-	demo ceilings	1	ls	@	=	
	-	demo closet	1	ls	@	=	
	-	demo flooring	1	ls	@	=	
		Subtotal					**$ 5,250.00**
05000	2	**Structural Steel**					
	-	7th floor steel work	1	ls	@	=	
	-	Fireproof patching	1	ls	@	=	
		Subtotal					**$ 18,000.00**
06000	6	**Millwork**					
	-	Install existing cabinets	1	ls	@	=	
	-	Furnish and install new countertop with support legs	8	lf	@	=	
		Subtotal					**$ 2,260.00**
08000	11	**Doors / Frames / Hardware**					
	-	new 3x7 solid core wood doors, hm frames and hardware	3	un	@	=	
	-	new lead lined hollow metal frame units	2	un	@	=	
	-	new door closers	2	un	@	=	
	-	new door viewer	1	un	@	=	
		Subtotal					**$ 7,250.00**
08000	12	**Glass and Glazing**					
	-	Lead lined glass	2	un	@	=	
		Subtotal					**$ 19,500.00**
09650	13	**Drywall / Framing / insulation / tape walls**					
	-	walls	144	sf	@	=	
	-	misc. patching	1	ls	@	=	
	-	Lead lined walls	480	sf	@	=	

Bid PKG#		ITEM	QTY	UNIT		Labor & Material
		Subtotal			**$**	**16,990.00**
09700	**14**	**Ceilings**				
		patchwork to existing 2x2				
	-	acoustical grid system	1	ls @	=	
		Subtotal			**$**	**2,950.00**
09680	**15**	**Flooring**				
	-	floor patchwork	1	ls @	=	
	-	floor repair misc patch	1	ls @	=	
	-	wall base repair / patch	1	ls @	=	
		Subtotal			**$**	**2,750.00**
09900	**16**	**Painting**				
	-	walls	1	ls @	=	
	-	door frames	1	ls @	=	
		Subtotal			**$**	**3,150.00**
15000	**19**	**HVAC Systems**				
		Subtotal			**$**	**9,500.00**
15400	**21**	**Plumbing Systems**				
	-	demo sink	1	un @		
		Subtotal			**$**	**1,350.00**
15500	**22**	**Fire Alarm work**				
		Subtotal			**$**	**950.00**
160000	**23**	**Electrical**				
		Subtotal			**$**	**9,500.00**
	23	**Telephone / Data Work**				
		Subtotal			**$**	**1,500.00**
	24	**Final Cleaning Service**				
	-	upon completion of project	1	ls @		
		Subtotal			**$**	**1,150.00**
	25	**Reimbursables / General Requirements**				
	-	Field supervision	1	ls @	=	

Bid PKG#	ITEM	QTY	UNIT		Labor & Material
-	Temporary protection / barriers	1	ls	@ =	
-	Daily cleaning site / dust control	1	ls	@ =	
-	Trash Removal / dumpster	1	un	@ =	
	Subtotal				**$ 8,250.00**

Sub - Total	**$ 110,300.00**
PCC O.H. & P. (10%)	**$ 11,030.00**
Sub - Total	**$ 121,330.00**
CT Sale/Use tax 6.35% (on Gen Condition, Fee, Permit cost)	**$ 1,319.53**
Building Permit Cost	**$ 1,500.00**
Grand Total	**$ 124,149.53**

EXHIBIT A

July 3, 2014

John Seibyl, MD
President
Molecular Neuroimaging, LLC
60 Temple Street
Suite 8A
New Haven, Connecticut 06510

Sales Agreement # 070214.6SL

Reconditioned Siemens Biograph HI-REZ 6 PET/CT

Biograph HI-REZ 6 PET/CT

The Siemens Biograph HI-REZ 6 is a whole-body PET/CT scanner ideal for oncology imaging and diagnosis. The Siemens Biograph HI-REZ 6 PET/CT produces excellent images that show detailed anatomy and biological processes with just one noninvasive procedure.

Features of the Siemens Biograph HI-REZ 6 PET/CT

70 cm gantry aperture and unique reinforced cantilever designs
50 cm scan FOV in all scan modes
Slice thickness: 1, 1.25,2,3,5,6,8 and 10mm, Spiral CT, and Dynamic
Syngo Acquisition Workplace and MI Workplace
Integrated panel display
Maximum patient weight of 204 kg (450 lbs)
Pico-3D ultra fast electronics for decreased dead time and high signal-to-noise
Three-dimensional display of organs with a large axial view
Easy-to-use scan protocols
LCD flat screen monitor

Price:	$465,000
Delivery:	December 1, 2014 or as agreed between the Parties
Payment Terms:	30% ($139,500) as per terms with signed Letter of Understanding above.

35% ($162,750) is due if the Biograph HI-REZ 6 is chosen as the preferred PET/CT. Such payment will be made on or before September 30, 2014.

35% ($162,750) is due at the completion of installation and training.

Validity: September 30, 2014.

Site Preparation: The preparation of the site to accept the Biograph HI-REZ 6 PET/CT scanner (the "Equipment") and all costs associated with such preparation are the responsibility of MNI alone. Installation/placement shall include Equipment set up and start-up. Any requirement for riggers and/or special moving equipment to allow for the safe movement of the Equipment into the building shall be the responsibility of MNI. Any deviation from the original manufacturer's site preparation guidelines which results in Equipment operating problems or damage to the Equipment may result in a forfeiture of Warranty.

Training: Operational training is **not** applications training. Operational training is provided for the technologist so that he/she can become familiar with the operation of the Equipment hardware and software and the environmental requirements for the Equipment. Such training shall not exceed three (3) contiguous days.

Other Preparation: All radioactive material (other than radioactive sources supplied with the scanner) needed to test and calibrate the equipment shall be supplied by MNI.

Warranty: A one-year limited warranty is included with the Equipment described herein. The warranty shall begin at the time of completion of installation or after first use of the Equipment, whichever is earlier, and shall include the replacement of parts that are found to be defective where such defect is not the cause of an action/s taken by MNI or others in MNI's employ. Labor, travel and emergency visits are included.

Should Marquis or its agents determine, in its sole discretion that the defect is as a result of MNI's improper operation of the Equipment or from the improper preparation of or maintenance of the Equipment facility then MNI shall be responsible for all costs (travel, labor and parts) associated with the repair. Such repair costs shall be invoiced and are due immediately upon receipt of invoice by MNI.

Failure to properly operate or maintain the Equipment facility or Equipment during the Warranty period shall result in suspension of or revocation of any remaining Warranty. Should Marquis or its agents determine that MNI has not practiced reasonable caution when housing, operating or maintaining the Equipment then written notice shall be delivered to MNI with the reasons for the revocation or suspension of the Warranty.

Taxes, Fees & Other Costs:

All taxes and fees that may be incurred by MNI for the installation and operation of the equipment are the responsibility of MNI and are not included in the price quoted herein. Any taxes, fees or permits or any other item required to ship, import, install or operate the equipment are the responsibility of MNI.

All approvals, licenses, permits required for installation and use are the responsibility of MNI. Marquis takes no responsibility for securing any governmental approval for the installation or operation of the Equipment. Any permitting required and the cost of such permitting or levied fees are the responsibility of MNI.

Force Majeure:

Notwithstanding any other provision, and in addition to all conditions and exclusions set forth, Marquis will not be liable for any delay or default in performing any obligations caused by events beyond Marquis's control, including (by way of example and not by way of limitation) acts of God, acts of third parties, acts of MNI (or any of MNI 's employees, agents, or representatives), acts of civil or military authorities, fires, floods, and other similar or dissimilar natural causes, riots, wars, sabotage, vandalism embargoes, labor disputes, strikes, lockouts, lack or shortage of transportation, labor, materials, supplies, fuel, power or water, delays in receiving any permits or licenses, delays caused by any laws, regulations, proclamations, ordinances, or any government action or inaction, delays caused by contractors and subcontractors, and any other cause or condition beyond Marquis's control. In the event of any such delay or default, the time for performance of the obligations of Marquis will be extended for such a time, as Marquis in its sole discretion deems reasonable.

Limitation of Liability:

IN NO EVENT SHALL EITHER PARTY BE LIABLE TO THE OTHER PARTY OR TO ANY THIRD PARTY FOR ANY INDIRECT, PUNITIVE, INCIDENTAL, SPECIAL OR CONSEQUENTIAL DAMAGES, IN CONNECTION WITH THE TRANSACTIONS UNDER THIS AGREEMENT, EVEN IF SUCH PARTY HAS BEEN ADVISED OF THE POSSIBILITY

OF SUCH DAMAGES AND REGARDLESS OF THE LEGAL OR EQUITABLE THEORY (CONTRACT, TORT OR OTHERWISE) UPON WHICH THE CLAIM IS BASED.

THE LIMITATIONS SPECIFIED IN THIS SECTION SHALL NOT APPLY TO A PARTY'S INDEMNIFICATION FOR BREACHES OF ANY CLAUSES WITHIN THIS SALES AGREEMENT OR COMPLIANCE WITH ALL APPLICABLE LAWS.

Governing Law & Jurisdiction: This Agreement and the parties' actions under this Agreement shall be governed by and construed under the laws of the State of Louisiana, without reference to conflict of law principals. The parties hereby expressly consent to the jurisdiction and venue of the Federal and State courts within the State of Louisiana. Each party hereby irrevocably consents to the service of process in any such action or proceeding by the mailing of copies hereof by registered or certified mail, postage prepaid, to such part at its address set forth in this Agreement, such service to become effective thirty (30) days after such mailing.

Severability: If any portion of this contract shall be held to be invalid or unenforceable for any reason, the remaining provisions shall continue to be valid and enforceable. If a court finds that any provision of this contract is invalid or unenforceable, but that by limiting such provision, it would become valid and enforceable, then such provision shall be deemed to be written, construed, and enforced as so limited.

Waiver: The failure of either party to enforce any provision of this contract shall not be construed as a waiver or limitation of that party's right to subsequently enforce and compel strict compliance with every provision of this contract.

Miscellaneous: This Agreement constitutes the entire Agreement between the parties and supersedes any previous agreement, understanding or order between the parties. Should the terms and conditions of any purchase order issued in connection with this Agreement conflict with the terms contained in this Agreement, the terms of this Agreement will prevail. No modification or waiver of the terms of this Agreement will be binding unless made in writing and signed

by both parties. This Agreement and any amendment or modification to this agreement are subject to Marquis Medical, LLC written acceptance at our home office in the state of Louisiana. This Agreement will be governed by and interpreted in accordance with the laws of the state of Louisiana. Marquis Medical, LLC will be permitted to assign this Agreement or any of Marquis Medical, LLC rights and obligations under this Agreement to any affiliate of Marquis Medical, LLC. If a dispute arises from or relates to this contract or the breach thereof, and if the dispute cannot be settled through direct discussions, the parties agree to endeavour first to settle the dispute by mediation administered by the American Arbitration Association under its Commercial Mediation Procedures before resorting to arbitration. Any unresolved controversy or claim arising from or relating to this contract or breach thereof shall be settled by arbitration administered by an arbitrator from the American Arbitration Association in accordance with its Commercial Arbitration Rules in Baton Rouge, Louisiana, and judgement on the award rendered by the arbitrator may be entered in any court having jurisdiction thereof. If all parties to the dispute agree, a mediator involved in the parties' mediation may be asked to serve as the arbitrator.

The Parties hereby acknowledge and agree that any and all terms, conditions, covenants and provisions of this Agreement have been negotiated in good faith and from equal bargaining positions with each other and that the Parties' respective legal counsel have reviewed and counselled each party respecting same and further agree that the rule of Contract Law which states that any ambiguity or contradictions between the terms shall be resolved against the drafter of such agreement, is hereby waived and shall not apply to the interpretation of this Agreement.

Please signify your acceptance of the terms stated herein with your signature:

For BUYER, Molecular Neuroimaging, LLC:	For SELLER, Marquis Medical, LLC	Date:
_____	_____	July 3, 2014
Name:	Joseph Sciarra	
Title:	Principal	

EXHIBIT B

July 3, 2014

John Seibyl, MD
President
Molecular Neuroimaging, LLC
60 Temple Street
Suite 8A
New Haven, Connecticut 06510

Sales Agreement # 070214.16SL

Reconditioned Siemens Biograph HI-REZ 16 PET/CT

Biograph HI-REZ 16 PET/CT

The Siemens Biograph HI-REZ 16 is a whole-body PET/CT scanner ideal for oncology imaging and diagnosis. The Siemens Biograph HI-REZ 16 PET/CT produces excellent images that show detailed anatomy and biological processes with just one noninvasive procedure.

Features of the Siemens Biograph HI-REZ 16 PET/CT

70 cm gantry aperture and unique reinforced cantilever designs
50 cm CT Transverse FOV
LSO Detector Technology
4.5 ns coincidence window
Static and multi-bed acquisition modes
100s spiral scan times

Estimated Tube Count	216852
Estimated Gantry Count	1.3542E+006
Dura Akron Q Tube Age	7/13/12

Software Options

HIS/RIS	3D SSD	MIP Image	MPR	Cap 3D Volume
Care Bolus	Care Dose	Syngo	High Speed	Image Fusion Basic
Image Fusion LM	Scan Voltage 100KV	Cardio Turbo Recon	Routine Speed	Sensation 16
Extended FOV	PET Application			

STATE OF CONNECTICUT

DEPARTMENT OF PUBLIC HEALTH

Office of Health Care Access

December 4, 2014

VIA FAX ONLY

Kimberly Fabrizio
Sr. Director of Regulatory Affairs and Quality Assurance
Molecular Neuroimaging, LLC
60 Temple Street
New Haven, CT 06510

RE: Certificate of Need Application, Docket Number: 14-31965-CON
Molecular Neuroimaging, LLC
Acquisition of a Positron Emission Tomography/Computed Tomography Scanner for Research Study

Dear Ms. Fabrizio:

On November 18, 2014, the Office of Health Care Access ("OHCA") received your Certificate of Need ("CON") application filing on behalf of Molecular Neuroimaging, LLC ("Applicant") proposing to acquire a Positron Emission Tomography/Computed Tomography ("PET/CT") scanner, with a total associated cost of $600,000.

OHCA has reviewed the CON application and requests the following additional information pursuant to General Statutes §19a-639a(c).

1. Provide any available documentation, such as a letter of interest, from Bank of America demonstrating your approval to borrow the funds needed to purchase the proposed PET/CT scanner.

2. On page 5 of the application, the Applicant indicates that demand for PET imaging as a research tool is growing. Please provide scholarly articles or other evidence to support that assertion. Additionally, please include a brief overview of each source submitted and how it demonstrates that need.

3. On page 10 of the CON application, the Applicant states that it will begin contracting new studies after the purchase, delivery and installation of the new scanner. Based on the above:

    a. How many, if any, pending or proposed studies do you have that would incorporate the proposed scanner? Provide information on these studies as you did

in Appendix G of your application.

b. Provide a detailed explanation of all assumptions used in the calculation of the projected volume for the new scanner.

4. Please elaborate on each of your responses of "N/A" for application questions 6(a)-(d), regarding your current and projected patient population mix. In particular, clearly explain why the Applicant states that Medicaid issues are "not applicable" to the proposed scanner.

In responding to the questions contained in this letter, please repeat each question before providing your response. Paginate and date your response, i.e., each page in its entirety. Information filed after the initial CON application submission (e.g., completeness response letter, prefile testimony, late file submissions and the like) must be numbered sequentially from the Applicant's document preceding it. Please begin your submission using Page 202 and reference "Docket Number: 14-31965-CON." Submit one (1) original and two (2) hard copies of your response. In addition, please submit a scanned copy of your response, in an Adobe format (.pdf) including all attachments on CD. If available, a copy of the response in MS Word should also be copied to the CD.

Pursuant to Section 19a-639a(c) of the Connecticut General Statutes, you must submit your response to this request for additional information not later than sixty (60) days after the date that this request was transmitted. Therefore, please provide your written responses to OHCA no later than **February 2, 2015**, otherwise your application will be automatically considered withdrawn. If you have any questions concerning this letter, please feel free to contact me by email or at (860) 418-7007.

Sincerely,

A. Veyberman

Alla Veyberman
Health Care Analyst

* * * COMMUNICATION RESULT REPORT (DEC. 4. 2014 3:42PM) * * *

FAX HEADER:

TRANSMITTED/STORED : DEC. 4. 2014 3:41PM FILE MODE	OPTION	ADDRESS	RESULT	PAGE
789 MEMORY TX		912035081503	OK	3/3

REASON FOR ERROR
E-1) HANG UP OR LINE FAIL
E-2) BUSY
E-3) NO ANSWER
E-4) NO FACSIMILE CONNECTION

STATE OF CONNECTICUT
OFFICE OF HEALTH CARE ACCESS

FAX SHEET

TO: KIMBERLY FABRIZIO

FAX: 203.508.1503

AGENCY: MNI

FROM: OHCA

DATE: 12/04/14 **Time:**

NUMBER OF PAGES: 2
(including transmittal sheet

Comments:
Docket Number: 14-31965

PLEASE PHONE IF THERE ARE ANY TRANSMISSION PROBLEMS

Phone: (860) 418-7001 Fax: (860) 418-7053

410 Capitol Ave., MS#13HCA
P.O.Box 340308
Hartford, CT 06134

MNI
Molecular NeuroImaging, LLC

60 Temple Street • Suite 8A • New Haven, Connecticut 06510 • Phone: 203.401.4300 • Fax: 203.789.8037 • www.mnimaging.com

December 22, 2014

State of Connecticut Department of Public Health
Office of Health Care Access
410 Capitol Ave., MS#13HCA
P.O. Box 340308
Hartford, CT 06134

RE: Certificate of Need Application Response, Docket Number: 14-31965-CON

Dear Alla Veyberman,

Please find enclosed Molecular NeuroImaging, LLC's responses to the letter received on 04 December 2014 in reference to Docket Number: 14-31965-CON. Enclosed in this response are the requested original and two hard copies of the response, as well as a PDF and MS Word version of the response contained on CD.

This response is pertaining to the request for Molecular NeuroImaging, LLC to aquire a Positron Emission Tomography/ Computed Tomography Scanner for use in Research studies. If you have any questions concerning this response or the initial application, please feel free to contact me by email (kfabrizio@mnimaging.com) or at (203) 401-4313.

Sincerely,

Kimberly Fabrizio
Sr. Director of Regulatory Affairs and Quality Assurance

1. Provide any available documentation, such as a letter of interest, from Bank of America demonstrating your approval to borrow the funds needed to purchase the proposed PET /CT scanner.

Please see the attached reference section for the letter of interest from Bank of America dated 11 June 2014.

2. On page 5 of the application, the Applicant indicates that demand for PET imaging as a research tool is growing. Please provide scholarly articles or other evidence to support that assertion. Additionally, please include a brief overview of each source submitted and how it demonstrates that need.

Clinicaltrials.gov yields a hit of 2,163 clinical research studies[1] containing a PET Imaging component. 923 of these clinical studies are currently open for enrollment and the use of PET PET imaging in these trials could be as inclusion criteria, efficacy/safety evaluation of new therapeutics under development or as an evaluation of novel PET imaging ligands to serve as potential biomarkers in future therapeutic trials. There has been a tremendous increase in the use of PET imaging in clinical trials aimed at evaluating the diagnosis or treatment modalities for neurodegenerative disorders thus resulting in an increase in demand for PET camera time at our imaging research center. The use of amyloid PET imaging is only recently become integrated into the diagnostic formulations for probable and possible Alzheimer's disease and used for inclusion criteria in the research setting now in nearly all therapeutic research trials for Alzheimer's disease. There are numerous peer-reviewed articles citing the current utility of the PET imaging in research and a sampling of these are referenced in the table provided below.

The FDA has also recognized the increased use of PET Drugs in clinical research in 2012 in an "FDA Update" presentation delivered by Dr. Dwaine Rieves, Director, Division of Medical Imaging Products (since retired). Several FDA initiatives surrounding the use of PET Drug research were outlined in this presentation including the creation of the *Medical Imaging Drugs Advisory Committee (MIDAC)* and a "PET Drug Website[2]". In addition, the FDA recognized the need to provide a standardized guidance to research organizations on how to submit an Investigational New Drug Application for PET Drugs (Investigational New Drug Applications for Positron Emission Tomography (PET) Drugs, December 2012), therefore further supporting the increased research into PET Drug research. All of these FDA supported initiatives provides validation for the increase use of PET Imaging in clinical research.

In 2013, the Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging convened the Amyloid Imaging Taskforce (AIT) in order to better define the use of PET Imaging in Amyloid detection, as it relates to Alzheimer Disease research ("The Appropriate use

criteria for amyloid PET: A report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association.").

Although the Centers for Medicare & Medicaid Services (CMS) determined in 2013 that there is "sufficient evidence that the use of PET Aβ imaging is promising in two scenarios: (1) to exclude Alzheimer's disease (AD) in narrowly defined and clinically difficult differential diagnoses, such as AD versus frontotemporal dementia (FTD); and (2) to enrich clinical trials seeking better treatments or prevention strategies for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors". Both of these factors accepted by the CMS support the use of PET Imaging in clinical research.

[1](http://www.clinicaltrials.gov/ct2/results?term=PET+Imaging&recr=&rslt=&type=&cond=&intr=&titles=&outc=&spons=&lead=&id=&state1=&cntry1=&state2=&cntry2=&state3=&cntry3=&locn=&gndr=&rcv_s=&rcv_e=&lup_s=&lup_e=)

[2] http://www.fda.gov/drugs/developmentapprovalprocess/manufacturing/ucm085783.htm

The following articles have been attached to this response to support the increased demand for PET Imaging as a research tool.

PDF Article	Abstract to be included
Reference 1: Appropriate use criteria for amyloid PET: A report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association, **Alzheimer's & Dementia; 2013; 1-15.**	"Positron emission tomography (PET) of brain amyloid B is a technology that is becoming more available, but its clinical utility in medical practice requires careful definition. To provide guidance to dementia care practitioners, patients, and caregivers, the Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging convened the Amyloid Imaging Taskforce (AIT). The AIT considered a broad range of specific clinical scenarios in which amyloid PET could potentially be used appropriately. Peer-reviewed, published literature was searched to ascertain available evidence relevant to these scenarios, and the AIT developed a consensus of expert opinion. Although empirical evidence of impact on clinical outcomes is not yet available, a set of specific appropriate use criteria (AUC) were agreed on that define the types of patients and clinical circumstances in which amyloid PET could be used. Both appropriate and inappropriate uses were considered and formulated, and are reported and discussed here. Because both dementia care and amyloid PET technology are in active development, these AUC will require periodic reassessment. Future research directions are also outlined, including diagnostic utility and patient-centered outcomes."
Reference 2: **Dr. Dwaine Rieves, MD; 2012 FDA Update, March 5, 2013**	*Focus on Imaging Drugs…* • Drug approvals & labeling actions • Standardization guidance • Medical Imaging Drug Advisory Committee (MIDAC) • Positron Emission Tomography (PET) topics
Reference 3: Decision Memo for Beta Amyloid Positron Emission Tomography in Dementia and Neurodegenerative Disease **(CAG-00431N), September 2013**	A. The Centers for Medicare & Medicaid Services (CMS) has determined that the evidence is insufficient to conclude that the use of positron emission tomography (PET) amyloid-beta (Aβ) imaging is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member for Medicare beneficiaries with dementia or neurodegenerative disease, and thus PET Aβ imaging is not covered under §1862(a)(1)(A) of the Social Security Act ("the Act"). B. However, there is sufficient evidence that the use of PET Aβ imaging is promising in two scenarios: (1) to exclude Alzheimer's disease (AD) in narrowly defined and clinically difficult differential diagnoses, such as AD versus frontotemporal dementia (FTD); and (2) to enrich clinical trials seeking better treatments or prevention strategies for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors.
Reference 4: *Review Article*	PET based tools can improve the early diagnosis of Alzheimer's disease (AD) and differential diagnosis of dementia. The importance of identifying individuals at risk of developing dementia among people with

A Survey of FDG- and Amyloid-PET Imaging in Dementia and GRADE Analysis, **Hindawi Publishing Corporation, BioMed Research International; Vol 2014, Aricle ID 785039**	subjective cognitive complaints or mild cognitive impairment has clinical, social, and therapeutic implications. Within the two major classes of AD biomarkers currently identified, that is, markers of pathology and neurodegeneration, amyloid- and FDG-PET imaging represent decisive tools for their measurement. As a consequence, the PET tools have been recognized to be of crucial value in the recent guidelines for the early diagnosis of AD and other dementia conditions. The references based recommendations, however, include large PET imaging literature based on visual methods that greatly reduces sensitivity and specificity and lacks a clear cut-off between normal and pathological findings. PET imaging can be assessed using parametric or voxel-wise analyses by comparing the subject's scan with a normative data set, significantly increasing the diagnostic accuracy. This paper is a survey of the relevant literature on FDG and amyloid-PET imaging aimed at providing the value of quantification for the early and differential diagnosis of AD. This allowed a meta-analysis and GRADE analysis revealing high values for PET imaging that might be useful in considering recommendations.
Reference 5: Molecular imaging insights into neurodegeneration: Focus on Tau PET radiotracers **J Nucl Med 2014; 55(6):871-874**	"Neurodegenerative diseases are characterized by progressive dysfunction and neuronal death, showing specific protein inclusions at autopsy. In vivo detection of these key proteins, namely amyloid-β, tau, α-synuclein, and trans-active response DNA binding protein 43 kDa, is possible by means of molecular neuroimaging techniques, such as PET. The development of selective PET radiotracers targeting these proteins is critical for early and accurate diagnosis and for the successful development of disease-modifying therapies. Selective PET radiotracers for amyloid-β are already available, and potential tau tracers are emerging as new-generation biomarkers. An overview of the tau-PET radiotracer development scenario, focusing on tracers that are presently being examined in humans, is presented."
Reference 6: Perspective on future role of biological markers in clinical therapy trials of Alzheimer's disease: a long-range point of view beyond 2020 **Biochm Pharmacol 2014;88:426-449**	"Recent advances in understanding the molecular mechanisms underlying various paths toward the pathogenesis of Alzheimer's disease (AD) has begun to provide new insight for interventions to modify disease progression. The evolving knowledge gained from multidisciplinary basic research has begun to identify new concepts for treatments and distinct classes of therapeutic targets; as well as putative disease-modifying compounds that are now being tested in clinical trials. There is a mounting consensus that such disease modifying compounds and/or interventions are more likely to be effectively administered as early as possible in the cascade of pathogenic processes preceding and underlying the clinical expression of AD. The budding sentiment is that ''treatments'' need to be applied before various molecular mechanisms converge into an irreversible pathway leading to morphological, metabolic and functional alterations that characterize the pathophysiology of AD. In light of this, biological indicators (including PET imaging markers) of pathophysiological mechanisms are desired to chart and detect AD

	throughout the asymptomatic early molecular stages into the prodromal and early dementia phase. A major conceptual development in the clinical AD research field was the recent proposal of new diagnostic criteria, which specifically incorporate the use of biomarkers as defining criteria for preclinical stages of AD. This paradigm shift in AD definition, conceptualization, operationalization, detection and diagnosis represents novel fundamental opportunities for the modification of interventional trial designs. This perspective summarizes not only present knowledge regarding biological markers but also unresolved questions on the status of surrogate indicators for detection of the disease in asymptomatic people and diagnosis of AD."
Reference 7: The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease **Alzheimer's & Dementia 2011; 7:263-69**	"The National Institute on Aging and the Alzheimer's Association charged a workgroup with the task of revising the 1984 criteria for Alzheimer's disease (AD) dementia. The workgroup sought to ensure that the revised criteria would be flexible enough to be used by both general healthcare providers without access to neuropsychological testing, advanced imaging, and cerebrospinal fluid measures, and specialized investigators involved in research or in clinical trial studies who would have these tools available. We present criteria for all-cause dementia and for AD dementia. We retained the general framework of probable AD dementia from the 1984 criteria. On the basis of the past 27 years of experience, we made several changes in the clinical criteria for the diagnosis. We also retained the term possible AD dementia, but redefined it in a manner more focused than before. Biomarker evidence (including amyloid PET imaging) was also integrated into the diagnostic formulations for probable and possible AD dementia for use in research settings. The core clinical criteria for AD dementia will continue to be the cornerstone of the diagnosis in clinical practice, but biomarker evidence is expected to enhance the pathophysiological specificity of the diagnosis of AD dementia. Much work lies ahead for validating the biomarker diagnosis of AD dementia."
Reference 8: Positron emission tomography molecular imaging for drug development **Br J Clin Pharmacol 2012; 73(2):175-86**	Human in vivo molecular imaging with positron emission tomography (PET) enables a new kind of 'precision pharmacology', able to address questions central to drug development. Biodistribution studies with drug molecules carrying positron-emitting radioisotopes can test whether a new chemical entity reaches a target tissue compartment (such as the brain) in sufficient amounts to be pharmacologically active. Competition studies, using a radioligand that binds to the target of therapeutic interest with adequate specificity, enable direct assessment of the relationship between drug plasma concentration and target occupancy. Tailored radiotracers can be used to measure relative rates of biological processes, while radioligands specific for tissue markers expected to change with treatment can provide specific pharmacodynamic information. Integrated application of PET and magnetic resonance imaging (MRI) methods allows molecular

	interactions to be related directly to anatomical or physiological changes in a tissue. Applications of imaging in early drug development can suggest approaches to patient stratification for a personalized medicine able to deliver higher value from a drug after approval. Although imaging experimental medicine adds complexity to early drug development and costs per patient are high, appropriate use can increase returns on R and D investment by improving early decision making to reduce new drug attrition in later stages. We urge that the potential value of a translational molecular imaging strategy be considered routinely and at the earliest stages of new drug development.

3. On page 10 of the CON application, the Applicant states that it will begin contracting new studies after the purchase, delivery and installation of the new scanner. Based on the above:

a. How many, if any, pending or proposed studies do you have that would incorporate the proposed scanner? Provide information on these studies as you did in Appendix G of your application.

In the below table, MNI has pulled as much proposed research for 2015 together to support the need for a second PET Camera, as proposed in our certificate of need application.

Study Description	Research Target	Patient Population	PET Scans Potential by Study May – Dec 2015
Tau Tracer Development Program Selectivity (various tracers)/Jan 2015	Tau	Alzheimer	18
Tau Tracer Development Program Tau Pathologies (various tracers)/Spring 2015	FTD, PSP, CBD	Neurodegenerative disorders	12
NIH Grant: “Program for Innovative PET Radioligand Development and Application – a translational toolbox for treatments for Mental Health”/Spring 2015	Various	Neurodegenerative and Psychiatric Disorders	16
Huntington’s Disease MNI65X and FMH3 Tracer Development Program/Spring 2015	Various	Huntington’s Disease	50 - 60
Tau Development	Tau	Mild/Moderate Alzheimer’s	24-31
Cholesterol Metabolism RO Study MNI79X/Spring 2015	Cholesterol marker	Alzheimer	10-14

MNI79X Test/Retest Study MNI79X Tracer/Jan 2015	PDE2A Inhibitor	Alzheimer	10 – 20
Dosimetry Study MNI79X/Sping 2015	PDE2A Inhibitor	Neurodegenerative disorders	10
Imaging agent development for A2A project/Summer 2015	Various A2A	Neurodegenerative and Psychiatric Disorders	10 - 20
HDACE2 Inhibitor Program/Summer 2015	HDACE2	Neurodegenerative and Psychiatric Disorders	10 - 20
Michael J. Fox Foundation Biomarker Development Program/Spring 2015	Synuclien	Neurodegenerative Disorders	10 - 20
GBA Imaging agent development project/Fall 2015	GBA	Neurodegenerative and Psychiatric Disorders	10 - 20
Current Total Estimated Scans Under Pending Studies Above			**190 - 261**
Estimated Scans using historical closing rate of 70%			**133 – 183**
Estimated New Study Scans Anticipated to be performed on existing PET camera			**60 – 90**
Estimated Scans to be performed on newly acquired PET-CT Camera			**73 – 93 (our mid-line estimate is 80 scans used in the CON application)**

b. Provide a detailed explanation of all assumptions used in the calculation of the projected volume for the new scanner.

To develop a multi-year estimate of utilization of the new PET scanner, we used the following methodology:

a. We developed our base year 2015 estimate of scans from our current prospect list of new studies to yield an estimate of 10 scans/month (80 for 2015 in total) expected on the new scanner during the partial year period May – December 2015 (please refer to chart of prospective studies, above).

b. For the YoY growth in utilization for future years beyond 2015, we reviewed the historical utilization growth rates from our existing PET scanner, and adjusted this

historical data to incorporate information from our recent experience and market knowledge.

The increase from 2015 to 2016 reflects a 60% growth in scanner utilization rate. Our initial YoY growth for our current scanner in the year after initial use was approximately 40%. Given the much larger volume of potential studies at this point in time compared the potential studies we had when we initiated our first PET scanner, we are confident our current growing momentum will continue and support a YoY growth of 60% between the utilization rates in 2015 and 2016.

For YoY growth in 2017 and beyond, we expect a lower rate of approximately 18% and lower as we begin to approach full utilization of the camera.

4. Please elaborate on each of your responses of "N/ A" for application questions 6(a)-(d)˜ regarding your current and projected patient population mix. In particular, clearly explain why the Applicant states that Medicaid issues are '"not applicable" to the proposed scanner.

MNI has responded that the Medicaid issues are not applicable to this proposed camera, as we are not a Medicaid reimbursable or POS entity. MNI conducts research only and neither MNI nor its researchers are registered to receive payment from Medicaid or any other insurers for any clinical services, including PET Imaging that would be conducted on the proposed camera.

Bank of America

June 11, 2014

Molecular Neuroimaging, LLC
60 Temple Street, Ste. 8A
New Haven, CT 06510

RE: Credit Application Number: 048969

Bank of America, N.A. ("BANA") is pleased to confirm its willingness to provide an Equipment Line of Credit to **Molecular Neuroimaging, LLC** ("Client") as further detailed in the attached "Exhibit A" which is made a part hereof and the terms of which are incorporated herein by reference, subject to the following:

DOCUMENTATION:	Client shall execute and deliver all transaction documents, in form and substance satisfactory to BANA, and satisfy all conditions required by BANA.
LINE AMOUNT:	An amount not to exceed **$750,000.00** ("BANA's Cost") which may with BANA's prior consent include related soft costs such as freight, installation and taxes paid up-front by BANA not exceeding 20% of BANA's Cost, but may not exceed the fair market value of the Equipment. BANA's Cost for used Equipment may be subject to verification by an independent third party appraiser at Client's expense.
LINE FEE:	(.25%), Payable upon the Draw
UTILIZATION PERIOD EXPIRATION DATE:	The latest date for any funding shall be **April 4, 2015**.
FINANCIAL COVENANTS:	All financial covenants shall apply.

The commitment of BANA to enter into this transaction is based on the current business, management, and financial condition of Client and Guarantors, if any. Accordingly, this approval is further subject to the condition that there does not occur any material adverse change in the business, current management, or financial condition of Client or any Guarantor, in BANA's sole determination.

To activate the Line, you must acknowledge your acceptance of the terms and conditions of this approval by signing below and returning this letter to my attention no later than thirty (30) business days after the date hereof. If we have not received your acceptance by that date, the approval set forth herein will terminate. My address is:

Bank of America, N.A.
2059 Northlake Parkway, 3rd Floor North
Tucker, GA 30084

Thank you for allowing Bank of America, N.A. to make this transaction available to you. If you have any questions, please call me at (678) 287-2842.

Sincerely,

Darnett Lue

Darnett Lue
Documentation Officer

cc: Ian Williams, VP, Leasing Sales Officer

Molecular Neuroimaging, LLC hereby agrees to the terms and conditions set forth herein.

By: _____

Printed Name: JACK MARIOTTI

Title: CFO

Date: 12 JUN 2014

"EXHIBIT A"
EQUIPMENT LINE TERMS

Date:	April 4, 2014
Client:	Molecular Neuroimaging, LLC (Borrower / Lessee depending upon transaction structure)
Guarantor(s):	John P. Seibyl and Kenneth L. Marek
Line Amount:	$750,000.00
Expiration of Line:	April 4, 2015
Line Fee:	(.25%); Payable upon the Draw
Equipment:	To be specified by Client and acceptable to Lender/Lessor. Soft costs (i.e., freight, installation, up-front taxes), if any, must be expressly approved by Lender/Lessor and may not exceed 20% of actual Equipment cost.
Collateral:	Advances under this Line may also be secured by any collateral pledged to the Lender/Lessor under any other loan or lease you have or may obtain from Lender/Lessor or any of its affiliates.
Financing Term:	Up to 60 months. Actual financing term will depend on Equipment financed and is subject to approval by Lender/Lessor.
Pricing:	This Line is non-revolving and non-renewable and will expire as of the Expiration date shown above. Remaining availability of funding under the Line will reduce by the amount of each draw made. Pricing for each draw under the Line will be based upon Lender/Lessor's assessment of market conditions at the time of each draw and locked in for the duration of its Financing Term at the time of such draw (each draw may have a different rate). Draws under this Line may be funded by Banc of America Leasing & Capital, LLC or Bank of America, N.A. (Lender/Lessor).
End of Term Option:	Dependent upon transaction structure selected by Client and approved by Lender/Lessor.
Market Disruption:	Notwithstanding anything contained herein to the contrary, in the event any material change shall occur in the financial markets after the date hereof, including but not limited to any governmental action or other event which materially adversely affects the extension of credit by banks, leasing companies or other lending institutions, Lessor may withdraw this Line or modify any of the terms and conditions thereof, including without limitation the Pricing described herein.
Transaction Costs:	All transaction costs, if any, will be paid by Client. An additional

one-time set-up charge of $250.00 will be waived if Client agrees to automatic deduction of payments.

Activation and Funding:

1. To activate this Line, sign and return the accompanying approval letter to the Documentation Officer noted in such letter.
2. To request funding under the Line, provide copies of vendor invoice(s) to the Documentation Officer shown below.
3. Documentation for your initial draw under the Line may include a Master Agreement, under which Schedules or Notes would be executed for each request. Minimum Schedule amount is $25,000.00.
4. Multiple vendor invoices may be combined under a single Schedule.
5. Upon completion, execution, and return of funding documentation we will pay you (with proof of payment) or your vendor(s) directly. Minimum invoice amount is $5,000.00.

Documentation Officer:

Darnett Lue
2059 Northlake Parkway,
3 North
Tucker, GA 30084

Darnett.E.Lue@baml.com
V: 678.287.2842
F: 404.965.9764

ELSEVIER

Alzheimer's & Dementia ■ (2013) 1–15

Alzheimer's & Dementia

Appropriate use criteria for amyloid PET: A report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association

Keith A. Johnson[a], Satoshi Minoshima[b], Nicolaas I. Bohnen[c], Kevin J. Donohoe[d], Norman L. Foster[e], Peter Herscovitch[f], Jason H. Karlawish[g], Christopher C. Rowe[h], Maria C. Carrillo[i,*], Dean M. Hartley[i], Saima Hedrick[j], Virginia Pappas[j], William H. Thies[i]

[a]*Departments of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[b]*Department of Radiology, University of Washington, Seattle, WA, USA*
[c]*Departments of Radiology and Neurology, University of Michigan, and VA Ann Arbor Healthcare System, Ann Arbor, MI, USA*
[d]*Beth Israel Deaconess Medical Center, Boston, MA, USA*
[e]*Department of Neurology, University of Utah, Salt Lake City, UT, USA*
[f]*PET Department, NIH Clinical Center, National Institutes of Health, Bethesda, MD, USA*
[g]*Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
[h]*Department of Nuclear Medicine and Centre for PET, Austin Health, Victoria, Australia*
[i]*Division of Medical and Scientific Relations, Alzheimer's Association, Chicago, IL, USA*
[j]*Society of Nuclear Medicine and Molecular Imaging, Reston, VA, USA*

Abstract Positron emission tomography (PET) of brain amyloid β is a technology that is becoming more available, but its clinical utility in medical practice requires careful definition. To provide guidance to dementia care practitioners, patients, and caregivers, the Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging convened the Amyloid Imaging Taskforce (AIT). The AIT considered a broad range of specific clinical scenarios in which amyloid PET could potentially be used appropriately. Peer-reviewed, published literature was searched to ascertain available evidence relevant to these scenarios, and the AIT developed a consensus of expert opinion. Although empirical evidence of impact on clinical outcomes is not yet available, a set of specific appropriate use criteria (AUC) were agreed on that define the types of patients and clinical circumstances in which amyloid PET could be used. Both appropriate and inappropriate uses were considered and formulated, and are reported and discussed here. Because both dementia care and amyloid PET technology are in active development, these AUC will require periodic reassessment. Future research directions are also outlined, including diagnostic utility and patient-centered outcomes.

Keywords: Guidelines; AUC; Imaging; Amyloid; MCI; Alzheimer's; PET; Florbetapir; Biomarker; Beta-amyloid; Dementia; Radiopharmaceutical

1. Introduction

Research progress in Alzheimer's disease (AD) and molecular imaging during the past decade has made it possible to detect human brain amyloid β (Aβ) deposition during life using positron emission tomography (PET). Parallel progress has improved our understanding of Aβ as an important and therapeutically targetable component of AD pathology. Although Aβ plaques are one of the defining pathological features of AD, many otherwise normal elderly people have elevated levels of Aβ, as do patients with clinical syndromes other than AD dementia. The potential clinical utility of Aβ PET therefore requires careful consideration so that its role may be identified and placed in the proper

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*Corresponding author. Tel.: +312 335 5722; Fax: +866 741 3716.
E-mail address: maria.carrillo@alz.org

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clinical context. The Society of Nuclear Medicine and Molecular Imaging (SNMMI) and the Alzheimer's Association (AA) have jointly developed this article to assist in the appropriate use of this class of PET radiopharmaceuticals. The primary goal of this article is to provide health care practitioners with the information necessary to provide their patients with optimal care while also considering the cost-effective use of limited health care resources.

2. Background

With the advent of carbon-11 (C-11)-labeled Pittsburgh compound B (PiB), Aβ—or amyloid PET—emerged as a major element in a transformation of AD research that emphasized the development of biomarkers that could potentially facilitate drug development [1]. Intense efforts were directed at assessing the amyloid status of individuals with AD dementia as well as those with prodromal and preclinical stages of disease, and the technology was adopted rapidly worldwide, albeit largely in specialized research centers. More recently, amyloid PET has been used increasingly in clinical trials for AD therapeutics. Because the short 20-minute half-life of C-11 limits routine clinical use of PiB as a result of the need for an onsite cyclotron, amyloid-binding radiopharmaceuticals labeled with longer lived fluorine-18, with a 110-minute half-life, were developed and commercialized for wide availability. One such compound, [F-18]florbetapir, achieved approval by the U.S. Food and Drug Administration in April 2012. The European Medicines Agency's Committee for Medicinal Products for Human Use recommended marketing authorization for [F-18]florbetapir in October 2012.

To develop this article, the Amyloid Imaging Taskforce (AIT), consisting of experts in the fields of imaging, neurology, and dementing diseases was assembled by the AA and SNMMI to review the available literature and develop consensus recommendations for the clinical use of these promising new radiopharmaceuticals. At the time of this review, experience with clinical amyloid PET imaging is limited. Most published studies to date have been designed to validate this technology and understand disease mechanisms rather than to evaluate applications in clinical practice. As a result, published data are available primarily from highly selected populations with prototypical findings rather than from patients with comorbidities, complex histories, and atypical features often seen in clinical practice. Despite these limited clinical use data, the members of the task force concluded that the proven sensitivity and specificity of the new radiopharmaceuticals for brain amyloid, and the known association between brain Aβ deposition and AD suggest these new radiopharmaceuticals could potentially be helpful in the workup and diagnosis of patients with cognitive impairment.

Translation of research findings to clinical populations poses substantial challenges. Unlike research subjects, clinical patients can exhibit a wide range of medical and psychiatric problems. Indeed, the prevalence of mixed-cause dementia increases with advancing age and is frequently seen in clinical practice [2]. As the population ages, individuals are increasingly likely to inquire about the usefulness of amyloid PET imaging in a variety of circumstances that are unlikely to be addressed in the scientific literature. In addition, as amyloid imaging agents become more well-known and longitudinal data accumulate, patients and referring physicians may request amyloid PET imaging for individuals who are asymptomatic. Colleagues may also ask for advice about using amyloid PET imaging for purposes such as screening someone with a family history of AD dementia, or for use in patients already carrying a diagnosis of a non-AD dementia.

When the peer-reviewed literature is incomplete, as is often the case, expert opinion can be valuable, particularly when considering numerous practical clinical issues and ethical concerns that largely remain overlooked in the design and discussion of published clinical trials. There is often diagnostic uncertainty resulting from the complexities of patient history as well as the inconsistencies in examination results. Incorporating amyloid imaging into clinical decision making may help to narrow a differential diagnosis and simplify some of the complexities inherent in evaluating patients with mild cognitive impairment (MCI) and dementia.

Although identifying potential benefits, the AIT concluded that an amyloid PET report will not constitute and is not equivalent to a clinical diagnosis of AD dementia. Imaging is only one tool among many that clinicians should use judiciously to manage patients. Amyloid PET imaging does not substitute for a careful history and examination. Indeed, the history and examination are required to understand the clinical context necessary to incorporate imaging results into clinical decision making. The diagnosis of dementing diseases has implications that resonate beyond the patient to include family members, particularly those who are genetically related. We hope that these recommendations will be relevant to many patients, even when published evidence may be lacking.

As with most guidelines, the health care provider has to make the ultimate judgment regarding the care of each individual patient. The AIT sought to assist this process and identified the following general sequence of events with which amyloid PET could be used according to the appropriate use criteria (AUC) set forth here: (*i*) evaluation by a dementia expert to assess the need for diagnostic testing, possibly to include amyloid PET, if the AUC are met; (*ii*) referral to a qualified provider of amyloid PET services; (*iii*) performance, interpretation, and reporting of the amyloid PET result according to established standards; (*iv*) incorporation of the PET result into the clinical assessment process by the dementia expert; and (*v*) disclosure of the PET result by the dementia expert to the patient and caregivers, along with discussion of the result and its management consequences. The health care provider must bear in mind that amyloid imaging does not make a diagnosis of AD, and by itself does not determine that a patient's cognitive impairment is a result of AD pathology.

3. Methods

The AIT formulated AUC for amyloid PET imaging using procedures similar to those used by groups such as the American College of Cardiology Foundation [3]. The process used (*i*) identification of potential indications/nonindications, (*ii*) evidence assessment and rating, (*iii*) group rating of potential indications/nonindications, (*iv*) discussion and revoting, and (*v*) writing. Three AIT subcommittees were established: the Indication Subcommittee, the Literature Subcommittee, and the Evidence Review Subcommittee (see Appendix A).

3.1. Possible indications and nonindications of clinical scenarios

The Indication Subcommittee, consisting of practicing dementia specialists and imaging experts, discussed 115 potential indications and nonindications based on multiple clinical and nonclinical scenarios with variables including symptoms, clinical setting, clinical context, evidence of cognitive deficit, family history, knowledge of AD genetic risk, and age. This process is described in Appendix B. Based on the consensus discussion, the Indication Subcommittee consolidated potential indications and nonindications into 14 scenarios that were subsequently incorporated in a data extraction form used for the evidence assessment (described later).

3.2. Evidence review and analytical framework

The Literature Subcommittee used a search strategy as established by the American Academy of Neurology and the Institute of Medicine of the National Academy of Sciences to identify relevant literature. The process is described in Appendix C. The AIT deliberated on the choice of literature screening criteria, and a decision was made on the basis of the types of evidence ultimately needed to establish clinical utility of amyloid PET. The ultimate goal was to determine whether there is evidence that using amyloid PET leads to clinically meaningful improvement in outcomes or is useful in medical or personal decision making. Because direct evidence linking amyloid PET to health outcomes is currently lacking, the AIT evaluated existing literature according to a possible chain of evidence consisting of three key questions, adapted from the scheme of Fryback and Thornbury [4]:

The first question deals with technical efficacy (analytical validity or technical test performance). This class of evidence reflects the stability, adequacy, and reproducibility of the test itself, and includes both the image data and the qualitative image interpretation. Proof of technical efficacy includes reproducibility of specific amyloid PET acquisition procedures and protocols under standardized conditions, which must be established separately for each amyloid tracer and must be applicable to the range of PET instrumentation in common use. In addition, the implementation of amyloid PET requires a qualitative read of images, so that evidence of standardized interpretation protocols that lead to acceptable levels of interrater agreement must be considered. These standards and procedures are ultimately the province of the professional certifying organizations, such as the American Board of Nuclear Medicine, but reports have already appeared from the Alzheimer Disease Neuroimaging Initiative [5] and phase 2 industry-sponsored trials [6] that describe standardized acquisition protocols for F-18-labeled PET ligands as well as interpretation standards. Such standards and procedures have been implemented by the commercial developers of the F-18 amyloid PET ligands, and evidence of validation is required by the U.S. Food and Drug Administration before approval for clinical use is granted. Additional analytical validity data should be acquired relating to test–retest and longitudinal stability and change. Web-based instruction programs for readers of F-18 amyloid PET have been developed and validated, and should be completed successfully by all imaging specialists prior to reading of clinical amyloid PET (see Image Quality and Reporting).

The second question deals with diagnostic accuracy (clinical validity) based on an autopsy truth standard. Considerable progress has been achieved to establish clinical validity using histopathology-to-image comparisons. As with other elements of validation, each tracer and its associated interpretation protocol must be assessed separately. The AIT elected to include longitudinal clinical studies as ancillary evidence of clinical or diagnostic validity when the design included a baseline amyloid PET followed by clinical evaluation and assessment of longitudinal decline or conversion in clinical status, according to accepted clinical diagnostic criteria [7,8].

The third question deals with clinical utility based on a change in management (including change in diagnostic evaluation) and associated improved clinical outcomes. This is the most challenging component of the analytical framework, and the evidence for a change in clinical management based on amyloid PET is not yet available. With only medications to treat symptoms currently available and no disease-modifying treatment yet proved, the clinical utility of a diagnostic test to alter patient management and result in a quantifiable benefit is very difficult to establish. However, an accurate diagnosis of the cause of cognitive impairment is often critically important for a practitioner to select appropriate treatments and avoid inappropriate interventions. Furthermore, different dementing diseases have distinctive courses, complications, and comorbidities that alter nonpharmacological management and treatment recommendations. Although psychological, social, economic, and family outcome variables, including value of knowing, can be identified as potentially altering management, the data supporting specific outcomes for amyloid PET are not yet available.

Multiple searches were performed using the National Institutes of Health's PubMed. The Literature Subcommittee reviewed the list for inclusion and identified a subset of documents by abstract analysis. Documents not relevant to the clinical use of amyloid PET were eliminated based on the primary focus of the reported study and data presented in the document. In addition, to ensure appropriate documents were captured during the initial search and review, the AIT performed a backward review to cross-check the literature included in seminal amyloid imaging reviews with those included in the AIT's initial assessment. For the backward review, the AIT used the bibliographies of Klunk [9], Villemagne and colleagues [10], and Laforce and Rabinovici [11].

The Literature Subcommittee developed a data extraction form for evidence assessment. The Evidence Review Subcommittee conducted evidence assessment in two steps. During the phase I review, valid documents identified during the initial review process were assigned to a pair of reviewers (a dementia specialist and an imaging specialist). Each reviewer scored the documents using the data extraction form. Other documents that met the preliminary inclusion criteria, as indicated by both reviewers, but that received low scores by both reviewers, or mixed scores, or strong reviewer comments for further assessment were also identified as documents to be discussed. During the phase II review, additional documents were identified through the backward review and an updated search, as well as new papers in press. A second round of reviews was conducted identical to phase I. The final inclusion criteria were that the document must contain data of one of two types—either PET–histopathology correlation or PET correlation with longitudinal clinical follow-up. After the review, co-chairs of the AIT reviewed the findings of both phase I and phase II and presented a final list of 23 documents that satisfied the final inclusion criteria and these were presented to the full AIT [5,12–33]. These documents were used as the literature-based evidence for rating the AUC outlined by the Indication Subcommittee.

3.3. Rating of the AUC

The group rating of potential indications/nonindications was conducted using a rating sheet by individual voting AIT members without knowledge of other members' rating results. Fourteen scenarios proposed by the Indication Subcommittee were consolidated to 10 possible indications/nonindications by defining a preamble that applies to all indications/nonindications. The rating sheet included (*i*) the final 10 possible indications/nonindications, (*ii*) the amount of qualified evidence determined by the evidence assessment, and (*iii*) individual documents that relate to each indication/nonindication. Based on the presented evidence and individual AIT members' opinions, the AIT members were asked to rate each indication/nonindication with Appropriate, Uncertain, or Inappropriate. A nonvoting AIT member summarized the rating results.

4. Definitions

The following terms are used in the AUC:

Dementia expert *a physician experienced in the assessment and diagnosis of dementia*. The AUC depend heavily on the training, experience, and clinical judgment of the dementia experts ordering the test and their application of the published, standardized clinical criteria for MCI and AD (as defined in this list). Expertise in applying these criteria is typically acquired through formal training and clinical experience in neurology, psychiatry, and geriatric medicine; however, not all physicians in these disciplines are dementia experts.

Alzheimer's disease (AD) *the pathological process reflected in specific postmortem histopathological criteria [34], which is frequently but not necessarily associated with a characteristic dementia syndrome [7,8]*. The AD pathological process differs conceptually and is uncoupled from the dementia syndrome with which it is associated, as evidenced by the very long preclinical or asymptomatic period preceding the dementia syndrome. The AUC specifically refer to clinical criteria for AD dementia that have recently emerged from the National Institute on Aging–Alzheimer's Association work group [7] and the international work group [8]. Core clinical criteria for AD dementia identify the specific conditions and circumstances under which a dementia expert may determine whether amyloid PET can be used appropriately. Although the two international work groups used different terms—probable AD and typical AD—the underlying principles are quite similar, and either nomenclature may be applied to support the conclusion that amyloid PET would or would not be appropriate (criterion 4).

Probable AD dementia *a clinical syndrome meeting the core clinical criteria specified in the National Institute on Aging–Alzheimer's Association work group report [9] (also see [35])*

Possible AD dementia *a clinical syndrome meeting the core clinical criteria for AD dementia in terms of the nature of the cognitive deficits for AD dementia, but either (i) has a sudden onset of impairment or demonstrates insufficient historical detail or objective documentation of progression, or (ii) has an etiologically mixed presentation because of evidence of vascular or Lewy pathology [7]*

Mild cognitive impairment (MCI) *a clinical syndrome meeting the published core clinical criteria for MCI [36–38] (also see [8,35,39]).* Although considerable debate and evolution of the criteria for MCI continue, general agreement exists about the core features. Briefly, these include (*i*) concern about a change in cognition, (*ii*) impairment in one or more cognitive domains, (*iii*) preservation of independence in functional activities, but (*iv*) not demented [38]. The application of these criteria and their use in determining whether amyloid PET would be appropriate is in the hands of the dementia expert.

Table 1
F-18 beta-amyloid PET radiopharmaceuticals compared to C-11 PiB

Ligand compared with C-11 PiB	Subjects	Correlation of binding between ligands	Diagnostic performance
Florbetapir [55]	12 AD patients, 14 cognitively normal control subjects	Composite cortical binding correlation $r = 0.78$, $P < .001$	Group discrimination florbetapir area under the curve = 0.90 vs PiB = 1.0.
Florbetapir [56]	24 MCI subjects, 8 healthy control subjects	Composite cortical binding correlation $\rho = 0.95$, $P < .001$, slope = 0.60	97% classification agreement using derived cut points
Flutemetamol [48]	20 AD patients, 20 MCI subjects	Composite cortical binding correlation $r = 0.905$, slope = 0.99	100% concordance of individual subject visual scan categorization between ligands
Florbetaben [57]	10 AD patients, 10 healthy control subjects	Composite cortical binding correlation $r = 0.97$, $P < .0001$, slope = 0.71	100% concordance of individual subject visual scan categorization between ligands
NAV4694 [53]	7 AD patients, 3 patients with frontotemporal dementia, 10 MCI subjects, 25 healthy control subjects	Composite cortical binding correlation $r = 0.99$, $P < .0001$, slope = 0.95	100% concordance of individual subject visual scan categorization between ligands

Abbreviations: PiB, Pittsburgh compound B; AD, Alzheimer's disease; MCI, mild cognitive impairment.

Amyloid positivity/negativity *the determination by an imaging expert that the amyloid PET scan indicates the presence or absence of Aβ plaque.* The imaging expert is a nuclear medicine specialist or radiologist with specific training in the interpretation of amyloid PET. The amyloid PET data must be technically adequate and must be acquired at a fully qualified and certified facility (see Image Quality and Reporting). The protocol for the qualitative read that determines positivity or negativity must be standardized (e.g., [5]) and must conform to a specific guideline provided by the manufacturer if it is available.

5. PET Aβ radiopharmaceuticals

Although a number of Aβ PET radiopharmaceuticals have been reported with human data [40–43], currently there are five that are in use at multiple sites to image Alzheimer pathology in vivo. Among these, [C-11]-(2-[4-methyl-amino phenyl]-1,3-benzothiazol-6-ol), or PiB, was the first to be described and is the most extensively studied [1]. PiB, a neutral analog of the histological dye thioflavin T, has been evaluated with respect to clinical syndromic and postmortem histopathological correlation over approximately 10 years in several clinical populations and in healthy control subjects. Histopathological correlation data demonstrate the association between PiB PET and postmortem assessment of Aβ pathology [12–14,23,30,44–47].

The short 20-minute half-life of C-11 limits routine clinical use because of the need for an onsite cyclotron, whereas the 110-minute half-life of F-18-labeled PET ligands allows incorporation of PET into routine clinical practice, as has occurred with [F-18]fluorodeoxyglucose (FDG) in clinical oncology. Several F-18-labeled Aβ PET radiopharmaceuticals have been developed, including [F-18]3'-F-PiB (flutemetamol) [25], [F-18]AV-45 (florbetapir) [15], [F-18]-AV-1 or [F-18]-BAY94-9172 (florbetaben) [49,50], and [F-18]-AZD4694 or NAV4694 [51–53]. Postmortem histopathology-to-PET correlations have been published for florbetapir [15], and biopsy histopathology-to-PET correlations have been published for flutemetamol [54].

F-18 ligand PET data have been compared quantitatively with C-11 PiB data acquired in the same subjects with respect to cortical binding, linear regression slope, and diagnostic classification performance (Table 1). Wolk and colleagues [55] performed PiB and florbetapir PET imaging in 14 cognitively normal adults and 12 AD patients and showed that both ligands displayed highly significant group discrimination and correlation of regional uptake. Landau and associates [56] compared PiB with florbetapir and found the data were correlated at $r = 0.95$ and a slope of 0.60, and that resulting cut points yielded classification agreement in 97% of cases evaluated. A correlation with PiB of $r = 0.905$ and a slope of 0.99 was reported for flutemetamol in 20 AD patients and 20 MCI patients, in which the concordance of visual reads between ligands was 100% [48]. Villemagne and coworkers [57] compared PiB with florbetaben in 10 healthy control subjects and 10 patients with AD and reported the correlation to be $r = 0.97$, the slope to be 0.71, and the concordance between ligands to be 100%. Rowe and colleagues [53] compared NAV4694 with PiB in seven patients with AD, three patients with frontotemporal dementia (FTD), 10 patients with MCI, and 25 healthy control subjects and found the correlation to be $r = 0.99$, the slope to be 0.95, and classification concordance to be 100%. These findings are consistent with a high correlation of these [F-18]-labeled ligands with PiB and they support the translation of PiB PET findings into the domain of these [F-18]-labeled radiopharmaceuticals. Comparison studies of one F-18 agent with another have not yet been reported.

6. Results of ratings

Ratings for each indication/nonindication were obtained from independent voting by eight AIT voting members, and the results were summarized by a nonvoting member. At the time of voting, each member was able to access qualified peer-reviewed documents that potentially concern each

possible indication, and ratings by AIT members of the quality of the evidence, based on the results of the literature review as described previously. For each indication, the number of supporting publications and the average quality of evidence were indicated on the voting sheet.

During the initial voting, four possible indications and seven possible nonindications were submitted for voting. Substantial disparities in voting results (30%–50%) were found for four potential indications. Each potential indication/nonindication was reviewed in subsequent AIT discussions, and uncertain language in the proposed AUC were clarified for revoting. During this process, two possible indications were combined into one indication, resulting in total three possible indications and seven possible nonindications.

In the revoting results, the ratings of Appropriate or Inappropriate were unanimous for possible indications 1 and 2, and possible nonindications 4 to 10. For indication 3, two voting members voted Uncertain whereas the other six voting members voted Appropriate.

6.1. Appropriate use criteria

Amyloid imaging is appropriate in the situations listed here for individuals with all of the following characteristics:

Preamble: (*i*) a cognitive complaint with objectively confirmed impairment; (*ii*) AD as a possible diagnosis, but when the diagnosis is uncertain after a comprehensive evaluation by a dementia expert; and (*iii*) when knowledge of the presence or absence of Aβ pathology is expected to increase diagnostic certainty and alter management.

1. Patients with persistent or progressive unexplained MCI
2. Patients satisfying core clinical criteria for possible AD because of unclear clinical presentation, either an atypical clinical course or an etiologically mixed presentation
3. Patients with progressive dementia and atypically early age of onset (usually defined as 65 years or less in age)

Amyloid imaging is inappropriate in the following situations:

4. Patients with core clinical criteria for probable AD with typical age of onset
5. To determine dementia severity
6. Based solely on a positive family history of dementia or presence of apolipoprotein E (*APOE*)ε4
7. Patients with a cognitive complaint that is unconfirmed on clinical examination
8. In lieu of genotyping for suspected autosomal mutation carriers
9. In asymptomatic individuals
10. Nonmedical use (e.g., legal, insurance coverage, or employment screening)

7. Discussion of individual indications

7.1. Preamble

The AIT considered whether to specify the patient characteristics for each indication separately, but recognized that there were several elements common to all appropriate indications and set these elements apart in a separate preamble. The preamble was intended to characterize all patients for whom the appropriate indications 1 to 3 apply.

The preamble restricts substantially the set of patients for whom amyloid imaging would be appropriate in several ways. First, the dementia expert, as defined earlier (Definitions), must evaluate the patient and determine that there is objective evidence of impairment. The objective evidence may be acquired and interpreted directly by the dementia expert in a detailed mental status examination or obtained from a separate neuropsychological assessment. Second, the expert should evaluate all available clinical evidence, including the history, physical and neurological examinations, and all available laboratory and neuroimaging data to consider the possible causes of the illness as well as potentially confounding circumstances such as depression, medication effects, and cerebrovascular, endocrine, or other medical disorders. This is because the presence of amyloid pathology in the brain, when considered in isolation, is insufficient to determine the cause of the impairment; rather, the presence of amyloid pathology is one factor among many that must be considered. The dementia expert must conclude on the basis of all available evidence that (*i*) the cause of the impairment is uncertain and (*ii*) that it could be explained on the basis of Aβ pathology (i.e., AD dementia or its prodromal stage must be in the differential diagnosis).

Last, the expert must conclude that a determination of either amyloid positivity or amyloid negativity would both increase the level of diagnostic certainty and alter the plan for patient management. Empirical evidence for the value of added certainty resulting from amyloid PET has not yet been reported; however, several patient-centered outcome studies are underway, and the following should serve to guide efforts of this type further. The AIT considered several situations in which the added certainty of amyloid PET could be useful to patients and caregivers, and could result in altered management. First, many patients with uncertain diagnoses undergo extensive and repeated testing that would be reduced if the diagnostic certainty were increased by amyloid PET. For others, however, it is also likely that amyloid negativity would require additional diagnostic testing as the dementia expert seeks to identify the underlying Aβ-negative cause of impairment. The relative utility of diagnostic tests should be evaluated further. Second, increased certainty of the diagnosis could provide a basis for earlier and more consistent drug treatment, avoidance of treatments unlikely to afford benefit, and improved monitoring for likely complications and adverse drug effects that are relevant to specific dementing diseases. In addition, improved diagnostic

certainty could provide more powerful motivation to make required lifestyle changes and difficult living transitions for which they are otherwise reluctant. Third, a more certain diagnosis can have profound social benefits to patients and families, who may need to identify the required resources and plan for future management. Minimizing diagnostic uncertainty can assist in bringing family members to a uniform understanding of the patient's condition and needs, facilitating the development of a unified plan of progressive support that best manages financial resources and maximizes quality of life.

Although learning the cause of dementia and the limited efficacy of available treatments may cause stress and anxiety for some, we believe that the value of knowing outweighs the disadvantages. Electing to manage dementing diseases without investigating the cause or with high levels of diagnostic uncertainty often contributes to inconsistent and poor quality of care. In any circumstance, patients and their families decide—on their own—whether to seek answers by electing or failing to seek care.

7.2. Indication 1 (appropriate): Patients with persistent or progressive unexplained MCI

This indication refers to a patient who satisfies all the criteria set forth in the preamble and is being evaluated for persistent or progressive cognitive impairment that is still mild (e.g., a patient with MCI as defined earlier). This means, in practice, that although impaired according to objective measures, the patient does not have "significant interference in the ability to function at work or in usual daily activities" (pg 265) [7] (also see [8]). In this circumstance, an amyloid PET finding of positivity would, on the basis of its known correspondence to brain Aβ, raise the level of certainty that the patient's mild impairment is on the basis of AD pathology and represents early AD dementia (see Definitions). However, it is important to emphasize again that not all patients with MCI would be appropriate for amyloid PET. Rather, amyloid PET would be appropriate only in those individuals who the dementia expert has concluded would benefit from greater certainty of the underlying pathology and whose clinical management would change as a result of this greater certainty.

The dementia expert should recognize that asymptomatic amyloid deposition is common in older (e.g., >75 years) individuals and may not be related to a patient's presenting symptoms. Furthermore, the dementia expert will need to consider in older individuals the possibility that amyloid positivity could be present but not the sole factor in causing the impairment and that comorbid conditions or pathologies such as vasculopathy could be present and could account for or significantly contribute to the observed impairment.

The prognostic value of amyloid PET for predicting future outcomes in MCI patients is under active investigation, and preliminary studies are suggestive but not complete. Initial reports suggest that the majority of patients with amnestic MCI, variously defined by neuropsychological evaluation, and a positive amyloid PET will progress to AD dementia, whereas the risk of progression to AD dementia is significantly lower in those who are amyloid negative. The available evidence to date has not definitively linked amyloid positivity in individual patients with a future time point when cognitive or functional deterioration can be predicted. Therefore, currently the use of amyloid PET to predict the trajectory of a patient's cognitive decline or the time to any specific outcome is not appropriate because published evidence is limited (see Further Research Questions).

A related, alternative scenario for this indication is a patient, also satisfying all the criteria set forth in the preamble, who is amyloid negative and therefore much less likely to be impaired on the basis of AD. The amyloid-negative scenario may, in practice, be the most frequently useful scenario in MCI, given the potential confound of age-associated Aβ, discussed earlier, among amyloid-positive individuals. Thus, in patients with MCI whose clinical picture may be complicated with potential vascular, traumatic, or medical causes of cognitive impairment, amyloid PET may find utility and could be used appropriately to exclude AD pathology effectively as a basis for the clinical syndrome.

7.3. Indication 2 (appropriate): Patients satisfying core clinical criteria for possible AD (i.e., atypical clinical course or etiologically mixed presentation)

This indication refers to a patient with an established dementia syndrome who is not typical with regard to presentation and clinical course, or to a patient who is considered to have a mixture of causal pathological processes. It is intended to explicitly exclude from the category of appropriate use the patient about whom there is little doubt of the underlying pathology because the onset, course, and examination findings are typical of AD dementia. It is, however, intended to include those patients for whom substantial uncertainty exists and for whom greater confidence would result from determining whether Aβ pathology is present or not present, as described next.

The AIT chose, here, to rely on the established concept of possible AD, specifically as it has been recently revised [7], and again to focus on the dementia specialist as the physician who would apply the criteria based on this diagnostic category. The restriction in this indication to patients with possible AD dementia is based on the well-established existence of patients about whom there is substantial doubt of whether the dementia is based on AD pathology. The sources of doubt are (*i*) the presence of an unusual course (e.g., sudden onset or episodic) or because the course cannot be established from the history or from retrospective cognitive test data, or (*ii*) the presence of a comorbid condition that confounds the interpretation of the clinical data, such as cerebrovascular disease, other neurological disease, other medical condition, or medication use that is affecting cognition and function. Amyloid PET is not useful in identifying the

possible confound of coexisting Lewy pathology (discussed later).

7.4. Indication 3 (appropriate): Patients with atypically young-onset dementia

Amyloid PET is appropriate in the scenario in which a relatively young patient (e.g., 50–65 years old, but possibly even younger) presents with a progressive impairment that has features of AD dementia as well as of a non-AD dementia. In the scenario covered by this indication, the dementia specialist is often called on to identify the cause of a devastating illness in such a patient, and to manage a complex and comprehensive evaluation. The purpose of the evaluation is to manage the symptomatic treatment rationally; make appropriate employment, driving, and lifestyle decisions; possibly refer the patient to clinical trials of candidate disease-modifying therapies; and to provide a basis for prognosis and planning for care. The presence or absence of AD pathology in this circumstance is frequently a critical component of the initial differential diagnosis, and it is well known from postmortem studies that clinical diagnosis based on history and examination is often wrong with regard to the presence of AD pathology [58].

7.5. Indication 4 (not appropriate): Patients with core clinical criteria for probable AD with typical age of onset

As mentioned earlier, the AIT identified seven circumstances or scenarios in which amyloid imaging would be inappropriate. The first is indication 4. The AIT recommended against the use of amyloid PET in cases in which core clinical criteria for probable AD dementia were satisfied [7], and there were typical history and examination findings, because the level of uncertainty would be low and the potential benefit from added information and the potential for altered management would be correspondingly low.

7.6. Indication 5 (not appropriate): To determine dementia severity

Data are lacking to support the use of amyloid imaging to determine the severity of any cognitive disorder. Thus far, the predominance of the evidence is that the level of Aβ burden measured with amyloid PET does not correlate well with severity of deficits in patients with dementia [10].

7.7. Indication 6 (not appropriate): Based solely on a positive family history of dementia or presence of APOE ε4

There are no data currently available that indicate that—based solely on family history or *APOE* genotype—that prognosis, course, or greater certainty in the cause of cognitive deficits is aided with amyloid PET imaging.

7.8. Indication 7 (not appropriate): Patients with a cognitive complaint that is unconfirmed on clinical examination

The significance of a cognitive complaint in an elderly person without deficits on examination is currently a topic of active investigation; however, there is insufficient evidence to suggest amyloid PET can aid prognostic judgments or relieve the concerns of such individuals. A negative amyloid PET scan today cannot exclude the possibility of AD dementia in the future.

7.9. Indication 8 (not appropriate): In lieu of genotyping for suspected autosomal mutation carriers

The use of amyloid PET in lieu of genotyping for suspected autosomal dominant mutation carriers is considered inappropriate. The optimal clinical evaluation in these cases is careful collection of a family history, followed (if appropriate) by genetic counseling prior to and after genetic testing for known mutations. Future use of amyloid PET in autosomal dominant mutation carriers could include determination of whether the amyloid deposition phase of their illness has begun. In the setting of a complete clinical evaluation, including serial neuropsychological testing, this information may be useful in identifying one disease-related milestone that, along with the genetic information, aids decision making.

7.10. Indication 9 (not appropriate): The clinical use of amyloid PET in asymptomatic individuals

The prognostic value of amyloid positivity in normal elderly individuals remains investigational (see Further Research Questions). There is a significant potential for patients and families to make inaccurate assumptions about risk and future outcomes on the basis of amyloid PET results. Currently, the potential harms outweigh the minimal benefits. The availability of proven preventative therapies would undoubtedly alter this judgment.

7.11. Indication 10 (not appropriate): Nonmedical usage

The AIT did not find any evidence to support the utility of amyloid PET in a context outside of a diagnostic evaluation to determine the cause of cognitive impairment. In particular, no evidence supported a role for amyloid imaging to inform physicians when they are consulted on legal-, disability-, and employment-related matters. These include assessing competency, screening for insurability, or assessing employability or the ability to perform activities of daily living such as driving, piloting an aircraft, or making financial decisions.

8. Limitations of amyloid PET in clinical evaluation

A major limitation of amyloid PET to support a diagnosis of AD dementia is the high prevalence of amyloid positivity in normal older individuals. Population-based studies are only beginning to be reported, but estimates of age-specific positivity rates for amyloid PET are less than 5% in those 50 to 60 years old, 10% in those 60 to 70 years old, 25% in those 70 to 80 years old, and more than 50% in persons aged 80 to 90 years [59,60]. This high age-associated prevalence means that the causality of Aβ for a patient's clinical syndrome cannot be established with amyloid PET by itself without considering the prior probability of positivity based solely on age. The dementia expert should consider the possibility, prior to ordering amyloid PET, that incidental, age-related amyloid detection may not be related to or relevant to the presenting symptoms of a patient.

Another major caveat is that a positive amyloid scan can also be seen in not only AD, but also in other medical conditions. For example, amyloid PET is frequently positive in dementia with Lewy bodies [61,62]. Amyloid imaging detects both fibrillar amyloid found in blood vessels (cerebral amyloid angiopathy) and interstitial fibrillar amyloid in plaques. Imaging cannot distinguish between amyloid angiopathy and parenchymal fibrillar plaques [32], and both are highly prevalent in the elderly, with or without dementia. Although usually associated with interstitial amyloid plaques, in rare cases amyloid angiopathy can occur alone [63]. Occasionally, amyloid angiopathy unaccompanied by typical pathological features of AD can cause progressive dementia [64,65]. More commonly, amyloid angiopathy can become clinically manifest as a cause of cerebral hemorrhage, and in such cases carries a high risk of recurrence [66,67]. It is important to emphasize that amyloid positivity does not establish the diagnosis of AD or differentiate it from Aβ disorders such as dementia with Lewy bodies and cerebral amyloid angiopathy.

It is important to note several clinical circumstances in which amyloid PET would not be expected to be useful. First, it would not add any useful information in differentiating disorders that are not associated with Aβ, such as the various FTD syndromes. Second, amyloid PET would not be expected to detect the rare forms of AD in which ligand binding is greatly reduced as a result of unusual forms of Aβ [14,68]. The appropriate use of amyloid PET requires knowledge of all relevant findings of clinical evaluations, laboratory tests, and imaging, relating how each component of the accumulated evidence should be weighed. Thus, clinical amyloid PET should be performed in the context of a comprehensive evaluation undertaken by a clinician with expertise in evaluating cognitive neurodegenerative disorders.

The AIT did not consider other proposed diagnostic biomarkers for AD and therefore did not draw any conclusions with regard to the relative value of amyloid PET compared with cerebrospinal fluid, magnetic resonance imaging, and FDG-PET (see Further Research Questions).

The AIT considered broader social and psychological implications of amyloid status determination. Although empirical data have not yet been evaluated, the AIT concluded that certain steps should probably be taken by the dementia expert to avoid psychological harm to patients and families that could follow after the initial disclosure of amyloid status. These steps include pretest counseling about the emotional and social implications of both a positive and a negative amyloid PET. Implications in the realms of legal and insurance status, including health, life, and long-term care, and employment ramifications are even less well understood at this time, and policymakers should consider whether existing laws such as the Americans With Disabilities Act provide adequate protection for these patients. Notably, the U.S. Genetic Information Nondiscrimination Act applies only to genetic tests.

9. Amyloid PET and anticipated impact on patient care

Although published data concerning amyloid PET results and impact on patient care outcome are extremely limited, amyloid PET is likely to contribute to better patient care under specific circumstances. These are described in the following three domains.

9.1. Change in medication management

Greater physician confidence in the diagnosis of or exclusion of AD can result in better medication management. An amyloid-positive PET result that raises confidence in the diagnosis of AD is likely to result in earlier and appropriate use of specific medications for symptomatic treatment of AD, such as acetylcholinesterase inhibitors and memantine. In contrast, a plan to commence or continue medications developed for the treatment of AD, such as the acetylcholinesterase inhibitors and possibly memantine, in patients with a negative amyloid scan may be inappropriate. However, there are no studies to date that have assessed the value of these medications in amyloid-negative persons with a clinical phenotype suggestive of AD. Furthermore, there is some evidence that acetylcholinesterase inhibitors can benefit patients with vascular dementia [69]. Exclusion of AD should result in consideration of alternate diagnoses including depression, and in some cases of patients with atypical cognitive impairment who are amyloid negative, it may be appropriate to undertake a trial of antidepressant medication.

9.2. Change in ordering other tests

An amyloid PET cannot answer all diagnostic questions that are encountered during clinical dementia evaluation. It can, however, reduce the use of other tests that are burdensome to patients and their caregivers. For example, a positive amyloid PET result may obviate repeat imaging for the

purpose of establishing a clinical diagnosis of dementia whereas a negative amyloid PET result may guide clinicians to order tests that can help differentiate amyloid-negative dementing disorders. Amyloid PET may reduce the use of neuropsychological testing for the purpose of clinical diagnosis.

9.3. Value of knowing

Under the circumstances outlined previously, the results of amyloid PET will increase physician confidence in the clinical diagnosis and allow better planning for patients and caregivers. The following data are from a survey conducted by the Harvard School of Public Health on the public perceptions and awareness of AD [70]. The poll was commissioned by Alzheimer Europe through a grant provided by Bayer. These data and facts can be found at http://www.hsph.harvard.edu/news/press-releases/2011-releases/alzheimers-international-survey.html [71] and http://www.alz.org/documents_custom/public-health/value_of_knowing.pdf [72].

Survey Summary

1. Nearly 89% of Americans say that if they were exhibiting confusion and memory loss, they would want to know if the cause of the symptoms was AD.
2. Of those aged 60 years and older, 95% say they would want to know if they had AD.
3. More than 97% say that if they had a family member exhibiting problems with memory loss, they would want him or her to see a doctor to determine whether the cause was AD.
4. The convergence of evidence from numerous sources indicates that as many as half of people with dementia have never received a diagnosis.
5. A formal diagnosis allows individuals and their caregivers to have access to available treatments, build a care team, participate in support services, and enroll in clinical trials.
6. Participating in planning early in the disease process allows individuals with AD to create advance directives regarding their care and finances so that their wishes can be carried out when they are no longer cognitively able to make such decisions.
7. Early diagnosis also allows individuals with the disease and their caregivers to manage medications more effectively, receive counseling, and address driving and safety issues in advance.
8. Undertaking the diagnostic process early potentially allows cognitive impairment to be reversed in some people. For nearly one in every four individuals who reported to a memory clinic with cognitive problems, their cognitive impairment was the result of a reversible cause, such as depression or a vitamin B12 deficiency.

10. Image quality and reporting

The clinical value of amyloid PET imaging is entirely dependent on the quality of the images and accuracy of interpretation. Amyloid PET imaging is technically challenging and should be performed only when there is strict attention to quality control. Clinical PET scanning is widely available, but the experience of PET facilities with brain imaging is quite variable. Amyloid imaging is an evolving modality; therefore, image interpretation criteria, the clinical significance of positive and negative scans, and technical imaging considerations are evolving. The following recommendations are based on current knowledge and may require modification in the future.

The safe performance and accurate interpretation of amyloid imaging require physician training as described in the nuclear medicine program requirements of the Accreditation Council for Graduate Medical Education or the equivalent. All nuclear medicine examinations should be performed under the supervision of and interpreted by a physician certified in nuclear medicine or nuclear radiology by the American Board of Nuclear Medicine or the American Board of Radiology in the United States or equivalent organizations outside the United States.

The individual performing the scan must be familiar with brain anatomy and must have adequate specific training in amyloid PET interpretation. Training specific to the interpretation of amyloid imaging such as provided by the manufacturer of the radiopharmaceutical (if available) should be completed, and preferably augmented by training programs offered by professional societies such as the SNMMI and the European Association of Nuclear Medicine.

Imaging procedures should be performed by a qualified nuclear medicine technologist with appropriate training and certification. All nuclear medicine examinations should be performed by a qualified nuclear medicine technologist who is registered/certified in nuclear medicine by the Nuclear Medicine Technology Certification Board, the American Registry of Radiologic Technologists, or equivalent organizations outside the United States. The nuclear medicine technologist should work under the supervision of a physician with qualifications outlined previously. Imaging should be performed in an imaging facility certified by the Intersocietal Commission for the Accreditation of Nuclear Laboratories, the American College of Radiology, or other equivalent accrediting agency. A procedure guideline for amyloid PET is currently being developed by the SNMMI and European Association of Nuclear Medicine.

Results of amyloid PET imaging should be communicated to the referring physician by the imaging physician by way of a written report according to a standard diagnostic imaging practice as outlined in the SNMMI General Imaging Guideline. The final reading should indicate whether Aβ was found to be present (amyloid positive) or was not found to be present (amyloid negative; see Definitions). Indeterminate

results may arise as a result of technical or physiological factors and should be reported as such.

The report should not confound amyloid positivity with AD dementia (i.e., it should not, by itself, advance or rule out a diagnosis of AD dementia). The dementia specialist should then communicate with patients and family members after comprehensive review of the clinical assessment and test results.

11. Further research questions

11.1. Prognosis in healthy individuals and in patients with MCI

The AIT recognized that studies suggest amyloid imaging may have a role in stratifying patients into their risk of developing cognitive decline and that, someday, as longitudinal research studies accumulate data, amyloid imaging may become useful to predict future clinical conditions, such as the risk of developing cognitive decline or of transitioning into clinical states such as MCI or dementia [10,16,21,24,27,33,73,74]. However, these studies need further replication and their results analyzed in a pooled meta-analysis [75]. Therefore, at this point, data are simply incomplete to support using amyloid imaging to provide prognostic information to persons with AD risk factors such as age, family history of dementia, *APOE* ε4 status, genetic mutation carrier status, and cognitive complaint that is unconfirmed on clinical examination, or to asymptomatic persons.

Recent data from longitudinal studies of normal elderly cohorts with positive amyloid scans show a very slow rate of decline in memory function and suggest that the process of amyloid accumulation may extend for 20 years before dementia is apparent [10,17]. These studies also have shown considerable variation in the rate of amyloid accumulation among individuals. The proportion of healthy elderly persons with a positive amyloid scan who will develop dementia in their lifetime is not known at this time. For this reason, scanning for amyloid in an asymptomatic person in the absence of an effective disease-modifying therapy is discouraged.

11.2. Amyloid PET in the context of other biomarkers and diagnostic tests

Multiple imaging modalities and fluid biomarkers have been investigated in clinical and research contexts. Brain FDG-PET has been used in a clinical setting and can be diagnostically useful in certain circumstances when a characteristic pattern of hypometabolism is detected for specific neurodegenerative disorders [76,77]—in particular, differentiation of dementing disorders in which amyloid PET are similarly positive (such as AD vs DLB) or negative (such as subtypes of FTD). Cerebrospinal fluid measures of amyloid peptides and tau have been investigated extensively and applied to research populations for the purpose of establishing the presence of AD pathology [7,38,78,79], and these fluid assays are beginning to be used in clinical settings. Combined use of presynaptic dopaminergic and amyloid imaging is now being studied as a diagnostic stratification approach to aid differential diagnosis of AD, DLB, and FTD [80]. However, the effective use of these diagnostic tests in relation to amyloid PET should be investigated further in the context of patient outcome, benefit, and resource use.

11.3. Computer software to assist image interpretation

Computer-aided analysis software for amyloid imaging is under development, and several programs are available for use in the clinic. These programs can provide quantitative information about the amount of radiopharmaceutical in different brain regions, and may be particularly valuable for sites with limited experience in the reading of amyloid scans and to provide more information than a binary visual read. However, more research is required to validate their use in the clinical environment and demonstrate that they improve the accuracy of clinical reports.

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*These articles were used as the literature-based evidence for rating the AUC outlined by the Indications Subcommittee.

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Appendix A: Task force members and literature reviewers

Task Force Members (alphabetical)
Co-chairs (alphabetical)
Keith Johnson, MD
Satoshi Minoshima, MD, PhD (Co-chair)
Voting Members (alphabetical)
Nicolaas Bohnen, MD, PhD
Kevin Donohoe, MD
Norman Foster, MD
Peter Herscovitch, MD
Keith Johnson, MD
Jason Karlawish, MD
Satoshi Minoshima, MD, PhD
Christopher Rowe, MD
Scientific Advisor
William Klunk, MD, PhD
Society of Nuclear Medicine and Molecular Imaging (SNMMI) and the Alzheimer's Association (AA) AA Members (alphabetical)
Maria Carrillo, PhD (AA)
Dean Hartley, PhD (AA)
Saima Hedrick, MPH (SNMMI)
Kristi Mitchell MPH (Avalere Health)
Virginia Pappas, CAE (SNMMI)
William Thies, PhD (AA)
Indication Subcommittee (alphabetical)
Norman L. Foster, MD (chair)
Jason Karlawish, MD
Christopher Rowe, MD
Literature Subcommittee (alphabetical)
Nicolaas Bohnen, MD, PhD (chair)
Peter Herscovitch, MD
Kristi Mitchell MPH
Literature Reviewers (alphabetical)
Nicolaas Bohnen, MD PhD
Michael Devous, PhD
Kevin Donohoe, MD
Alex Drezga, MD
Karl Herholz, MD, FRCP
Peter Herscovitch, MD
Keith Johnson, MD
Jason Karlawish, MD
Satoshi Minoshima, MD, PhD
Gil Rabinovici, MD
Christopher Rowe, MD
Victor Villemagne, MD
David Wolk MD

Appendix B: Indications Subcommittee

The Expert Work Group consisted of three experienced clinicians, two geriatric cognitive neurologists, and a geriatrician. The developed guidelines for appropriate and inappropriate clinical use are based on available literature as well as expert opinion using a modified Delphi procedure. The first task of the work group was to individually rate appropriateness of 115 different clinical scenarios based on the seven variables listed in Table B1. Beginning with the premise that there is value in determining the cause of cognitive impairment, each expert weighed the potential clinical value of amyloid positron emission tomography (PET) against the expense and potential for misuse. After reporting the outcome of these individually considered judgments, the experts came to a consensus about each of the scenarios and used these conclusions to draw generalizations that should be applicable to many different scenarios.

A second task of the work group was to consider the utility of amyloid PET in eight situations when syndrome classification would be clinically important (e.g., delirium vs dementia) and in 40 clinically relevant differential diagnosis decision points (e.g., Alzheimer's disease vs. frontotemporal dementia). Last, the three clinicians jointly reviewed 10 actual anonymous clinical cases to test whether the guidelines they constructed accurately reflected their own clinical judgment in real rather than strictly theoretical situations. All voting members of the Amyloid Imaging Taskforce (AIT) reviewed and discussed the expert-recommended guidelines, and comments were elicited from within and beyond the work group. The final expert guidelines reflect this entire process and are the joint opinion of the entire AIT.

In our deliberations, we assumed that the expert clinician would receive an interpretation of the amyloid PET study as either positive or negative (see Definitions). Briefly, a positive amyloid PET result typically means that the image demonstrates uptake of amyloid radiotracer in the gray matter. In contrast, a negative scan means that the image does not demonstrate any gray matter cortical uptake that is above the level of nonspecific binding. The determination of cortical uptake relies on inspection of the contrast between white matter and cortical gray matter. In white matter, imaging ligands are routinely visible not as a result of their binding to amyloid, but as a result of lipophilic interactions with myelin. Some ligands have greater white matter uptake than others. Histopathology-to-PET correlation studies relating amyloid PET results with neuropathological measures of amyloid plaques, using both immunohistochemistry or silver staining, have shown that if amyloid plaques are none or sparse, amyloid imaging ligand-specific binding in cortical gray matter is very low and reflects nonspecific (i.e., nonamyloid) binding. In contrast, when histopathological evidence at autopsy confirms the presence of frequent amyloid plaque, the amyloid image contrast between white and gray is greatly reduced or visually undetectable.

The AIT did not consider the potential impact on the appropriate use criteria of the use of quantitative PET data (i.e., data from automated numerical measurements of specific ligand binding). Quantitative measurement entails image processing steps in which specific brain regions are identified in each individual data set for the purpose of comparing regional cortical tracer uptake with an unaffected reference

region. Currently, there is insufficient published data to recommend a specific implementation of amyloid PET quantitation that could be identified in the appropriate use criteria.

Table B1
Variables considered in constructing clinical presentation scenarios

Scenario variable	Variations considered
Symptoms	None
	Memory or cognitive complaint
Clinical setting	Nonmedical
	PCP
	Specialist
	Dementia specialist
Clinical context	Not applicable (nonmedical)
	Initial assessment
	Full evaluation, AD not suspected
	Full evaluation, AD suspected
Evidence of deficit on examination	Not applicable (nonmedical)
	None
	Yes
	MCI
	Mild—moderate dementia
	Severe dementia
Family history of AD or apolipoprotein E ε4 positive	Negative
	Positive
AD genetic mutation carrier	Negative
	Positive
	Not applicable
Age	<65 years
	>65 years

Abbreviations: PCP, Primary Care Physician; AD, Alzheimer's disease; MCI, mild cognitive impairment.

Appendix C: Literature Subcommittee and evidence review

The Literature Subcommittee used a search strategy as established by the American Academy of Neurology and the National Institutes of Medicine to identify relevant literature. Multiple searches were performed using the National Institutes of Health's PubMed in which 408 publications were initially identified. Literature search limits and parameters were as follow: human, English, and publication date January 01, 2002 to the present. The search terms determined to be the most useful for identifying the pertinent literature were (*i*) Florbetapir and AV-45, or Amyvid; (*ii*) PiB or Pittsburgh compound B; (*iii*) flutemetamol or AV1; (*iv*) F-18 FDDNP or F18 FDDNP; and (*v*) florbetaben or 8F-BAY94-9172.

Using the PubMed–generated list of 408 documents, the Literature Subcommittee reviewed the list for inclusion and identified a subset of documents by abstract analysis. Documents not relevant for clinical use of amyloid positron emission tomography (PET) were eliminated based on the primary focus of the study and data presented in the document. These include radiochemistry study, in vitro study, animal toxicity study, biodistribution study, image and kinetic analysis study, dosimetry study, pathophysiological investigation, correlational study, study with a small number of subjects, surrogate marker study in therapeutic trials, and review and editorial commentary. In addition, to ensure appropriate documents were captured during the initial search and review, the Amyloid Imaging Taskforce (AIT) performed a backward review to cross-check the literature included in seminal amyloid imaging reviews with those included in the AIT's initial assessment. For the backward review, the AIT used the bibliographies of Klunk [8], Villemagne and colleagues [9], and Laforce Rabinovici [10].

The Literature Subcommittee developed a data extraction form for the evidence assessment. The data extraction form included multiple questions and data extraction sections including whether the document addresses one or more of the potential indications/nonindications proposed by the Indication Subcommittee, individual data points for recalculation of the data, study design, study logistics, patient recruitment setting, inclusion/exclusion criteria, criteria used for diagnosis, inclusion of control subjects, subject characteristics, type of radiopharmaceuticals used, type of PET scanner used, method of PET interpretation and analysis, 2×2 data extraction for histopathologically confirmed study as well as mild cognitive impairment to Alzheimer's disease conversion study, a 19-point quality score of the document, the American Academy of Neurology Level of Evidence for a Diagnostic Study Article, the American Academy of Neurology Level of Evidence for a Prognostic Study Article, and whether the document addressed changes in physician confidence.

Appendix D: Relationships with industry and management of conflicts of interest

The Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging rigorously attempted to avoid any actual, perceived, or potential conflicts of interest (COIs) that might have arisen as a result of an outside relationship or personal interest of the writing committee members of the Amyloid Imaging Taskforce (AIT) or of external reviewers used to review specific documents. Both organizations reviewed their own industry relationship policies to ensure that the ensuing process adhered to both standards.

AIT members were required to provide disclosure statements of all relationships that might be perceived as real or potential COIs. These statements were reviewed and discussed by the AIT co-chairs, and were updated and reviewed by an objective third party at the beginning of every AIT meeting and/or teleconference. A table of disclosures for AIT members and external literature reviewers can be found in Table D1.

To adjudicate the COIs, the leadership from the Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging first determined the threshold for a real COI. After consulting with various experts and reviewing other policies used, the team defined COIs as the following: An individual who has relationships with industry, including consulting, speaking, research, and other

nonresearch activities that exceed $5000 in funding over the previous or upcoming 12-month period.

In addition, if external expert reviewers of the documents were either a principle investigator or other key study personnel on a study, their participation in the review would likely present a COI. All reviewers completed COI forms. Document authors and sponsors were identified and then cross-checked against reviewers' financial and intellectual COIs. Conflicted individuals were noted as unable to review documents in which there was a real COI present.

Of note, William Klunk, MD, co-invented the PiB-class and Chrysamine-G-class amyloid imaging agents, was appointed as an advisor to the AIT, contributing expertise as requested, but recused himself from any and all discussions that resulted in a vote among writing committee members.

Table D1
Table of relationships with industry and other entities for task force members and outside reviewers

Name	Reported relationships with industry or other entities
Bohnen, Nic	• None
Devous, Michael	• Avid Pharmaceuticals • Lilly Healthcare • Bayer (now Piramal Pharmaceuticals)
Donohoe, Kevin	• None
Drzezga, Alexander	• Avid Radiopharmaceuticals/Lilly Healthcare • Bayer Healthcare • GE Healthcare • Siemens Healthcare
Foster, Norman	• Bristol-Meyers Squibb • GE Healthcare • Janssen AI • Center for Health Improvement
Herholz, Karl	• GE Healthcare • Elan • Avid Radiopharmaceuticals/Lilly Healthcare
Herscovitch, Peter	• None
Johnson, Keith	• Siemens • Avid Radiopharmaceuticals/Lilly Healthcare • Janssen AI • Bayer • Navidea Biopharmaceuticals • Piramal Healthcare
Karlawish, Jason	• Alzheimer's Disease Cooperative Study (member)
Minoshima, Satoshi	• None
Rabinovici, Gil	• Avid Radiopharmaceuticals
Rowe, Christopher	• Bayer • GE Healthcare • AstraZeneca • Piramal Healthcare • Avid Radiopharmaceuticals/Lilly Healthcare • Navidea Biopharmaceuticals
Villemagne, Victor	• Bayer
Wolk, David	• Pfizer • GE Healthcare

Appendix E: Public commentary

The Amyloid Imaging Taskforce solicited information from all communities through the Society of Nuclear Medicine and Molecular Imaging and the Alzheimer's Association websites and by direct solicitation to members of these societies. The comments and input helped to shape the development of these appropriate use criteria and the consensus recommendation for the appropriate use of amyloid imaging for clinical indications of the detection of fibrillar amyloid in the brain.

2012 FDA Update

"High Country Meeting"

March 5, 2012/11:30 EST

Dwaine Rieves, MD
Director, Division of Medical Imaging Products

Focus on Imaging Drugs…

- **Drug approvals & labeling actions**
- **Standardization guidance**
- **Medical Imaging Drug Advisory Committee (MIDAC)**
- **Positron Emission Tomography (PET) topics**

2011/12 Imaging Drug Approvals & Labeling Actions

Gadobutrol Injection (Gadavist)	NDA (Bayer)
Fludeoxyglucose F18 Injection	ANDA (PETNET)
Perflutren lipid microsphere injectable suspension (Definity®)	NDA supplement (Lantheus)
Rubidium Rb 82 chloride injection (CardioGen-82®)	NDA supplement (Bracco)

Draft "Standardization" Guidance

Guidance for Industry
Standards for Clinical Trial Imaging Endpoints

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Final guidance targeted for October, 2012

Medical Imaging Drugs Advisory Committee (MIDAC)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. FDA–2011–N–0002]

Advisory Committee; Medical Imaging Drugs Advisory Committee; Reestablishment

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing the

Nominees undergoing vetting process

Positron Emission Tomography (PET) Drugs

"PET Drug Website"

Goto:

http://www.fda.gov/

In search box, place:

"PET Drug Manufacturing"

Updates, Guidances, Presentations, Historical Information…*and more*…

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

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Home | Food | Drugs | Medical Devices | Vaccines, Blood & Biologics | Animal & Veterinary | Cosmetics | Radiation-Emitting Products | Tobacco Products

Drugs

Home › Drugs › Development & Approval Process (Drugs) › Manufacturing

Development & Approval Process (Drugs)

- Manufacturing
- Drug Applications and Current Good Manufacturing Practice (CGMP) Regulations
- Positron Emission Tomography (PET)
- Questions and Answers on Current Good Manufacturing Practices (CGMP) for Drugs
- Current Good Manufacturing Practices for Drugs: Reports, Guidances and Additional Information

Resources for You

Positron Emission Tomography (PET)

Notice of FDA Exercise of Enforcement Discretion for PET Drugs

In 1997, Congress passed the Food and Drug Administration Modernization Act (Public Law 105-115) (the Modernization Act). Section 121 of the Modernization Act directed FDA to establish appropriate approval procedures and Current Good Manufacturing Practices (CGMP) for PET drugs. These procedures were published on December 9, 2009, triggering an implementation timeline. Under the requirements of section 121, within 2 years of that publication date, a new drug application (NDA) or abbreviated new drug application (ANDA) must be submitted for any PET drug marketed for clinical use in the United States.

Recently, FDA has received requests to extend the application submission deadline from and on behalf of some PET drug producers trying to comply with the regulation and application submission requirements. Some firms have expressed concern that if they are unable to submit their application by December 12, 2011, they will have to halt production of PET drugs for use in clinical care of patients. Further, although we do not anticipate any shortages of PET drugs after December 12, 2011, we are concerned that sole producers in isolated areas may halt production if their application has not been submitted and this could create a barrier to access in that particular area. Having considered these points, in addition to the fact that we have yet to issue the two instructive guidances for PET drug producers *(Investigational New Drug Applications for PET Drugs and FDA Regulation of PET Drug Products, Questions and Answers)* that are currently under development, FDA has decided to exercise enforcement discretion under the following circumstances until June 12, 2012.

For the next six months, until June 12, 2012, FDA does not intend to take enforcement action against a PET facility currently producing PET drugs for clinical use for a failure to submit a new drug application by December 12, 2011, provided that the facility complies with all other FDA requirements, including current good manufacturing practices (CGMPs). **FDA will not exercise enforcement discretion after June 12, 2012.**

PET Drug
Current Good Manufacturing Practices (cGMP)

Background

1997	**FDA Modernization Act** • Required cGMP for PET • 2 yrs post-publication NDA/ANDA for any PET drug in "clinical use"
2009 Dec	**FDA publishes PET Drugs cGMP** • NDA/ANDA due by 12/12/2011
2011 Dec	**Notice of enforcement discretion** • NDA/ANDA due by 06/12/2012 for any PET drug in "clinical use"

PET Presentations / Seminars

Pre-2009	Multiple presentations/draft info
2009 - 2011	FDA seminars at SNM Annual Meetings; Planned for 2012
2010 Apr	FDA PET Drug Workshop
2011 Mar	FDA PET Drug Public Meeting
2012 Jan	FDA webinar on cGMP… *Still available on the FDA "PET Drug website"*

PET Drug Guidances

2011	**PET Drug Applications:** **Content & Format for** **FDG F18 / Ammonia N13 / Fluoride F18**
2011	**Media Fills for Validation of Aseptic Preparations (draft)**
2012	**Questions and Answers…*new!***
2012	**Investigational New Drug (IND) Applications (draft)…*new!***

PET Drug Main Points

- **By June 12, 2012 must have submitted NDA/ANDA for any drug in "clinical use" *or* the "clinical use" must be under an IND**

- **By December 12, 2015 must have an approved NDA/ANDA or an effective IND for the "clinical use"**

"Use" Phrases

"CLINICAL use" – PET drug is a component of clinical care/not a systematic study of drug safety-efficacy

- under NDA/ANDA *or* IND

"INVESTIGATIONAL use"– PET drug is studied to determine its safety-efficacy

- under IND *or* exempted from IND

"RESEARCH use" – PET drug is a component of a research project under Radioactive Drug Research Committee (RDRC) approval

- RDRC approval (no exemption option)

"*Expanded Access IND Submission*"

Intended to provide clinical access to investigational drugs for diagnostic / therapeutic monitoring purposes

- **Can be an original IND submission or a submission to an existing IND**
- **Applies to limited situations defined by criteria**
- **May be submitted with a "Request to Charge"**

Expanded Access Criteria

1) **Patient(s) with serious or immediately life-threatening disease / condition**

2) **No comparable/satisfactory alternative "therapy"**

3) **Potential benefit justifies the potential risk of the clinical use**

4) **Provision of drug will not interfere with drug development for market approval**

Expanded Access (EA) Criteria Interpretation (p1 of 2)

1) **Regarding serious or immediately life-threatening disease or condition,**

   FDA allows for use of PET drug to help detect a serious / life-threatening disease even if the condition not actively manifest

2) **No comparable/satisfactory alternative therapy**

   Necessitates justification of why alternative drugs are not satisfactory (e.g., PET drug's unique metabolic assessment activity)

Expanded Access (EA) Criteria Interpretation (p2 of 2)

3) **Potential benefit justifies the potential risk of the clinical use**

   Based on available evidence/prior experience/dosage/consideration of population characteristics (e.g., pediatrics)

4) **Provision of the drug will not interfere with drug development for market approval**

   FDA anticipates EA will only apply in situation where NDA/ANDA not feasible

Expanded Access Pointers

- **Expanded Access Submission Process**
  - ❖ **explained in IND guidance**
- **Not appropriate when an NDA/ANDA is feasible**
  - ❖ **not appropriate for FDG F18, ammonia N 13, sodium fluoride F18**
- **Clinical use may continue during the 30 day review period based upon prior clinical use / otherwise, the sponsor will be contacted**
- **If plan to charge, need to submit a "Request to Charge" submission to the IND**

"Request to Charge"

- **A submission process unique to an IND**
  - ❖ **described in PET IND guidance**
- **Charging may be requested for either**
  - ❖ **a clinical trial / investigation or**
  - ❖ **Expanded Access**
- **Certain criteria must be met before FDA authorizes charging**

"Request to Charge" Review Criteria -- Clinical Trial --

- **Potential benefit of investigational drug provides significant advantage over available products**
- **Clinical trial data essential for marketing support**
- **Cost of drug extraordinary**
- **Describe proposed cost (only direct costs)**
- **Statement that CPA has reviewed/approved cost calculations**

"Request to Charge" Review Criteria -- Expanded Access Program --

- **Assurance that charging will not interfere with developing the drug for marketing**
- **Describe proposed cost to be charged a patient (direct costs for single patient; direct + indirect costs for other)**
- **Statement that CPA has reviewed/approved cost calculations**

Other Special PET Drug IND Considerations

- **Prior to December 12, 2015, an IND may not be necessary *if* the PET drug is the subject of a submitted NDA/ANDA**

- **After December 12, 2015, all investigational use of a PET drug must be under an IND unless the use is exempted from IND**

- **Many studies using FDG F18 are currently ongoing outside of IND**
  - ❖ **these uses may continue until 12/12/2015 *if* the FDG F18 is the subject of a submitted NDA/ANDA**

Thank you!

Q & A

PET Drug Web address:

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/Manufacturing/ucm085783.htm

or

Go to http://www.fda.gov/ and search for "PET Drug Manufacturing"

Decision Memo for Beta Amyloid Positron Emission Tomography in Dementia and Neurodegenerative Disease (CAG-00431N)

Decision Summary

A. The Centers for Medicare & Medicaid Services (CMS) has determined that the evidence is insufficient to conclude that the use of positron emission tomography (PET) amyloid-beta (Aβ) imaging is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member for Medicare beneficiaries with dementia or neurodegenerative disease, and thus PET Aβ imaging is not covered under §1862(a)(1)(A) of the Social Security Act ("the Act").

B. However, there is sufficient evidence that the use of PET Aβ imaging is promising in two scenarios: (1) to exclude Alzheimer's disease (AD) in narrowly defined and clinically difficult differential diagnoses, such as AD versus frontotemporal dementia (FTD); and (2) to enrich clinical trials seeking better treatments or prevention strategies for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors.

Therefore, we will cover one PET Aβ scan per patient through coverage with evidence development (CED), under §1862(a)(1)(E) of the Act, in clinical studies that meet the criteria in each of the paragraphs below.

Clinical study objectives must be to (1) develop better treatments or prevention strategies for AD, or, as a strategy to identify subpopulations at risk for developing AD, or (2) resolve clinically difficult differential diagnoses (e.g., frontotemporal dementia (FTD) versus AD) where the use of PET Aβ imaging appears to improve health outcomes. These may include short term outcomes related to changes in management as well as longer term dementia outcomes.

Clinical studies must be approved by CMS, involve subjects from appropriate populations, and be comparative and longitudinal. Where appropriate, studies should be prospective, randomized, and use postmortem diagnosis as the endpoint. Radiopharmaceuticals used in the PET Aβ scans must be FDA approved. Approved studies must address one or more aspects of the following questions. For Medicare beneficiaries with cognitive impairment suspicious for AD, or who may be at risk for developing AD:

1. Do the results of PET Aβ imaging lead to improved health outcomes? Meaningful health outcomes of interest include: avoidance of futile treatment or tests; improving, or slowing the decline of, quality of life; and survival.

2. Are there specific subpopulations, patient characteristics or differential diagnoses that are predictive of improved health outcomes in patients whose management is guided by the PET Aβ imaging?

3. Does using PET Aβ imaging in guiding patient management, to enrich clinical trials seeking better treatments or prevention strategies for AD, by selecting patients on the basis of biological as well as clinical and epidemiological factors, lead to improved health outcomes?

Any clinical study undertaken pursuant to this national coverage determination (NCD) must adhere to the timeframe designated in the approved clinical study protocol. Any approved clinical study must also adhere to the following standards of scientific integrity and relevance to the Medicare population.

a. The principal purpose of the research study is to test whether a particular intervention potentially improves the participants' health outcomes.

b. The research study is well supported by available scientific and medical information or it is intended to clarify or establish the health outcomes of interventions already in common clinical use.

c. The research study does not unjustifiably duplicate existing studies.

d. The research study design is appropriate to answer the research question being asked in the study.

e. The research study is sponsored by an organization or individual capable of executing the proposed study successfully.

f. The research study is in compliance with all applicable Federal regulations concerning the protection of human subjects found at 45 CFR Part 46. If a study is regulated by the Food and Drug Administration (FDA), it must be in compliance with 21 CFR parts 50 and 56.

g. All aspects of the research study are conducted according to appropriate standards of scientific integrity (see http://www.icmje.org).

h. The research study has a written protocol that clearly addresses, or incorporates by reference, the standards listed here as Medicare requirements.

i. The clinical research study is not designed to exclusively test toxicity or disease pathophysiology in healthy individuals. Trials of all medical technologies measuring therapeutic outcomes as one of the objectives meet this standard only if the disease or condition being studied is life threatening as defined in 21 CFR §312.81(a) and the patient has no other viable treatment options.

j. The clinical research study is registered on the ClinicalTrials.gov website by the principal sponsor/investigator prior to the enrollment of the first study subject.

k. The research study protocol specifies the method and timing of public release of all pre-specified outcomes to be measured including release of outcomes if outcomes are negative or the study is terminated early. The results must be made public within 24 months of the end of data collection. If a report is planned to be published in a peer reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors

(http://www.icmje.org). However a full report of the outcomes must be made public no later than three (3) years after the end of data collection.

l. The research study protocol must explicitly discuss subpopulations affected by the treatment under investigation, particularly traditionally underrepresented groups in clinical studies, how the inclusion and exclusion criteria effect enrollment of these populations, and a plan for the retention and reporting of said populations on the trial. If the inclusion and exclusion criteria are expected to have a negative effect on the recruitment or retention of underrepresented populations, the protocol must discuss why these criteria are necessary.

m. The research study protocol explicitly discusses how the results are or are not expected to be generalizable to the Medicare population to infer whether Medicare patients may benefit from the intervention. Separate discussions in the protocol may be necessary for populations eligible for Medicare due to age, disability or Medicaid eligibility.

Consistent with §1142 of the Act, the Agency for Healthcare Research and Quality (AHRQ) supports clinical research studies that CMS determines meet the above-listed standards and address the above-listed research questions.

All other uses are noncovered.

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Decision Memo

To: Administrative File: CAG-00431N
Beta Amyloid Positron Emission Tomography in Dementia and Neurodegenerative Disease

From: Louis Jacques, MD
Director, Coverage and Analysis Group

Tamara Syrek Jensen, JD
Deputy Director, Coverage and Analysis Group

James Rollins, MD, PhD
Division Director

Brijet Burton Coachman, MPP, MS, PA-C
Lead Analyst

Stuart Caplan, RN, MAS

Analyst

Rosemarie Hakim, PhD
Epidemiologist

Jeffrey Roche, MD, MPH
Medical Officer

Joseph Hutter, MD, MA
Lead Medical Officer

Subject: Final Decision Memorandum for: CAG-00431N
Beta Amyloid Positron Emission Tomography in Dementia and Neurodegenerative Disease

Date: September 27, 2013

I. Final Decision

A. The Centers for Medicare & Medicaid Services (CMS) has determined that the evidence is insufficient to conclude that the use of positron emission tomography (PET) amyloid-beta (Aβ) imaging is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member for Medicare beneficiaries with dementia or neurodegenerative disease, and thus PET Aβ imaging is not covered under §1862(a)(1)(A) of the Social Security Act ("the Act").

B. However, there is sufficient evidence that the use of PET Aβ imaging is promising in two scenarios: (1) to exclude Alzheimer's disease (AD) in narrowly defined and clinically difficult differential diagnoses, such as AD versus frontotemporal dementia (FTD); and (2) to enrich clinical trials seeking better treatments or prevention strategies for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors.

Therefore, we will cover one PET Aβ scan per patient through coverage with evidence development (CED), under §1862(a)(1)(E) of the Act, in clinical studies that meet the criteria in each of the paragraphs below.

Clinical study objectives must be to (1) develop better treatments or prevention strategies for AD, or, as a strategy to identify subpopulations at risk for developing AD, or (2) resolve clinically difficult differential diagnoses (e.g., frontotemporal dementia (FTD) versus AD) where the use of PET Aβ imaging appears to improve health outcomes. These may include short term outcomes related to changes in management as well as longer term dementia outcomes.

Clinical studies must be approved by CMS, involve subjects from appropriate populations, and be comparative and longitudinal. Where appropriate, studies should be prospective, randomized, and use postmortem diagnosis as the endpoint. Radiopharmaceuticals used in the PET Aβ scans must be FDA approved. Approved studies must address one or more aspects of the following questions. For Medicare beneficiaries with cognitive impairment

suspicious for AD, or who may be at risk for developing AD:

1. Do the results of PET Aβ imaging lead to improved health outcomes? Meaningful health outcomes of interest include: avoidance of futile treatment or tests; improving, or slowing the decline of, quality of life; and survival.

2. Are there specific subpopulations, patient characteristics or differential diagnoses that are predictive of improved health outcomes in patients whose management is guided by the PET Aβ imaging?

3. Does using PET Aβ imaging in guiding patient management, to enrich clinical trials seeking better treatments or prevention strategies for AD, by selecting patients on the basis of biological as well as clinical and epidemiological factors, lead to improved health outcomes?

Any clinical study undertaken pursuant to this national coverage determination (NCD) must adhere to the timeframe designated in the approved clinical study protocol. Any approved clinical study must also adhere to the following standards of scientific integrity and relevance to the Medicare population.

a. The principal purpose of the research study is to test whether a particular intervention potentially improves the participants' health outcomes.

b. The research study is well supported by available scientific and medical information or it is intended to clarify or establish the health outcomes of interventions already in common clinical use.

c. The research study does not unjustifiably duplicate existing studies.

d. The research study design is appropriate to answer the research question being asked in the study.

e. The research study is sponsored by an organization or individual capable of executing the proposed study successfully.

f. The research study is in compliance with all applicable Federal regulations concerning the protection of human subjects found at 45 CFR Part 46. If a study is regulated by the Food and Drug Administration (FDA), it must be in compliance with 21 CFR parts 50 and 56.

g. All aspects of the research study are conducted according to appropriate standards of scientific integrity (see http://www.icmje.org).

h. The research study has a written protocol that clearly addresses, or incorporates by reference, the standards listed here as Medicare requirements.

i. The clinical research study is not designed to exclusively test toxicity or disease pathophysiology in healthy individuals. Trials of all medical technologies measuring therapeutic outcomes as one of the objectives meet this standard only if the disease or condition being studied is life threatening as defined in 21 CFR §312.81(a) and the patient has no other viable treatment options.

j. The clinical research study is registered on the ClinicalTrials.gov website by the principal sponsor/investigator prior to the enrollment of the first study subject.

k. The research study protocol specifies the method and timing of public release of all pre-specified outcomes to be measured including release of outcomes if outcomes are negative or the study is terminated early. The results must be made public within 24 months of the end of data collection. If a report is planned to be published in a peer reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors

(http://www.icmje.org). However a full report of the outcomes must be made public no later than three (3) years after the end of data collection.

l. The research study protocol must explicitly discuss subpopulations affected by the treatment under investigation, particularly traditionally underrepresented groups in clinical studies, how the inclusion and exclusion criteria effect enrollment of these populations, and a plan for the retention and reporting of said populations on the trial. If the inclusion and exclusion criteria are expected to have a negative effect on the recruitment or retention of underrepresented populations, the protocol must discuss why these criteria are necessary.

m. The research study protocol explicitly discusses how the results are or are not expected to be generalizable to the Medicare population to infer whether Medicare patients may benefit from the intervention. Separate discussions in the protocol may be necessary for populations eligible for Medicare due to age, disability or Medicaid eligibility.

Consistent with §1142 of the Act, the Agency for Healthcare Research and Quality (AHRQ) supports clinical research studies that CMS determines meet the above-listed standards and address the above-listed research questions.

All other uses are noncovered.

II. Background

Definitions

The following radiopharmaceuticals are referenced in this decision memorandum (DM):

- Florbetapir is florbetapir F18 (or AV-45)
- Florbetaben is florbetaben F18 (or AV-1, or BAY-94-9172)
- Flutemetamol is flutemetamol F18 (or GE-067)
- FDDNP is FDDNP F18
- AZD4694 is AZD4694 F18 (or NAV4694)
- PIB is Pittsburgh Compound B C11
- FDG is fluoro-D-glucose F18

The terms "PET Aβ imaging," "amyloid-beta PET," "PET Aβ," "amyloid imaging," "amyloid PET," "Aβ imaging," "amyloid-beta imaging" and "beta-amyloid imaging" are used synonymously in the literature and in this DM.

Dementia

Dementia is a syndrome involving cognitive and behavioral impairment in an otherwise alert patient, due to a number of neurological diseases, alone or combined. It is not a specific cause or disease process itself. The impairment must involve a minimum of two domains (memory, reasoning, visuospatial abilities, language or personality behaviors); impact daily functioning; represent a decline from previous levels of functioning; not be explainable by delirium (a temporary state of mental confusion and fluctuating consciousness from various causes) or a major psychiatric disorder; and be objectively documented by a "bedside" mental status exam (e.g., the mini-mental status exam) or neuropsychological testing (McKhann 2011).

Mild cognitive impairment (MCI)

Increasingly, research has focused on early stages of cognitive impairment, which lie between the cognitive changes of normal aging and dementia. Mild cognitive impairment (MCI) is a syndrome in which persons experience memory loss (amnestic MCI) or loss of thinking skills other than memory loss (non-amnestic MCI), to a greater extent than expected for age, but without impairment of day-to-day functioning. The clinical work up for MCI is similar to that for AD and other causes of dementia (discussed below).

Individuals with MCI are at increased risk of developing dementia (whether from AD or another etiology), but many do not progress to dementia, and some get better. MCI has multiple subtypes, discussed in more detail later in this DM. These subtypes, and associated results from "bedside" mental status exams and neuropsychiatric testing, could, when combined with (1) other patient characteristics (e.g., age, genetics, cognitive reserve, comorbidities), and (2) biomarkers (for hypometabolism, plaque accumulation, synaptic dysfunction and neuronal loss), serve as the foundation for the development of objectively defined "risk pools," or subpopulations of individuals who are at risk of progressing from MCI or even pre-symptomatic states to AD (Petersen 1999 and 2009, Wolk 2009, Hughes 2011, Ward 2012, Landau 2012, Sachdev 2012).

Epidemiology, clinical criteria, causes and treatment

AD is an irreversible dementia characterized by progressive, relentless cognitive and functional decline. It is the number one cause of dementia in older Americans (age 65 and over), contributing to 60-80% of cases. Over 5 million older Americans (> 12.5%) have AD. This prevalence is expected to rise to 8.7 million by 2030, and could reach 13.8 million by 2050. AD is the 5th leading cause of death in older Americans (and the 7th leading cause of death overall). Older African-Americans are two times as likely to have AD (and other dementias) as older whites. Older Hispanics are 1.5 times as likely to have AD as older whites. Women are more likely to have AD than men, although this is in part because women live longer (NIA 2013, Brookmeyer 2011, CDC 2013, AA 2013).

Clinical criteria for diagnosing AD are informed by the NIA-AA 2011guidelines (McKhann 2011). Core clinical criteria for “probable AD” dementia must first meet the criteria for “all-cause” dementia described above. Additionally, there must be: (a) insidious onset; (b) documented worsening of cognition; (c) exclusion of major concomitant cerebrovascular disease (as most individuals with AD have some level of this as well); and (d) exclusion of alternative diagnoses (such as dementia with Lewy bodies (DLB), behavioral variant frontotemporal dementia (FTD), progressive aphasia or other neurological disease associated with dementia). A clinical diagnosis of “possible AD” dementia would meet the criteria for “probable AD” above, with the exception of having an “atypical course” (e.g., sudden rather than insidious onset) or an “etiologically mixed presentation.”

The first symptom of AD is usually memory loss (amnesia), due to synaptic dysfunction and loss of neurons in the hippocampus. This leads to impairment of reasoning, judgment, behavior and communication, as well as motor functions, as the disease spreads to other regions of brain. Rarely the initial (or “presenting”) symptoms can be nonamnestic, such as disturbances in language, visuospatial abilities or decision-making.

Most individuals with AD become symptomatic after age 60. Generally an indolent process, it is typically fatal within 8-10 years of onset but can be fatal anywhere between 2 and 20 years. Among 70-year-olds, 61% of those with AD die within a decade (compared to only 30% of those without AD) (NIA 2013, Dilworth 2008, AA 2013).

The underlying cause of AD remains unknown. The number one risk factor is age itself. Investigators hypothesize that a wide range of factors may contribute to its development, including genetic, metabolic, inflammatory, mitochondrial, environmental, and neuronal, to include both cytoskeletal (within the neuronal cell itself) and synaptic (the connectivity among cells) (ECRI 2012, Pimplikar 2010, Herrup 2010, Sperling 2011).

Currently, there is no effective treatment for AD. Existing interventions do not prevent, modify or cure the disease process. Some medications, such as memantine and cholinesterase inhibitors, can temporarily improve cognitive and neuropsychiatric symptoms in some patients with AD (as well as certain other dementias). Care is therefore primarily supportive and increases as functional impairment progresses, eventually leading to round-the-clock supervision which can be needed for years.

Diagnostic work-up, integration of biomarkers, and their shortcomings

The clinical work-up for patients presenting with symptoms of dementia or cognitive impairment, including MCI with possible AD, is extensive. It includes a medical history taken from the patient and from an informant who is well acquainted with the affected person, a physical examination comprising a mental status evaluation aided by quantitative scales and/or neuropsychological assessment, and laboratory testing and often structural neuroimaging such as MRI or CT to rule out other diseases. Clinical assessment is performed primarily using two sources: the National Institute on Aging and the Alzheimer's Association (NIA-AA) 2011 criteria, which updates the NINCDS-ADRA 1984 criteria to "incorporate more modern innovations in clinical imaging and laboratory assessment" (McKhann 2011); and the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criteria for dementia of the Alzheimer's type.

The innovations in "imaging and laboratory assessment" above refer to biomarkers. There are two types: those detecting amyloid-beta (Aβ) protein deposition; and those detecting downstream neuronal degeneration or injury (Jack 2011). Examples of the former type include: direct imaging of amyloid plaques in living brain with florbetapir, PIB and other agents; and decreased Aβ42 in cerebral spinal fluid (CSF), resulting from accumulation of this molecule in the brain. Examples of the latter type include: atrophy of hippocampus and entorhinal cortex on MRI, reflecting neuronal loss; increased total tau protein in CSF, which correlates with neuronal damage; and increased phosphorylated-tau (p-tau) in CSF, which correlates with formation of neurofibrillary tangles (NFTs) (Jack 2008, Sperling 2011, Hampel 2008, Mattsson 2009).

This distinction between amyloid deposition and neuronal degeneration becomes important in current theories of the role of amyloid in the development of AD (discussed below). Increasing use of biomarkers in clinical research has given rise to two new proposed classifications for AD in the NIA-AA 2011 criteria: "probable" or "possible" AD dementia "with evidence of AD pathophysiology."

These proposed classifications are explicit hypotheses to be assessed through further research. Currently, there are no established biological or neuroimaging markers for the diagnosis of AD or related disorders. Accordingly, the NIA-AA workgroup on dementia concludes that "the core clinical criteria for AD dementia will continue to be the cornerstone of the diagnosis in clinical practice, but biomarker evidence is expected to enhance the pathophysiological specificity of the diagnosis of AD dementia. Much work lies ahead for validating the biomarker diagnosis of AD dementia" (McKhann 2011).

Unfortunately, despite being the "cornerstone" of diagnosis, clinical assessment of AD remains poor. For example,

a review of 919 subjects with both clinical and neuropathologic (autopsy) data collected from the NIA-sponsored National Alzheimer's Coordinating Center Uniform Data Set between 2005-2010 demonstrated sensitivity of clinical diagnosis ranging from 70.9% to 87.3%, and specificity ranging from 44.3% to 70.8% (depending on the restrictiveness of the clinical criteria); this study also found that 39% of subjects with dementia not clinically diagnosed with AD actually had "minimum levels of AD histopathology" (Beach 2012). Other studies found the clinical diagnosis of AD by expert neurologists to be 81% sensitive and 70% specific compared to neuropathology (Knopman 2001, Grundman 2012).

Clinical diagnosis is poor because several other neurological diseases can mimic the dementia seen in AD, including cerebrovascular dementia, dementia with Lewy bodies (DLB), behavioral variant frontotemporal dementia (FTD), Parkinson's disease, Creutzfeld-Jakob disease, and normal pressure hydrocephalus (NPH). Accordingly, NIA-AA 2011 guidelines require exclusion of these diseases as one of the criteria for clinical diagnosis of "probable AD." Also, one or more of these diseases, most commonly vascular disease, co-exist in the majority of individuals with AD, as seen at autopsy (Schneider 2007). So there are relatively few patients with "pure" AD. Finally, it is not possible to measure the partial contributions of various coexisting diseases, identified either during life with imaging or biomarkers, or at autopsy, to a patient's symptoms of dementia.

Pathophysiology and the diagnostic gold standard for AD

The pathophysiological hallmarks of AD are Aβ plaques, neurofibrillary tangles (NFTs) of the protein tau, and neuronal dysfunction and loss. However, amyloid plaques are seen in other diseases, such as dementia with Lewy bodies, cerebral amyloid angiopathy, Parkinson's disease, Huntington's disease, and inclusion body myositis. Amyloid plaques can also be detected in cognitively normal older adults. Autopsy studies demonstrate that approximately 33% of older individuals (20-65% depending on age) who are cognitively normal have amyloid accumulation at levels consistent with AD pathology (Hulette 1998, Price 1999, Knopman 2003, Rowe 2010). Finally, amyloid is associated with physiologic processes of disease prevention or response, such as protection against oxidative stress, regulation of cholesterol transport, and anti-microbial activity (Guglielmotto 2010, Zou 2002, Yao 2002, Soscia 2010).

Because clinical diagnosis is poor, and amyloid pathology is seen in other diseases as well as in cognitively normal older persons, the "gold standard" for diagnosis requires both (a) the presence of moderate to frequent Aβ plaques and neurofibrillary tangles on autopsy, and (b) clinical documentation of progressive dementia during life (NIA-Reagan Institute 1997, Hyman 1997).

Competing views on the role of amyloid

Acknowledging that there are competing views on the role of amyloid in the pathophysiology of AD is key to interpreting the significance of trials on AD prognosis, diagnosis and clinical utility. It is widely accepted that the presence of amyloid plaques in human brain is virtually necessary for the diagnosis of AD. It is built into the postmortem diagnostic gold standard, and reflected in the FDA-approved label for florbetapir (Sperling 2011, NIA

-Reagan 1997, FDA 2012). However, whether a threshold level of amyloid plaques in a patient is sufficient for diagnosing AD is a subject of much debate. One hypothesis is that patients with symptoms of cognitive impairment and evidence of brain amyloid have AD, and it is just a matter of time before this manifests clinically as AD dementia.

A competing hypothesis is that "Aβ accumulation is necessary but not sufficient to produce the clinical manifestations of AD. It is likely that the cognitive decline would occur only in the setting of Aβ accumulation plus synaptic dysfunction and/or neurodegeneration" (Sperling 2011).

In this light, the NIA-AA criteria authors conclude that "at this point, it remains unclear whether it is meaningful or feasible to make the distinction between Aβ as a risk factor for developing the clinical syndrome of AD versus Aβ accumulation as an early detectable stage of AD because current evidence suggests that both concepts are plausible" (Sperling 2011).

PET Aβ imaging

PET is a minimally invasive diagnostic imaging procedure used to evaluate normal tissue as well as diseased tissues in conditions such as cancer, ischemic heart disease and some neurologic disorders. A ligand that binds to a given targeted substrate (e.g., Aβ plaque aggregates) is labeled with a radioisotope (e.g., fluorine F18). The injected radiopharmaceutical (or "tracer") emits positrons when it decays. PET uses a positron camera (tomograph) to measure the decay of such tracers within human tissue. The relative differences in the rate of tracer decay among anatomic sites provide biochemical information on the tissue being studied.

PET Aβ imaging detects amyloid plaque density in vivo in human brain. While several Aβ imaging agents exist, including Pittsburg compound B (PIB C11), and several F18 labeled agents (florbetapir; florbetaben; flutemetamol; AZD469; and FDDNP, which images both amyloid and tau), the longer half-lives of the F18-labelled agents render them more practical in clinical settings. As the only FDA-approved agent for PET Aβ imaging to date is florbetapir, it is the primary focus of our review.

III. History of Medicare Coverage

CMS did not previously cover PET Aβ imaging. FDG PET is nationally covered for either the differential diagnosis of FTD versus AD under specific requirements; or, its use in a CMS-approved practical clinical trial focused on the utility of FDG PET in the diagnosis or treatment of dementing neurodegenerative diseases. FDG PET for dementia and neurodegenerative diseases and other specific covered uses of particular PET radioactive tracers (N13 ammonia, Rb82 and F18 sodium fluoride (NaF-18) are found in detail in Section 220.6 of the National Coverage Determination Manual available at http://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/Downloads/ncd103c1_Part4.pdf.

A. Current Request

In July 2012 Lilly USA, LLC, manufacturer of the PET amyloid radiopharmaceutical florbetapir (Amyvid™), requested that CMS reconsider its non-coverage decision for PET scans and provide coverage for the use of PET amyloid imaging as a diagnostic test to "estimate amyloid neuritic plaque density in adult patients with documented cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment" (Requestor Letter, at http://www.cms.gov/medicare-coverage-database/details/nca-tracking-sheet.aspx?NCAId=265&fromdb=true).

B. Benefit Category

Medicare is a defined benefit program. An item or service must fall within a benefit category as a prerequisite to Medicare coverage (§1812 (Scope of Part A); §1832 (Scope of Part B) and §1861(s) (Definition of Medical and Other Health Services) of the Act. PET is considered to be within the following benefit category: other diagnostic tests §1861(s)(3) of the Act).

IV. Timeline of Recent Activities

October 9, 2012	CMS accepts the formal request for the coverage of PET Aβ imaging in the diagnosis of AD and other causes of cognitive decline. A 30-day public comment period begins.
November 8, 2012	The 30-day public comment period ends. CMS received 27 timely comments.
July 3, 2013	CMS posts the proposed decision memorandum for 30 days of public comment.
August 2, 2013	The public comment period on the proposed decision memorandum closes with 202 comments received.

V. FDA Status

The FDA has reviewed and approved one radiopharmaceutical for PET Aβ imaging, florbetapir (Amyvid™), in April 2012, to estimate Aβ neuritic plaque density in adult patients with cognitive impairment who are being evaluated for AD and other causes of cognitive decline. In the FDA-approved label for florbetapir there is no definition of "cognitive impairment," but the label does reference studies whose cognitively impaired patient populations range from MCI to dementia. The label states that although a negative florbetapir scan reduces the likelihood of AD, a positive florbetapir scan does not confirm the diagnosis of AD or any other cognitive disorder. This is because a positive florbetapir scan, which indicates the presence of moderate to frequent amyloid plagues in the brain, may be seen in persons with AD or other causes of cognitive decline as well as in persons with normal cognition.

The FDA-approved label for florbetapir indicates that it was not evaluated by the FDA as a screening tool to predict the development of dementia (including AD) or other cognitive disorders, nor to monitor the therapeutic response to treatment of these neurological conditions. Additionally, the label indicates that florbetapir images should only be interpreted by readers who successfully complete a special training program, which has been provided by the manufacturer through an in-person tutorial or electronic process. The FDA-approved label for florbetapir can be viewed in its entirety at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202008s000lbl.pdf

VI. General Methodological Principles

When making national coverage determinations, CMS evaluates relevant clinical evidence to determine whether the evidence is sufficient to support a finding that an item or service falling within a benefit category is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member. The critical appraisal of the evidence enables us to determine to what degree we are confident that: (1) the specific assessment questions can be answered conclusively; and (2) the intervention will improve health outcomes for beneficiaries. An improved health outcome is one of several considerations in determining whether an item or service is reasonable and necessary. A detailed account of the methodological principles of study design that CMS uses to assess the relevant literature on a therapeutic or diagnostic item or service for specific conditions can be found in Appendix A.

Public commenters sometimes cite the published clinical evidence and provide CMS with useful information. Public comments that provide information based on unpublished evidence, such as the results of individual practitioners or patients, are less rigorous and, therefore, less useful for making a coverage determination. CMS uses the initial comment period to inform its proposed decision. CMS responds in detail to the public comments that were received in response to the proposed decision when it issues the final decision memorandum.

VII. Evidence

A. Introduction

The purpose of this evidence review is to summarize the published literature on whether PET Aβ imaging is beneficial to patients with symptoms of AD. The evidence reviewed here includes the published medical literature as of August 31, 2013, on pertinent clinical trials, focusing on florbetapir, as it is the only clinically-relevant, FDA-approved PET Aβ imaging tracer. Additional supporting evidence from other studies and sources are cited below.

B. Summary of Evidence

1. Questions:

a. Is the evidence adequate to conclude that PET Aβ imaging improves meaningful health outcomes in beneficiaries who display signs or symptoms of AD?

b. Is the evidence adequate to conclude that PET Aβ imaging results inform the treating physician's management of the beneficiary to improve meaningful health outcomes? Those outcomes may include reasonably considered beneficial therapeutic management or the avoidance of unnecessary, burdensome interventions.

2. External Technology Assessment

CMS did not request an external technology assessment (TA) on this issue.

3. Internal technology assessment

Literature search methods

Literature searches performed on PubMed included combinations of the following terms: amyloid, beta-amyloid, PET imaging, dementia, Alzheimer's disease, neurodegenerative disorders, and mild cognitive impairment. Searches were also performed, using the same search terms, in ClinicalTrials.gov, the National Guideline Clearinghouse, the Cochrane Library, EMBASE, and other sources such as Trip Database.

Additional articles were selected from citations from key clinical trials, recent review articles, the NCD request, expert speaker talks at the MEDCAC meeting, MEDCAC panel members and public comments.

A review of the medical literature failed to reveal any pertinent meta-analysis or systematic reviews evaluating specifically the use of PET Aβ imaging in patients with signs and symptoms of AD. Although no randomized clinical trials were found exploring the use of PET Aβ imaging in this population, most studies found were prospective longitudinal studies. One study employed the use of a cross-sectional design (Landau 2012).

Prospective Longitudinal Studies

Wong D, Rosenberg P, Zhou Y, Kumar A, Raymont V, Ravert H, et al. In Vivo Imaging of Amyloid Deposition in Alzheimer's Disease using the Novel Radioligand [18F]AV-45 (Florbetapir F 18). J Nucl Med. 2010 June;51(6):913–920.

Wong and associates performed a study designed to explore brain imaging properties in cognitively healthy patients and those with AD by using PET florbetapir imaging. This open-label, multicenter, study involved 16 patients with Alzheimer's disease, as well as 16 cognitively healthy controls; both groups received florbetapir and PET imaging (in AD patients the mean age was 75.8 +/- 9.2, in healthy controls (HC) the mean age was 72.5 +/- 11.6). Patients with AD had to be greater than 50 years of age and have a probable diagnosis of AD according to NINCDS-ADRDA criteria, with a mini-mental status examination (MMSE) score between 10 and 24 inclusive. All healthy control subjects also had to be greater than 50 years of age, have no evidence of cognitive impairment by history and psychometric testing, and had to have an MMSE score of ≥ 29. Subjects who showed evidence of any other significant neurodegenerative or psychiatric disease on clinical examination or MRI, or clinically significant medical comorbidities, were excluded from the study. In the study, standard uptake values ratios (SUVR) were calculated using cerebellar grey matter as the primary reference region, and centrum semiovale white matter as an alternative reference region, and a parametric mapping approach employing the cerebellum as a reference region was used to calculate distribution/volume ratios (DVR).

Looking at the demographics of the two groups, though the baseline average MMSE was lower in the AD subjects than in the HC subjects (19.1 +/− 3.1 vs. 29.8 +/− 0.45), both groups were similar in age, weight, and education. A review of baseline data also revealed that there were a slightly higher proportion of males in the healthy control group than in the AD group (10/16 versus 8/16, respectively).

Results of the study revealed that accumulation of florbetapir tracer was found in cortical target areas such as the frontal cortex, temporal cortex and precuneus, areas that were expected to be high in amyloid deposition, while in healthy control subject tracer accumulation predominantly was distributed in the white matter areas. The cortical to cerebellar SUVR values remained much longer in AD patients than in healthy controls, reaching a plateau within 50 minutes. Using the 10 minute period from 50–60 minutes post administration as a representative sample, the cortical average SUVR for this period was 1.67 +/− 0.175 for patients with AD vs. 1.25 +/− 0.177 for healthy control subjects. The study also revealed that spatially normalized DVRs generated from PET dynamic scans were highly correlated with SUVR ($r = 0.58–0.88$, $p < 0.005$) and were significantly greater for AD patients than for healthy control subjects in cortical regions, but not in subcortical white matter or cerebellar regions.

The authors concluded that florbetapir PET imaging showed significant discrimination between clinically diagnosed AD patients and healthy control subjects using either a parametric reference region method (DVR) or a simplified SUVR method.

Camus V, Payoux P, Barré L, Desgranges B, Voisin T, Tauber C, et al. Using PET with 18F-AV-45 (florbetapir) to quantify brain amyloid load in a clinical environment. Eur J Nucl Med Mol Imaging. 2012 Apr;39(4):621-31. doi: 10.1007/s00259-011-2021-8. Epub 2012 Jan 18.

Camus and associates performed a prospective study to evaluate the clinical usefulness of florbetapir. The purpose of the study was to assess the feasibility of using PET imaging with florbetapir in three-level clinical settings to differentiate patients with mild to moderate AD or MCI patients from normal healthy control subjects in three PET centers. They also wanted to assess the safety of a florbetapir injection immediately after injection and during the follow-up period. Subjects included consecutive patients referred from the three participating memory clinics associated with the study center in France, and who met specific criteria as stated in the NINCDS-ADRDA criteria set for probable AD and DSM-IV criteria for Alzheimer's type dementia or diagnostic criteria for amnestic MCI. All participants had to be at least 55 years of age, be able to speak French fluently, have completed at least seven years of education and have neither unstable somatic disease nor psychiatric comorbidities. Healthy subjects who acted as controls were recruited through a community advertisement and evaluated in the same clinical settings.

The diagnosis of AD was confirmed using a mini-mental state examination (MMSE), as well as meeting the guidelines for global neuropsychological testing and an evaluation of verbal episodic memory (Free and Cued Selective Reminding Test, FCSRT), language (verbal fluency, naming, comprehension), gnosis, praxis, visuospatial functions and executive functions. Patients were excluded if they had any past or current symptomatic treatment with acetylcholinesterase inhibitors or memantine or had participated in any experimental study investigating Aβ-lowering agents. For MCI patients, a subjective memory complaint associated with isolated impairment in episodic memory had to be present, and assessed by a free recall total based on FCSRT. Healthy controls used in the study could not have any past history of or current major depressive episodes and/or antidepressant treatment, cognitive impairment in the diagnostic neuropsychological battery, memory complaints, or MRI brain scan abnormalities. A total of 46 subjects (20 men, 26 women, mean age 69.0 ± 7.6 years) were included in the study, including 13 AD patients, 12 MCI patients and 21 healthy control subjects. A brain MRI scan, a whole-body hybrid PET/CT scan and florbetapir PET imaging was performed on all subjects. PET images were assessed visually by blinded inspectors to any clinical information and quantitatively via the standard uptake value ratio (SUVR) in the specific regions of interest, which were defined in relation to the cerebellum as the reference region.

Results of the study revealed that the PET scan procedures were well tolerated, and no serious adverse events were reported during the immediate follow-up period, though at the 1-year follow-up, two patients did had medical problems unrelated to the study and were excluded from the analysis. The mean values of SUVR were higher in AD patients (median 1.20, Q1-Q3 1.16-1.30) than in healthy control subjects (median 1.05, Q1-Q3 1.04 -1.08; p = 0.0001) in the overall cortex and in all cortical regions (precuneus, anterior and posterior cingulate, and frontal median, temporal, parietal and occipital cortex). The MCI subjects also showed a higher uptake of florbetapir in the posterior cingulate cortex (median 1.06, Q1-Q3 0.97-1.28) compared with healthy control subjects (median 0.95, Q1-Q3 0.82-1.02; p = 0.03). Qualitative visual assessment of the PET scans showed a sensitivity of 84.6% (95% CI 0.55 – 0.98) and a specificity of 38.1% (95% CI 0.18 – 0.62) for discriminating clinically diagnosed AD patients from healthy control subjects; however, the quantitative assessment of the global cortex SUVR showed a sensitivity of 92.3% and specificity of 90.5% with a cut-off value of 1.122 (area under the curve 0.894).

Based on the results of the study, the authors felt that PET with florbetapir was suitable for routine use to improve the accuracy of AD diagnosis in the clinical setting, because the quantitative analyses showed a higher global SUVR and SUVR in several cortical regions (precuneus, anterior and posterior cingulate, frontal median, temporal, parietal and occipital cortex) in AD patients than in healthy control subjects. It also showed that the SUVR in the posterior cingulate and frontal median regions was significantly higher in AD patients than in MCI patients. The authors also note the following:

- the pattern of florbetapir cortical uptake found in the present study is similar to that found in previous studies conducted by Wong et al. and Clark et al.;
- the pattern also appears to be similar to those found with other amyloid-labeling compounds, such as PIB C11 and its flutemetamol F18-derived molecule, 11C-BF-227, FDDNP F18 and BAY94-9172 F18; and
- these patterns closely match the neuropathological stages of AD progression, which was strengthened by the high correlation found between florbetapir PET imaging and autopsy results.

The authors concluded that PET with florbetapir should become a routine clinical procedure because it improves the reliability of AD diagnosis and the detection of typical or atypical forms of pre-dementia stages, such as amnestic MCI and MCI associated with multi-domain deficits or neuropsychiatric symptoms (e.g., depression). But the authors also note that more studies testing the feasibility and tolerability of consecutive scans with florbetapir are needed to better document the accuracy of PET imaging with florbetapir in the AD diagnostic process at the dementia or pre-dementia stages, and that comparisons (or combinations) with other biomarkers, such as FDG PET, MRI and CSF dosages of tau and protein, are also needed.

Clark CM, Sneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA. Use of Florbetapir PET for Imaging Aβ Pathology. JAMA 2011 Jan 19;305(3):275-83.

Clark and associates performed a prospective clinical evaluation study to determine the qualitative and quantitative relationship between the florbetapir PET image and postmortem-amyloid pathology. This phase 3 multicenter study had two cohort groups. One group involved individuals at the end of life who consented to both florbetapir PET imaging and brain donation after death. In the other group, PET images were also obtained from younger individuals presumed to be free of brain amyloid to better understand the frequency of a false positive florbetapir PET image.

The study enrolled 152 individuals who were at least 51 years of age and approaching the end of their life, to obtain 35 postmortem brain evaluations from those who received PET imaging 12 months or less prior to death. Inclusion criteria for this group included a physician's assessment that the individual was likely to die within six months of study enrollment, absence of any known destructive lesion in the brain (e.g., stroke or tumor), and the individual's willingness to have florbetapir PET imaging followed by a brain autopsy at the time of death. The study also involved a second group of 74 young, cognitively normal, healthy individuals (aged 18-50 years). In both groups, physical, neurological, and cognitive evaluations that included assessments of memory, language, and constructional praxis were obtained.

Participants were imaged at 23 sites using clinical PET and PET/computed tomographic scanners, and florbetapir PET images were visually assessed by three board-certified nuclear medicine physicians, using a semi-quantitative score ranging from 0 (no amyloid) to 4 (high levels of cortical amyloid). A semi-automated quantitative analysis of the ratio of cortical to cerebellar signal (SUVR) also was performed for florbetapir PET images from all study participants. The main outcome measure of the study was correlation of florbetapir PET image interpretation (based on the median of 3 nuclear medicine physicians' ratings) and semi-automated quantification of cortical retention with postmortem Aβ burden, neuritic amyloid plaque density, and neuropathological diagnosis of Alzheimer disease in the first 35 participants autopsied (out of 152 individuals enrolled in the PET pathological correlation study). Autopsied brain tissue was obtained to identify and quantify Aβ aggregation using an automated immunostainer following established immunohistochemistry methods, and PET image quantification was performed using image processing and analysis software. Aβ neuritic plaque density was determined, and the mean density for both neuritic and diffuse plaques, using silver stain, was summarized by anatomical region using a 4-point semi-quantitative scale (0 = none, 1 = sparse, 2 = moderate, 3 = severe).

Also, a neuropathological diagnosis was made using standardized criteria as described by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and the National Institute on Aging (NIA) and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease (NIA/Reagan Institute criteria).

Results of the study revealed that there were significant correlations between the two measures of amyloid on florbetapir PET (SUVR versus semiquantitative visual score: 0.82 [95% CI, 0.64 - 0.87]; $p < .001$) and the two measures of amyloid aggregation at autopsy (immunohistochemistry vs. silver stain: 0.88 [95% CI, 0.76 - 0.94]; $p < .001$). The strengths of the inter-method correlations (e.g., PET visual read to immunohistochemistry) were similar to that for the intra-method correlations (e.g., PET visual read to PET SUVR, pathology immunohistochemistry to pathology plaque score). The study also revealed that 15 participants in the primary analysis autopsy cohort met pathological criteria for AD (CERAD: probable or definite AD; NIA/Reagan Institute criteria: intermediate to high likelihood of AD) and of these 15 participants, 14 had florbetapir PET scans that were interpreted as visually positive (median read 2), giving a sensitivity of 93% (95% CI, 68% - 100%). Finally, 14 participants in the autopsy cohort had low levels of Aβ aggregation on the postmortem examination and did not meet CERAD or NIA/Reagan Institute pathological criteria for AD. All 14 had florbetapir PET scans that read as negative, yielding a specificity of 100% (95% CI, 76.8% - 100%). The authors noted that the reviewers who read results for the florbetapir PET images agreed with the final autopsy with respect to the presence or absence of neuropathological criteria of AD in 28 of 29 cases.

The authors concluded that florbetapir PET imaging performed during life in this study correlated with the presence and density of Aβ at autopsy, and felt that this study provides evidence that a molecular imaging procedure can identify Aβ pathology in the brains of individuals during life.

Clark C, Pontecorvo M, Bench T, Bedell B, Coleman R, Doraiswamy P. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic Aβ plaques: a prospective cohort study. Lancet Neural 2012;11:669-78.

This second study by Clark and associates was a continuation of the 2011 discussed above. Like the original study, this prospective cohort study's purpose was to determine the qualitative and quantitative relationship between florbetapir PET imaging and postmortem-amyloid pathology. Patients who were alive at the end of the first study were followed up to autopsy, or for an additional year after the PET scan. Images and histopathological results from the original cohort study were used and extended to follow-up and were analyzed together to test the diagnostic accuracy of binary visual interpretation of florbetapir PET scans by comparison with the reference standard of neuritic plaque density at autopsy. The original study enrolled 152 individuals and obtained 35 postmortem brain evaluations from those who had received PET imaging 12 months or less prior to death. Autopsy results of the original Clark article was based on this cohort of 35 subjects.

The second Clark study used the same inclusion and exclusion criteria as the original study, as well as the same physical, neurological, and cognitive evaluations that included assessments of memory, language, and constructional praxis. The second study also had three board-certified nuclear medicine physicians read the florbetapir PET images, using a semi-quantitative score ranging from 0 (no amyloid) to 4 (high levels of cortical amyloid). And as before, a semi-automated quantitative analysis of the ratio of cortical to cerebellar signal (SUVR) was performed for florbetapir PET images from all study participants. Autopsied brain tissue was examined to identify and quantify Aβ aggregation, and neuritic plaque density was determined using a 4-point

semi-quantitative scale (0 = none, 1 = sparse, 2 = moderate, 3 = severe). The main outcome measure of the study was correlation of florbetapir PET image interpretation and semi-automated quantification of cortical retention with postmortem Aβ burden, and neuritic amyloid plaque density. The neuropathologic diagnosis of AD was made using standardized criteria as described by the CERAD and the National Institute on Aging (NIA) and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease (NIA/Reagan Institute criteria).

In the original Clark study, 35 participants died and had a postmortem exam. The remaining participants were followed up to 1 year, or a maximum of two years after the original PET scan. During this period an additional 24 autopsy results became available, leaving a combined total of 59 participants with a valid florbetapir PET scan and autopsy results within 24 months which comprised the primary efficacy analysis population. The mean age of this group was 79.4 years, and male as well as female genders were equally represented in this study. According to inclusion criteria, 12 subjects had no cognitive impairment, five had mild cognitive impairment that did not meet the criteria for dementia, 29 had AD, and 13 had other forms of dementia (e.g., dementia with Lewy bodies, Parkinson's disease dementia, frontotemporal dementia, unspecified dementia, and mixed dementia). The secondary efficacy analysis population, which consisted of patients in the 12 month autopsy cohort, had similar demographic and characteristics as the primary efficacy analysis population.

Results of the study revealed that 39 of the 59 patients included in the study in the primary efficacy analysis population had moderate or frequent neuritic plaques at autopsy and were categorized as positive for Aβ according to histopathological assessment. Most readers rated the florbetapir PET scans as positive in 36 of these 39 subjects, giving this a sensitivity rating of 92%. All 20 subjects with no or sparse neuritic plaque at autopsy were categorized as negative by the majority of readers of the florbetapir PET scan, resulting in a specificity of 100%. The overall accuracy for the primary efficacy analysis population was 95%. The sensitivity, specificity, and overall accuracy of the 46 participants included in the secondary efficacy analysis population were 96%, 100% and 98% respectively.

Visual semi-quantitative ratings of Aβ by use of florbetapir PET imaging showed a positive correlation with postmortem levels of Aβ measured via immunohistochemistry in subjects who had autopsies within two years of PET scan (Spearman $\rho = 0.76$; $p < 0.0001$), as well as subjects who had autopsies within one year of PET scan (Spearman $\rho = 0.79$; $p < 0.0001$). The authors concluded that the results of the study showed correlation between florbetapir PET imaging and postmortem amyloid burden, and the authors concluded that florbetapir might be useful for imaging of Aβ neuritic plaques in the brains of patients with cognitive impairment.

Fleisher AS, Chen K, Liu X, Roontiva A, Thiyyagura P, Ayutyanont N. Using Positron Emission Tomography and Florbetapir F 18 to Image Cortical Amyloid in Patients With Mild Cognitive Impairment or Dementia Due to Alzheimer Disease. Arch Neurol. 2011;68(11):1404-1411.

Fleischer and associates used multiple research imaging centers in their study to characterize quantitative florbetapir PET measurements of fibrillar Aβ burden in a large clinical cohort of participants with probable AD or mild cognitive impairment and older healthy controls. The study used pooled data from the four registered phase I and II trials of florbetapir PET imaging, using standard dosing of florbetapir and non-dynamic PET acquisitions. The study evaluated both continuous and binary measures of florbetapir PET activity to assess global differences between clinical diagnostic groups, to confirm expected patterns of regional distributions of fibrillar Aβ, and to determine proportions of positive scans using cut-off thresholds for global cortical florbetapir activity. During the

course of the study, researchers predetermined SUVR threshold levels for defining florbetapir PET positivity based on a previously reported study of expired end-of-life patients and a specificity cohort of young ApoE4 non-carriers.

The study involved a total of 210 participants who were 55 years of age or older, consisting of 82 cognitively normal volunteers, 60 individuals with MCI, and 68 individuals with probable AD. Florbetapir PET scans were taken of all participants, and they were required to have no subjective cognitive complaints as corroborated by an informant report, to have an MMSE score of 29 or greater, and to be cognitively normal based on psychometric testing. Participants with probable AD met NINCDS-ADRDA criteria for probable AD and had an MMSE score at screening in the range of 10 to 24. ApoE genotyping was performed as an optional procedure on 155 participants. Subjects were excluded if they had other current clinically relevant neurologic or psychiatric illnesses, were receiving any investigational medications, or ever received an anti-amyloid experimental therapy.

All participants underwent a florbetapir PET session that consisted of intravenous injection of florbetapir F 18, and a region of interest (ROI) analysis was performed on individual PET images. Cerebral–to–whole-cerebellar florbetapir standard uptake value ratios (SUVRs) were computed. The study compared mean cortical SUVRs, and a threshold of SUVRs greater than or equal to 1.17 was used to reflect pathological levels of amyloid associated with AD based on separate antemortem PET and postmortem neuropathology data from 19 end-of-life patients. Also a threshold of SUVRs greater than 1.08 was used to signify the presence of any identifiable Aβ because this was the upper limit from a separate set of 46 individuals 18 to 40 years of age who did not carry ApoE4. In this study florbetapir PET activity was the outcome measure of interest.

Results of the study revealed that all participant groups differed significantly in terms of mean [SD] cortical florbetapir SUVRs. Those with probable AD had a mean score of 1.39 [0.24], those with MCI had a mean score of 1.17 [0.27], and those who were older healthy controls (HC) had a mean score of 1.05 [0.16] ($p < 1.0 \times 10^{-7}$). In terms of percentage meeting levels of amyloid associated with AD by SUVR criteria the scores were 80.9% (AD), 40.0% (MCI) and 20.7% (HC) ($p < 1.0 \times 10^{-7}$). In terms of percentage meeting SUVR criteria for the presence of any identifiable Aβ the scores were 85.3% (AD), 46.6% (MCI) and 28.1% (HC) ($p < 1.0 \times 10^{-7}$). In older healthy controls, the percentage of florbetapir positivity increased linearly by age decile ($p = .05$). The study also revealed that for the 54 older health controls with available ApoE genotypes, ApoE4 carriers had a higher mean [SD] cortical SUVR than did non-carriers (1.14 [0.2] versus 1.03 [0.16]; $p = .048$). The authors felt that the results support the ability of florbetapir PET SUVRs to characterize amyloid levels in clinically probable AD, MCI, and older healthy control groups, using both continuous and binary quantitative measures of amyloid burden.

Doraiswamy P, Sperling R, Coleman R, Johnson K, Reiman E, Davis, M. Amyloid-β assessed by florbetapir F18 PET and 18-month cognitive decline: A multicenter study. Neurology 2012;79:1636–1644.

Doraiswamy and associates performed a prospective, multicenter, observational study to evaluate the prognostic utility of detecting Aβ pathology using florbetapir PET in older subjects at risk for progressive cognitive decline. In this study, 51 subjects with MCI, 69 clinically normal cognitively healthy controls, and 31 subjects clinically diagnosed with AD dementia who had previously received a florbetapir PET scan were enrolled. Patients with AD dementia met NINCDS-ADRDA criteria for probable AD and had MMSE scores less than or equal to 24. MCI subjects were presenting for an initial evaluation, or had received a diagnosis of MCI within the past year prior to the study. MCI participants had to be at least 50 years of age, had a complaint of memory or cognitive

impairment corroborated by an informant, had a clinical dementia rating (CDR) scale global rating of 0.5, and MMSE > 24 and no episodic memory cut-off was required. The healthy control subjects had to be at least 50 years of age, and were assessed clinically as cognitively normal, and had a CDR global of 0 and an MMSE of 29 or 30. Cognitively normal subjects were recruited approximately equally across age deciles (50–59, 60-69, 70–79, and equal to or greater than 80 years of age).

All subjects included in the study underwent a detailed medical history, physical and neurologic examinations, a clinical interview and laboratory evaluations; additionally an MRI was performed at screening or within six months prior to enrollment to rule out significant CNS lesions. Subjects were excluded if they had other relevant neuropsychiatric diseases, received anti-amyloid investigational drugs, were unable to complete psychometric testing, or had contraindications to PET. A battery of procedures was performed on all subjects including a clinical diagnostic interview and cognitive/functional testing including the CDR, MMSE, Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog; 11-item version), Wechsler Logical Memory (immediate and delayed recall), Digit-Symbol Substitution, Category Verbal Fluency (animals and vegetables), Alzheimer's Disease Cooperative Study–Activities of Daily Living Scale (ADCS-ADL), and Geriatric Depression Scale (GDS). ApoE genotyping was also performed.

Subjects underwent PET amyloid imaging using florbetapir. Three nuclear medicine physicians, blinded to clinical data, independently reviewed all PET images and rated each on both a semi-quantitative (0–4) and a binary qualitative scale (amyloid positive or amyloid negative) based on the pattern of tracer uptake in gray matter cortical areas. Cerebral-to-whole-cerebellar florbetapir standard uptake value ratios (SUVRs) were calculated using whole cerebellum as the reference region. The average of the SUVR across the six cortical target regions was used for analysis. Subjects who completed the initial PET scan were eligible to participate in the follow-up protocol which would determine whether florbetapir PET predicts progressive cognitive impairment at 36 months.

By the end of the study, of the 151 subjects (69 cognitively normal, 51 mild cognitive impairment, 31 AD) who entered the study, 97% of cognitively normal, 90% of MCI, and 87% of AD subjects completed the 18 months follow-up. The analysis revealed that in both MCI and cognitively normal patients, baseline Aβ positive scans were associated with greater clinical worsening on the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog ($p < 0.01$) and Clinical Dementia Rating–sum of boxes (CDR-SB) ($p < 0.02$). Analysis also revealed that MCI Aβ positive scans were associated with greater decline in memory, Digit Symbol Substitution (DSS) and MMSE scores ($p < 0.05$). And though MCI subjects had higher baseline SUVR, which was correlated with greater subsequent decline on the ADAS-Cog ($p < 0.01$), CDR-SB ($p < 0.03$), a memory measure, DSS, and MMSE ($p < 0.05$), Aβ positive MCI subjects tended to convert to AD dementia at a higher rate than Aβ negative subjects ($p < 0.10$).

The authors of the study felt that the results demonstrated that florbetapir amyloid imaging confirms that both cognitively normal subjects and subjects with MCI with higher levels of cortical Aβ on PET are at higher risk for future cognitive progression than individuals with lower levels of amyloid, after controlling for age and baseline cognitive performance. They felt that not only did the findings support the use of florbetapir PET as a predictive biomarker of cognitive decline in at-risk subjects, but also that amyloid PET may have predictive value in MCI for developing AD dementia. They concluded that florbetapir PET may help identify individuals at increased risk for progressive cognitive decline.

Grundman M. Pontecorvo M, Salloway S, Doraiswamy P, Fleisher A, Sadowsky C, et al. Potential Impact of Amyloid Imaging on Diagnosis and Intended Management in Patients With Progressive Cognitive Decline. Alzheimer Dis Assoc Disord 2012;00:000–000.

Grundman and associates performed a prospective study to determine the impact of amyloid imaging on the diagnoses and management of patients undergoing evaluation for cognitive decline, more specifically to determine whether knowledge of the presence or absence of moderate to frequent neuritic amyloid plaques, as assessed by a florbetapir PET scan, would alter a physician's diagnostic thinking and intended patient management. The study consisted of two roughly equal groups of patients: those who had completed a diagnostic evaluation for progressive cognitive decline/impairment within the previous 18 months (group A, n = 110), and those who were currently undergoing an evaluation (group B, n = 119), but presumably were at a point where the physician was interested in obtaining florbetapir PET scan information. For patients in the study undergoing diagnostic evaluation at entry, the investigator had the option of completing the evaluation and enrolling the patient in group A or enrolling the patient in group B and then considering additional evaluations after the PET scan had been obtained. Although there was no requirement that patients had to meet a specific level of cognitive impairment for inclusion in the study, only patients in whom a history of cognitive decline was documented were included. Exclusion criteria included patients who had a previous amyloid imaging scan or previous participation in a clinical trial of an amyloid targeting therapeutic agents (unless they were in the placebo group).

Screening and baseline studies were obtained, which consisted of a medical history including demographic features, history of cognitive decline, and a record of diagnostic tests performed as part of the standard practice clinical evaluation/diagnostic workup. Subjects also underwent the MMSE. The site physicians decided whether or not patients should be placed in group A (completed their diagnostic evaluation) or group B (still undergoing diagnostic evaluation). If the screening visit/pre-scan evaluation indicated a need for additional diagnostic testing, patients were always assigned to group B. At the end of the screening, physicians recorded the current diagnosis (group A), or working diagnosis (group B) for each patient. Diagnoses were classified as either:

- etiology due to AD (or most likely prodromal AD, or MCI due to AD, probable AD, atypical AD, Lewy body disease with AD/amyloid pathology, or mixed dementia with AD);
- non-AD etiology (most likely etiology is not AD, e.g., mild cognitive impairment of uncertain etiology, but not due to AD; or a specific non-AD etiology such as vascular dementia, frontotemporal dementia; Lewy body disease without AD pathology; primary progressive aphasia; metabolic, psychiatric, or medication-induced impairments); or
- indeterminate (syndromic) etiology, (where the clinician could describe a syndrome but could not provide a more specific etiology, e.g., progressive cognitive decline, mild cognitive impairment, or dementia of uncertain etiology).

For all participants in the study, the treating physicians had to provide results of diagnostic testing and a management plan using information available before florbetapir imaging. After subjects received imaging with florbetapir PET, the diagnosis and intended management at baseline were compared with those obtained after receiving the florbetapir PET scan result. For purposes of this study, a change from an indeterminate/uncertain etiology to a specific etiology (such as MCI due to AD) or a change from one etiologic category (due to AD/not due to AD) to the other was considered a change in diagnosis. A change within etiologic category (e.g., MCI due to AD changed to Dementia due to AD) was not considered a change in diagnosis.

A total of 229 subjects (group A, 48%, n = 110; group B, 52%, n = 119) were enrolled in the study and underwent florbetapir PET scans. The mean age of participants was 74.1 ± 8.1 years, 95% of the subjects were white, and 50.2% were male. With the exception of gender (p = 0.0202), there were no significant demographic differences between subjects who had previously completed a workup and diagnosis and those still undergoing a

workup. Of the study participants, 36% had dementia, and the remaining 64% had cognitive impairment not at the level of dementia; also 113 subjects were amyloid positive, while 116 were amyloid negative. Analysis of data revealed that after receiving the results of the florbetapir scan, post-scan diagnosis changed in 125 (54.6%) of 229 cases (95% CI, 48.1% - 60.9%). The scan had an impact on the classification for 37% of subjects with a pre -scan diagnosis indicating an etiology due to AD, 66% of subjects with an indeterminate pre-scan diagnosis, and 62% of subjects with a non-AD pre-scan diagnosis.

When looking at changes in confidence in terms of etiologic diagnosis at both the pre-scan and the postscan time points, the mean confidence level significantly increased after florbetapir PET by an average of 21.6% (95% CI, 18.3% - 24.8%; $p < 0.0001$. And in terms of intended management, there was a change in the overall management plan for 199 (86.9%) of 229 subjects (95% CI, 81.9% - 90.7%), especially when it came to intended medication management as a result of the scan. In 71 (31%) of 229 subjects (95% CI, 25.4% - 37.3%) florbetapir PET results led to an intended change in AD medications and in 17 (7.4%) of 229 patients (95% CI, 4.7% - 11.6%), the results led to an intended change in treatment with psychiatric medications (e.g., antidepressants, antianxiety medications, or antipsychotics).

The authors concluded that after receiving the results of the florbetapir scan, physicians made significant changes in their diagnoses and had increased diagnostic confidence. They also showed that treatment plans were modified after florbetapir imaging both for patients who were in the midst of their workup and for those with a complete workup.

Cross-sectional study

Landau S, Mintun MD, Joshi A, Koeppe R, Petersen R, Aisen P, et al. Amyloid Deposition, Hypometabolism, and Longitudinal Cognitive Decline. Ann Neurol 2012;72:578–586.

Landau and associates performed a study using longitudinal multisite data to examine the cross-sectional relationships between amyloid deposition, hypometabolism, and cognition, and the associations between amyloid and hypometabolism measurements, and retrospective, longitudinal cognitive measurements. In this study, 426 Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with an available florbetapir and MRI scan were enrolled (126 normal, 162 early mild cognitive impairment (EMCI), 85 late mild cognitive impairment (LMCI), 53 Alzheimer's disease (AD); 417 of these participants also had an FDG-PET scan acquired approximately concurrently with the florbetapir scan (average time between FDG-PET and florbetapir, < one week). Approximately 2/3 of the total sample were newly enrolled subjects who had no longitudinal follow-up, whereas approximately 1/3 were continuing normal (n = 76) and LMCI (n = 81) participants from ADNI 1 who were followed for an average of about four years prior to their florbetapir scans.

Inclusion as well as exclusion criteria were specified and followed. LMCI participants had the following characteristics: a subjective memory complaint, a Clinical Dementia Rating (CDR) of 0.5, and were classified as single- or multi-domain amnestic. The EMCI group differed from LMCI group only based on education-adjusted

scores for the delayed paragraph recall sub-score on the Wechsler Memory Scale–Revised Logical Memory II, such that EMCI subjects were intermediate between normal subjects and LMCI. Normal subjects had CDR scores of 0, and patients with AD met standard diagnostic criteria. The ADAS-cog16 was used in the cross-sectional analyses and well as the primary outcome measure in the longitudinal analyses (total score ranges from 0 to 70, with a higher score indicating poorer cognitive function). Changes in diagnostic status (e.g., remaining LMCI or converting to AD) were also assessed. In the study, ApoE genotypes were determined with blood samples in all except two EMCI subjects. PET image data were acquired based on ADNI protocol. The associations between concurrent florbetapir, FDG, and ADAS-cog measurements for the whole population and for each diagnostic group separately (normal, EMCI, LMCI, AD) were obtained; Spearman rank correlation coefficients were used for continuous variables to account for the non-normally distributed nature of florbetapir and ADAS-cog, and chi-square tests were used for dichotomous variables. For participants with longitudinal data, associations between independent variables (florbetapir and FDG PETs) and longitudinal ADAS-cog change were explored using linear mixed effects models.

Results of the study revealed that 29% of normal subjects, 43% of EMCI patients, 62% of LMCI patients, and 77% of AD patients were categorized as florbetapir positive, and florbetapir was negatively associated with concurrent FDG and ADAS-cog in both MCI groups. The longitudinal analysis also revealed that florbetapir-positive subjects in both normal and LMCI groups had greater ongoing ADAS-cog decline than those who were florbetapir negative, though in normal subjects, florbetapir positivity was associated with greater ADAS-cog decline than FDG, whereas in LMCI, FDG positivity was associated with greater decline than florbetapir.

The authors concluded that, although both hypometabolism and Aβ deposition were detectable in normal subjects and all diagnostic groups, Aβ showed greater associations with cognitive decline in normal participants. In view of the minimal cognitive deterioration overall in this group, the authors felt that the study suggested that amyloid deposition has an early and subclinical impact on cognition that might precede metabolic changes. They also concluded that at moderate and later stages of disease (LMCI/AD), hypometabolism becomes more prominent and more closely linked to cognitive decline.

Additional Studies submitted during the Second Comment Period - (July 3, 2013 – August 2, 2013)

Johnson KA, Sperling RA, Gidicsin RA, et al. Florbetapir (F18-AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging. Alzheimer's & Dementia. 30 January 2012:1-12.

Johnson and associates used florbetapir to perform a study to assess amyloid burden, using visual as well as quantitative measures (Johnson, Sperling, Gidicsin, et. al 2012). This multi-center, phase II investigation included 45 patients with AD, 60 patients with MCI, and 45 apparently normal healthy patients in the control group. Results of the study revealed that florbetapir PET imaging was rated visually amyloid positive in 76% of AD patients, 38% of MCI patients, and 14% of HCs. Also 84% of AD patients, 45% of MCI patients, and 23% of HCs were classified as amyloid positive using the quantitative threshold. It also revealed that amyloid positivity and mean cortical amyloid burden were associated with age and apolipoprotein E ε4 carrier status.

The authors acknowledged that the percentage of subjects rated positive, particularly for the AD and MCI groups, was less than in some previous studies using other PET amyloid tracers, and gave several explanations (e.g., the percentage of subjects who were APOE ε4 carriers in the current study (40% of MCI patients and 53% of AD patients) was lower than in previous APOE ε4-enriched multicenter research studies; the selection criteria may have contributed to the lower observed rate of amyloid-positive cases). They also noted that some of the image readers in the study appeared to be more conservative in their interpretation, and potentially less sensitive to the presence of tracer accumulation/amyloid pathology in comparison with the quantitative analysis, and even noted that one reader did show a higher overall rate of positivity than the others. Finally, a post-mortem examination, required for the gold standard diagnosis of AD, was not part of the study.

Zannas AS, Doraiswamy PM, Shpanskaya KS, et al. Impact of 18F-florbetapir PET imaging of β-amyloid neuritic plaque density on clinical decision-making. Neurocase. 14 May 2013:1-8.

Zannas and associates performed a case series study; the objective was to determine if clinical management changed based on the results of florbetapir PET imaging (Zannas et.al 2013). The study involved 11 cognitively impaired subjects. Clinician surveys were done before and after PET scanning to document the impact of amyloid imaging on the diagnosis and treatment plans. All patients had dementia or MCI as a pre PET diagnosis. Of the patients involved in the study, four were felt to have AD as the etiology; the rest were suspected of having depression, vascular disease or another etiology. Results of the study were mixed. It revealed that in five cases, the florbetapir test was negative, leading to a change in diagnosis in four patients, and a change in treatment in two cases. In six cases, the test was positive leading to a change in diagnosis in four patients and a change in treatment plan in three of these cases. But the authors were also able to document cases were patients were suspected of having MCI or depression, and even though their test were positive for florbetapir, there was no change in management. Also the authors noted a case of an MCI patient that was kept on cholinesterase inhibitors treatment despite a negative test. None of the patients were followed longitudinally long enough in order to have a post mortem examination of the brain—the gold standard for the diagnosis of AD.

Choi SR, Scheider JA, Bennett BA, et al. Correlation of amyloid PET ligand florbetapir F 18 (18F-AV-45) binding with β-amyloid aggregation and neuritic plaque deposition in postmortem brain tissue. Alzheimer Disease and Associated Disorders. 2012 January;26(1):8–16.

Choi and associates studied the ability of florbetapir F 18 to accurately identify and quantify amyloid aggregates in human autopsy brain tissue (Choi et. al. 2013). The purpose of their study was to determine the relationship between florbetapir F 18 tissue retention as measured by autoradiography (ARG) and the localization of amyloid plaques using double-labeling studies. They also wanted to determine the correlation between the intensity of the florbetapir ligand signal and β-amyloid deposition. In the study the postmortem brain tissue of 40 subjects suffering with varying degrees of neurodegenerative pathology was assessed using florbetapir F 18 autoradiography (subjects chosen to represent a range of pathologic diagnoses including subjects free of pathology, subjects with AD, subjects with vascular dementia and subjects with progressive supranuclear palsy), and later correlated with β-amyloid identified utilizing silver staining, thioflavin S staining, and immunohistochemistry.

The study was able to demonstrate that there was a strong correlation between the density of in vitro florbetapir F 18 binding in human autopsy tissue, and that there was a strong correlation between the density of in vitro florbetapir F 18 binding and the density of β-amyloid. The authors also noted that the intensity of the florbetapir

F 18 signal in human autopsy sections was correlated with the degree of ligand binding in regional brain homogenates; and that florbetapir F 18 does not bind to neurofibrillary tangles in human postmortem tissue.

Though the authors concluded that florbetapir F 18 can be used as an amyloid PET ligand to identify the presence of AD pathology in patients with signs and symptoms of progressive late-life cognitive impairment, they provided little information on the degree of correlation of florbetapir F 18 in patients with conditions other than AD (e.g., subjects free of pathology, subjects with vascular dementia and subjects with progressive supranuclear palsy).

4. MEDCAC

A Medicare Evidence Development and Coverage Advisory Committee (MEDCAC) meeting was convened on the role of PET Aβ imaging in dementia and neurodegenerative disease on January 30, 2013. The purpose was to seek the expert panel's input on whether the published evidence identified patient characteristics that would predict improved health outcomes for patients who undergo PET Aβ imaging. The panel voted on a series of questions using a 1-5 confidence scale (with 1 representing low or no confidence; 3, intermediate confidence; and 5, high confidence).

A key question for the panel was: How confident are you that there is adequate evidence to determine whether PET imaging of brain beta amyloid changes health outcomes (improved, equivalent or worsened) in patients who display early symptoms or signs of cognitive dysfunction? The average score of voting panel members was below an intermediate level (2.17 out of 5).

The record of the MEDCAC meeting is available on the CMS website. We hereby incorporate it into the administrative record of this NCD by reference. (http://www.cms.gov/medicare-coverage-database/details/medcac-meeting-details.aspx?MEDCACId=66).

5. Evidence-based guidelines

We searched the National Guideline Clearinghouse (www.guidelines.gov) and the Internet more generally for relevant guidelines.

Keith A. Johnson, Satoshi Minoshimab, Nicolaas I. Bohnen, Kevin J. Donohoe, Norman L. Foster, Peter Herscovitch, Jason H. Karlawish, Christopher C. Rowe, Maria C. Carrillo, Dean M. Hartley, Saima Hedrick, Virginia Pappas, William H. Thies. Appropriate use criteria for amyloid PET: A report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association. First published January 28, 2013, doi: 10.2967/jnumed.113.120618 J Nucl Med March 1, 2013 jnumed.113.120618

Given that PET Aβ imaging "is a technology that is becoming more available," the Amyloid Imaging Taskforce (AIT) formed jointly by the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association, sought "to provide guidance to dementia care practitioners, patients, and caregivers" on its appropriate use.

A summary of the AIT's appropriate use criteria appears below:

"Amyloid imaging is appropriate in the situations listed here for individuals with all of the following characteristics: Preamble: (i) a cognitive complaint with objectively confirmed impairment; (ii) AD as a possible diagnosis, but when the diagnosis is uncertain after a comprehensive evaluation by a dementia expert; and (iii) when knowledge of the presence or absence of Aβ pathology is expected to increase diagnostic certainty and alter management.

1. Patients with persistent or progressive unexplained MCI
2. Patients satisfying core clinical criteria for possible AD because of unclear clinical presentation, either an atypical clinical course or an etiologically mixed presentation
3. Patients with progressive dementia and atypically early age of onset (usually defined as 65 years or less in age)

Amyloid imaging is inappropriate in the following situations:

4. Patients with core clinical criteria for probable AD with typical age of onset
5. To determine dementia severity
6. Based solely on a positive family history of dementia or presence of ApoE4
7. Patients with a cognitive complaint that is unconfirmed on clinical examination
8. In lieu of genotyping for suspected autosomal mutation carriers
9. In asymptomatic individuals
10. Nonmedical use (e.g., legal, insurance coverage, or employment screening)"

6. Professional Society Position Statements

A handful of nuclear medicine and physician professional societies, and AD/dementia organizations commented on the PET Aβ proposed decision memo, which we responded to in the Public Comment section below. These comments can be viewed in their entirety at: http://www.cms.gov/medicare-coverage-database/details/nca-view

-public-comments.aspx?NCAId=265.

7. Expert Opinion

We sought and received expert opinion through the MEDCAC process. We also received expert opinion during our public comment period.

8. Public Comments

A. *Initial Comment Period: October 9, 2012 – November 8, 2012*

CMS received 27 timely public comments during the first public comment period. Twenty-six out of 27 commenters supported Medicare coverage of PET Aβ scans in the diagnostic context of suspected dementia. Of the supporting commenters, a few wrote that Aβ imaging agents should not be covered for screening of asymptomatic patients, patients without documented cognitive decline, or patients whose AD diagnosis could be confirmed without a PET Aβ scan. Another supportive commenter stated that the meaning of a positive or negative PET Aβ scan, as outlined in the FDA-approved label, should be fully communicated by providers to patients.

The non-supportive commenter argued that research on Aβ imaging agents (particularly Amyvid™ (florbetapir), as the only FDA-approved Aβ imaging agent to date) is too limited, and does not demonstrate a beneficial impact on clinical management of dementia and on health outcomes. This commenter did, however, support the use of Amyvid™ in clinical trials.

Comments came from the following sources:

- 1 (4%) comment came from physicians;
- 7 (26%) comments came from the pharmaceutical and PET imaging industry;
- 5 (18%) comments came from medical imaging societies and specialty groups;
- 9 (33%) comments came from researchers or persons at academic institutions;
- 1 (4%) comment came from the health insurance industry;
- 1 (4%) comment came from research hospitals;
- 2 (7%) comments came from Alzheimer's societies (USAgainstAlzheimer's and Alzheimer's Foundation of America); and
- 1 (4%) comment came from members the general public who did not identify a further affiliation.

B. *Second Comment Period: July 3, 2013 – August 2, 2013*

CMS received 202 timely public comments on the proposed decision. Many of the public comments we received cited unpublished evidence such as data presented at conferences and the results of individual practitioners or patients (often on behalf of family members and caregivers). CMS took into consideration all public comments. We respond in detail to major themes in the public comments below.

The public commenters raised eight key concerns. Several commenters:

(1) raised concerns regarding the CMS standard for making a reasonable and necessary determination for diagnostic tests;
(2) believed that CMS should cover amyloid PET to help differentiate frontotemporal dementia (FTD) from AD since CMS has covered FDG PET for this use;
(3) suggested that the final decision should more closely reflect the recommendations by expert consensus panels;
(4) state or imply that a PET amyloid scan gives an accurate, positive diagnosis of AD;
(5) claimed that dementia specialists could make an accurate positive diagnosis of AD when integrating the result of an amyloid PET scan.
(6) suggested that because the FDA approved the amyloid PET agent florbetapir (Amyvid™), CMS should cover a diagnostic test using that agent;
(7) believed that our proposed decision permitting coverage only in certain qualifying clinical studies would be inconsistent with the National Alzheimer's Project Act (NAPA); and/or
(8) believed the proposed decision would be too onerous and restrictive and would limit access to this new technology.

We address the above concerns in detail in our response to the comments.

CMS standard for making a reasonable and necessary determination for diagnostic tests

Comment
Several commenters believe that evidence of "improved health outcomes" should not be a factor for a coverage determination on amyloid PET.

Response
We disagree. Section 1862(a)(1)(A) of the Act states that no payment may be made for items or services "which are not reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member." When making national coverage determinations, CMS evaluates relevant clinical evidence to determine whether the evidence is of sufficient quality to support a finding that an item or service that falls within a benefit category is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member. This critical appraisal of the evidence enables us to

determine whether: 1) the specific assessment questions can be answered conclusively; and 2) the investigational item or service will improve health outcomes for patients. An improved health outcome is one of several considerations in determining whether an item or service is reasonable and necessary.

Specifically with regard to diagnostic tests, the Medicare regulations at 42 CFR § 410.32(a) state in part, that "…diagnostic tests must be ordered by the physician who is treating the beneficiary, that is, the physician who furnishes a consultation or treats a beneficiary for a specific medical problem and who uses the results in the management of the beneficiary's specific medical problem." Thus, we looked for evidence demonstrating how the treating physician uses the result of beta amyloid PET imaging for the management of a patient with suspected AD.

In evaluating diagnostic tests, Mol and colleagues (2003) reported: "Whether or not patients are better off from undergoing a diagnostic test will depend on how test information is used to guide subsequent decisions on starting, stopping, or modifying treatment. Consequently, the practical value of a diagnostic test can only be assessed by taking into account subsequent health outcomes." When a proven, well established association or pathway is available, intermediate health outcomes may also be considered. For example, if a particular diagnostic test result can be shown to change patient management and other evidence has demonstrated that those patient management changes improve health outcomes, then those separate sources of evidence may be sufficient to demonstrate positive health outcomes from the diagnostic test.

A diagnostic test would not be expected to directly change health outcomes. Rather, a diagnostic test affects health outcomes through changes in disease management brought about by physician actions taken in response to test results. Such actions may include decisions to treat or withhold treatment, to choose one treatment modality over another, or to choose a different dose or duration of the same treatment. To some extent the usefulness of a test result is constrained by the available treatment options. Unfortunately the data are silent on health outcomes, and do not establish that the treating physicians appropriately base patient management on the PET test result. Most studies have focused on test characteristics and have not considered health outcomes. We believe that health outcomes are more persuasive than test characteristics.

We generally consider the evidence in the hierarchical framework of Fryback and Thornbury (1991) where Level 2 addresses diagnostic accuracy, sensitivity and specificity of the test; Level 3 focuses on whether the information produces change in the physician's diagnostic thinking; Level 4 concerns the effect on the patient management plan, and Level 5 measures the effect of the diagnostic information on patient outcomes. CMS has generally found evidence of efficacy at Level 5 more persuasive to support unconditional coverage. We believe that coverage supported by that level or higher evidence results in the greatest benefit for Medicare beneficiaries.

The expectation that a diagnostic test will produce relevant information that informs physician management is well established in the practice of medicine and is also reflected in our regulation (42 CFR 410.32). Accordingly, we ask: Does the test lead the physician to reconsider the pre-test treatment plan and make appropriate modifications in light of the test result? Such actions may include decisions to treat or withhold treatment, to choose one treatment modality over another, or to choose a different dose or duration of the same treatment. There is no persuasive evidence that amyloid PET testing produces relevant information for these purposes.

Specifically for amyloid PET, and as discussed in the analysis and discussions sections of this decision memorandum, there is no convincing evidence that the scan changes physician management of the patient in a meaningful manner (e.g., there is no convincing benefit to Medicare beneficiaries). However, we believe there is promising evidence to cover amyloid PET under coverage with evidence development (CED) and that the test has a high potential to provide a significant benefit to Medicare beneficiaries in the future. Per the CED guidance document, when the evidence is inadequate to determine that the item or service is reasonable and necessary under section 1862(a)(1)(A), Medicare coverage may be extended to patients enrolled in a clinical research study. In this case, AHRQ and CMS are supporting research under section 1862(a)(1)(E). For the readers' convenience, the 2006 CED Guidance Document is available at http://www.cms.gov/determinationprocess/downloads/CED.pdf

We believe that beneficiaries would benefit from the use of the amyloid PET scan to enrich clinical trials and help find better treatments or prevention strategies for AD.

CMS covered FDG PET to differentiate frontotemporal dementia (FTD) from AD

Comment
Several commenters claimed that because CMS currently covers FDG PET to help differentiate frontotemporal dementia (FTD) from AD, amyloid PET should also be covered because they believe it is a similar technology for the same diagnosis and that amyloid PET is a better diagnostic tool. They ask that amyloid PET should be similarly covered, without CED.

Response
In 2004 CMS issued an NCD to cover FDG PET scans for either the differential diagnosis of frontotemporal dementia (FTD) and Alzheimer's disease (AD) under specific requirements; OR, its use in a CMS-approved practical clinical trial focused on the utility of FDG PET in the diagnosis or treatment of dementing neurodegenerative diseases (see NCD manual, section 220.6.13). FDG PET is a fundamentally different – not a similar – technology. FDG PET measures the physiological process of metabolism, while amyloid PET looks at the anatomical burden of amyloid plaques.

The proposed clinical use of the amyloid PET scan to differentiate FTD from AD leverages the power of a negative scan to help exclude AD, which is consistent with the FDA-approved label and our own detailed assessment. However, the evidence for the scan's possible clinical utility comes from very small, or yet to be published studies. In response to our concern about the small sample sizes, the lead author of one such study wrote in the public comments that he has soon-to-be published data expanding this patient pool from 12 to 25 subjects, with consistent results. It is encouraging to hear that the data will be published and we look forward to reviewing the data. However, we note that 25 subjects is still a very small sample size in light of reports that over five million Americans age 65 and over have AD. (https://www.alz.org/downloads/facts_figures_2012.pdf)

We also note that many of the commenters appear to assume that amyloid PET is a better tool than FDG PET for differentiating FTD from AD. This may or may not be true – the evidence is not clear – and there is at least some evidence that FDG PET is actually better (as another distinguished PET researcher argues in the public comments, citing peer-reviewed publications). While outside the scope of this NCD, we encourage further study, involving prominent researchers on amyloid PET and FDG PET alike, to help build the evidence base, and determine which of multiple potentially useful tests should be used, when alone or in combination, and for which particular subpopulations (recall that FTD has multiple subtypes, and one algorithm may not fit all of them).

The differentiation of FTD from AD may be one clinical use where CED leads to earlier and broader coverage than would otherwise be accomplished. In addition, our goal under CED is to facilitate the development of additional evidence that will assist practitioners and beneficiaries in determining the best management strategy for patients with suspected AD, based on the results of amyloid PET imaging. We are eager to see new and greater published evidence that amyloid PET could help resolve other such narrowly defined and clinically difficult differential diagnoses, where use of the scan may prove to offer tangible benefits to the patient. Health outcomes of interest, again, include, but are not limited to, any of the following: avoiding inappropriate and potentially harmful medications; avoiding futile or burdensome treatments or tests; improving, or slowing the decline of, quality of life; and survival.

Recommendations by expert consensus panels

Comment
Numerous commenters stated we should accept the recommendations of the AIT (Amyloid Imaging Taskforce) consensus panel regarding the appropriate use of amyloid PET.

Response
The persuasiveness of expert opinion is constrained by the available evidence. Depending on the evidence, expert opinion may vary from conjectural to conclusive. While we recognize and respect the expertise of the AIT panelists, we believe that significant questions still remain open, and that CED can help develop the right studies to answer them.

Furthermore, we also recognize the expertise of another relevant expert consensus panel that we convened on January 30, 2013 – the MEDCAC (Medicare Evidence Development & Coverage Advisory Committee). As noted earlier, the MEDCAC proceeding is available on the CMS website and we refer the reader there for a more detailed account. The MEDCAC:

- *includes experts not only on the clinical subject at hand, but also on biostatistics, epidemiology and ethics; and it taps experts from various clinical disciplines – cardiology, surgery, internal medicine – to broaden perspectives on, and experience with, evidence development more generally;*
- *is not sponsored by industry or any particular organization; and*
- *includes external expert speakers who provide transparent and critical views during deliberations.*
- *conducts its deliberations in a public forum.*

A key question for the MEDCAC panel was: How confident are you that there is adequate evidence to determine whether amyloid PET imaging of brain beta amyloid changes health outcomes (improved, equivalent or worsened) in patients who display early symptoms or signs of cognitive dysfunction? The mean score of voting panel members was 2.17 (on a scale of 1 to 5, where 1 represents "low confidence," 5 represents "high confidence," and 3 represents "intermediate confidence").

Although the MEDCAC did not find sufficient evidence for CMS to support outright coverage of amyloid PET, this comment by one guest panel member – "coverage with evidence development would help fill in a lot of very substantial questions" – echoed comments by multiple panel members (See part 00279 lines 10 – 20 of the MEDCAC transcript available at http://www.cms.gov/Regulations-and-Guidance/Guidance/FACA/Downloads/id66d.pdf). We note that the MEDCAC panel does not actually vote on whether they think CMS should pursue CED.

The MEDCAC also considered the recommendations of the AIT and others during its deliberations. These two credible expert panels – the AIT and the MEDCAC – produced differing consensuses. This highlights the limitation of consensus panels: if you change panel members, you might well change the consensus. That's why, in the well-established process of scientific evaluation, evidence must be evaluated to determine the strength of the consensus opinion (see Appendix A).

As for the AIT, we acknowledge the difficulty in crafting recommendations in light of the limitations of the currently available evidence. We have recognized numerous points the AIT makes and have included those in this decision where appropriate. This includes the AIT July 2013 update that dementia specialists are better equipped to order such scans than other types of physicians.

We continue to believe based on our review of the published, peer-reviewed medical literature that the evidence gaps for amyloid PET, and AD biomarkers generally, as noted in the AIT's publication as well as in the 2011 NIA-AA series of guidelines, are consistent with the current CMS decision for CED (see our discussion of biomarkers in the Background and Analysis sections). For example, the AIT does not identify objectively-defined subpopulations of patients with cognitive impairment for which the scan (alone or combined with other tests) may be more or less appropriate. Yet there are many subtypes of MCI, and some (e.g., amnestic MCI) may be more relevant than others. Furthermore, there is evidence that the same level of amyloid burden detected by a scan may mean something very different in say, a 66 year-old compared to an 86 year-old (e.g., Le Couteur 2013, Laforce 2011). Yet the AIT is silent about such potentially important distinctions.

Widespread clinical use of the scan both in many types of patients with unexplained MCI, and to make a positive diagnosis of AD (despite insufficient evidence on the clinical meaning of a positive scan) has great potential to lead to over-diagnosis of AD. Such misdiagnosis of AD portends real harm to our beneficiaries (La Couteur 2013), and this must be considered in our coverage decision. Therefore, we believe CED is appropriate to encourage more studies that will benefit Medicare beneficiaries by answering some of these outstanding questions.

Diagnosis of Alzheimer's disease

Comment
Numerous commenters state or imply that an amyloid PET scan gives an accurate, positive diagnosis of Alzheimer's disease. The commenters further claim that such use is consistent with the FDA-approved labeling.

Response
We disagree with the commenters. An amyloid PET scan does not give an accurate positive diagnosis of AD, and a claim that it does is inconsistent with the FDA-approved label. Moreover, the FDA Medical Review of florbetapir PET notes that there are two pathophysiological hallmarks of AD which contribute to the gold-standard diagnosis – neurofibrillary tangles of the protein tau, and neuritic amyloid plaques and the amyloid PET scan detects only one of these. Finally, the scan does not distinguish between diffuse and neuritic amyloid plaques, and the significance of this lack of distinction remains unclear.

The positive diagnosis of AD requires not only both of these pathophysiological hallmarks, but also clinical documentation of progressive dementia, and exclusion of other diseases as the cause of the dementia. Because presence of neuritic amyloid plaques is one of the requirements for diagnosing AD, exclusion of the same excludes that diagnosis. Accordingly, the FDA-approved label states that a negative scan "is inconsistent with" a diagnosis of AD.

However, the presence of additional elements are required for the diagnosis of AD, and it is not clear that a certain threshold of amyloid definitively predicts these other elements. The FDA-approved label for Amyvid™ does not make any similar statement on the meaning of a positive scan. Moreover, the FDA notes that similar amyloid levels "may also be present in patients with other types of neurologic conditions as well as older people with normal cognition." In other words, a positive scan is not necessarily consistent with a diagnosis of AD. This conclusion is consistent with the 2011 NIA-AA consensus guidelines which state that although the presence of amyloid plaques is "necessary," it is not necessarily "sufficient," for diagnosing AD. More importantly, this conclusion – that the meaning of a positive scan is unclear – is consistent with the evidence that appears in published clinical studies discussed in the Evidence section.

Integrating the amyloid PET scan in diagnosing AD

Comment
Many commenters claim that dementia specialists could make an accurate positive diagnosis of AD when integrating the result of an amyloid PET scan.

Response

Prior to the publication of our proposed decision memorandum (PDM), the industry-sponsored Grundman (2012) study was the sole prospective study exploring the impact of scan results on physicians' diagnosis of AD, as well as their subsequent intended clinical management. The Grundman study design assumes that physicians can use the scan to make an accurate diagnosis, but does not demonstrate that they can (as there is no reference to a gold standard diagnosis of AD in the study); nor do any prior research studies demonstrate this.

Studies prior to Grundman 2012 do not report predictive values of the test for AD. The published data are limited to sensitivity (Sn) and specificity (Sp) values for the detection of amyloid alone. Yet these (Sn and Sp) are not the most clinically meaningful values for a diagnostic test. In the case of amyloid PET, while a "negative" test appears to minimize the risk of AD, the meaning of a "positive" test for any particular individual with unexplained cognitive impairment is unclear, and this again could lead to over-diagnosis of AD.

Positive and negative predictive values for AD are more useful than Sn and Sp – they can tell you the meaning of scan results for particular patients who belong to risk-stratified populations – but unfortunately no currently available study presents data on these values. Predictive values for AD mathematically include not only computations for Sn and Sp, but also the quantitative prevalence of disease in objectively-defined patient subpopulations or "risk pools." But the risk pools for AD are themselves not yet even defined in the literature. Because predictive values corresponding to a "positive" or "negative" test result vary, depending on the "risk pool" the patient objectively falls into, test results absent such values have no clinical meaning for an individual patient.

Since the publication of our PDM, studies similar to Grundman (e.g. Zannas 2013) have emerged, but present similar limitations and thus inclusive results. (Please see further discussion in Section VIII: CMS Analysis, below.)

FDA approval and CMS coverage.

Comment
Several commenters stated that CMS should automatically cover amyloid PET scans because the FDA approved the PET amyloid agent florbetapir (Amyvid™).

Response
FDA premarket review and CMS national coverage determinations differ significantly. Each process operates under different statutory standards and each asks different questions to meet its respective mandates. The FDA premarket review generally assesses the safety and effectiveness of these medical products. Even within FDA's

review processes, there are differences in types of evaluation depending upon the application under consideration (for example, premarket approval applications (PMAs) must meet standards different from premarket clearance (510(k)).

CMS serves a different function by providing health insurance to protect the nation's aged and disabled persons from the substantial burdens of illness. Under section 1862(a)(1) of the Act, CMS makes determinations regarding the coverage of specific items and services. In short, CMS must make multiple decisions: It must decide what items and services it can and should pay for; how it should accomplish the payment; and how much to pay.

CMS' evaluation of medical products depends on the type of request. For most NCDs, CMS evaluates whether a medical product or service is reasonable and necessary to diagnose or treat an illness or injury affecting the Medicare population. This evaluation includes review of appropriate outcomes data, such as whether the product provides improved, equivalent, or complementary health outcomes in the Medicare population as compared to alternative treatments or diagnostics already covered by the program. CMS may also evaluate medical product indications that have not been approved or cleared by FDA, so-called unapproved or off-label uses as found in 75 FR 57045, pages 57045 -57048 available at http://www.gpo.gov/fdsys/pkg/FR-2010-09-17/pdf/2010-23252.pdf

In the case of amyloid PET, FDA limited its evaluation essentially to the safety and efficacy of the radiopharmaceutical agent itself – florbetapir (Amyvid™) – that is used in the diagnostic imaging test. We discussed in previous responses to comments CMS statutory and regulatory authority for reviewing items and services for the purposes of Medicare coverage.

National Alzheimer's Plan Act (NAPA)

Comment
Some commenters claimed that CMS' proposed decision to cover amyloid PET under CED is inconsistent with NAPA. The commenters state that NAPA supports the coverage of amyloid PET scan as a diagnostic tool for AD.

Response
We believe covering amyloid PET scans under CED supports NAPA. In fact, NAPA's Strategy 2.B, "Ensure Timely and Accurate Diagnosis," was intended in part to further the NIH-CMS work on early detection using "assessment tools that can be used to detect cognitive impairment."

NAPA seeks the development of tools that can help ensure an "accurate diagnosis" of AD, but this particular test – amyloid PET – has not been demonstrated to do this. This finding is based on our independent evidence review,

and is consistent with the FDA-approved label and supporting FDA Medical Review of florbetapir, as well as technology assessments by other scientific bodies (TEC 2013, EMA 2013). We believe supporting amyloid PET under CED is the best decision for beneficiaries and practitioners. It is our belief that if the appropriate CED trials are completed CED will give information on where this new technology will be most useful in the diagnosis and treatment of AD. This NCD is consistent with and supportive of the NAPA goals in the following ways:

CED supports NAPA's strategy 1.B "Expand Research Aimed at Preventing and Treating Alzheimer's Disease." By CMS requiring CED for the coverage of amyloid PET scans we support any study that meets the criteria outlined in section I. As stated previously, we believe CED is necessary to ensure that beneficiaries are receiving the best care. Based on our review of the evidence, including MEDCAC input, we believe that amyloid PET will be available to Medicare beneficiaries in the context of clinical studies. It is CMS' belief that these studies are necessary to determine the best use of this diagnostic test.

This decision is consistent with NAPA's Strategy 1.C, "Accelerate Efforts to Identify Early and Presymptomatic Stages of Alzheimer's Disease." As discussed in the analysis and discussion section of this decision memorandum, CMS-approved studies done under CED should help better define subpopulations at risk for developing AD. This is an important question not only for this Medicare population and amyloid PET, but aligns with other ongoing research efforts (e.g. the large, multicenter, NIH-funded Alzheimer's Disease Neuroimaging Initiative).

Coverage with evidence development (CED)

Comment
Several commenters expressed concern that CED requirements would be too onerous and restrictive. Comments believed that CMS would only approve a single study. Some commenters also stated that no study could answer all of the questions posed in the proposed decision.

Response
We do not believe CED has to be onerous or unduly restrictive. There appear to be many misperceptions about how CED for amyloid PET could be designed. Under this NCD, there are potentially many studies that could meet the CED study criteria outlined in section I of this decision memorandum. CMS is not limited to approving only a single study; any number of studies can be approved as long as the study meets the NCD criteria. Further, a study does not have to attempt to answer all CED questions asked in this NCD, but could focus on any aspect of one or more of the questions (which appear in the Section I: Final Decision).

We stated that these studies should be prospective, randomized, and have autopsy as an endpoint, only when appropriate. The specific clinical study protocol is determined by the nature of the question being asked, and the likely sources of bias and confounding, and we will evaluate the protocols as they are submitted to determine which CED studies appropriately meet the criteria specified in the NCD. In addition, an approved study that meets the NCD criteria might synergize with, or piggy-back on, existing research efforts. Studies might be integrated, involving enrollment in companion or parallel studies. And they might employ newer methods such as "adaptive"

or "pragmatic" clinical trial designs.

Comment
Some commenters asked whether a study approved under this NCD could use newer analytical methods to churn on large amounts of cohesive clinical data gathered from use of the scan in "real patients" in "real clinical settings."

Response
We think it is possible. This would be consistent with the vision for the future of research articulated in chapter 6 of the Institute of Medicine's recent report, "Best Care at Lower Cost: The Path to Continuously Learning Health Care in America" (IOM 2012). We recognize that such data could help not only to close basic evidence gaps, but also to establish "generalizability" of the technology – evidence that beneficial outcomes could be sustained outside the clinical trial setting – as access rolls out to potentially hundreds of thousands of patients (as has actually happened in a prior CED). We encourage submission of clinical research designs that incorporate this vision.

Additional Evidence

Comment
Some commenters provided additional evidence that was not included in the bibliography of the proposed decision memo as sufficient for coverage of amyloid PET in dementia and neurodegenerative disease without CED.

Response
We appreciate the additional references provided in the public comments. We found the three published clinical trials on florbetapir relevant to this NCA and referenced them in the Evidence Section above and the Analysis Section.

Full text public comments without PHI can be viewed at http://www.cms.gov/medicare-coverage-database/details/nca-view-public-comments.aspx?NCAId=265

VIII. CMS Analysis

National coverage determinations (NCDs) are determinations by the Secretary of Health and Human Services ("the Secretary") of whether a particular item or service is covered nationally by Medicare, under §1869(f)(1)(B)

of the Act.

In order to be covered by Medicare, an item or service must fall within one or more benefit categories contained within Part A or Part B, and must not be otherwise excluded from coverage. Moreover, §1862(a)(1) of the Act in part states that, with limited exceptions, no payment may be made under Part A or part B for any expenses incurred for items or services:

- which are not reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member (§1862(a)(1)(A)) of the Act; or

- in the case of research conducted pursuant to section 1142, which is not reasonable and necessary to carry out the purposes of that section (§1862(a)(1)(E)) of the Act.

Section 1142 of the Act describes the authority of the AHRQ. Under section 1142, research may be conducted and supported on the outcomes, effectiveness, and appropriateness of health care services and procedures to identify the manner in which diseases, disorders, and other health conditions can be prevented, diagnosed, treated, and managed clinically.

Section 1862(a)(1)(E) of the Act allows Medicare to cover under CED certain items or services where additional data gathered in the context of clinical care would further clarify the impact of these items and services on the health of Medicare beneficiaries. The 2006 CED guidance document is available at www.cms.gov/determinationprocess/downloads/ced.pdf.

Questions:

a. Is the evidence adequate to conclude that PET Aβ imaging improves meaningful health outcomes in beneficiaries who display signs and symptoms of AD?

b. Is the evidence adequate to conclude that PET Aβ imaging results inform the treating physician's management of the beneficiary to improve meaningful health outcomes? Those outcomes may include reasonably considered beneficial therapeutic management or the avoidance of unnecessary, burdensome interventions.

In the following pages we note the limitations of specific published studies and ultimately our overall conclusions about the body of evidence.

Wong D, Rosenberg P, Zhou Y, Kumar A, Raymont V, Ravert H, et al. In Vivo Imaging of Amyloid Deposition in Alzheimer's Disease using the Novel Radioligand [18F]AV-45 (Florbetapir F18). J Nucl Med. 2010 Jun; 51(6): 913–920.

Wong and associates performed a prospective, open-label, multicenter, brain imaging study to test the pharmacokinetics of the tracer florbetapir and its safety for patients. They concluded that florbetapir PET imaging could discriminate between AD patients and healthy control subjects. But as noted by the authors, there were a number of limitations of the study. The study was small, and 6 of 32 (19%) of planned subjects were not included in the primary analysis due to technical failures during the scanning process. There was limited evaluation of imaging protocols and test efficacy. Also, due to the open-label study design, interpreters could have been biased in reporting results as they were not blinded. Despite its limitations, this study was a stepping stone to efficacy studies (e.g., Clark 2011 and 2012), which used autopsy, not clinical diagnosis, as the gold standard.

Camus V, Payoux P, Barré L, Desgranges B, Voisin T, Tauber C, et al. Using PET with 18F-AV-45 (florbetapir) to quantify brain amyloid load in a clinical environment. Eur J Nucl Med Mol Imaging. 2012 Apr;39(4):621-31. doi: 10.1007/s00259-011-2021-8. Epub 2012 Jan 18.

Camus and associates performed a prospective study and concluded that florbetapir PET was "a safe and suitable biomarker for AD that can be used routinely in a clinical environment." A number of limitations were noted by the authors, including a small sample size (n = 46), and selection bias due to the significantly older age in the MCI group than in the AD and healthy control groups. The authors were also concerned about the short half-day training sessions as well as the low specificity of the visual PET scan assessment, which could result in a high false positive rate, but suggested ways to improve these, such as improving and lengthening the duration of training, increasing the spatial resolution of tomographs, and adopting semiautomatic or automatic quantification methods or software. Finally, clinical diagnosis was used as the reference standard in this study, instead of the postmortem gold standard as used in other studies (Clark 2011, Clark 2012).

Clark CM, Sneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA. Use of Florbetapir PET for Imaging Aβ Pathology. JAMA 2011 Jan 19;305(3):275-83.

The 2011 study by Clark and associates concluded that overall Aβ burden assessed in vivo with florbetapir PET imaging correlates with histopathological assessments at autopsy. The authors acknowledged a number of limitations of the study. First, the sample size of the autopsy cohort was small (n = 35, of which six subjects were used to validate the protocol). Second, the non-autopsy cohort, used to determine the likelihood that a florbetapir PET image could falsely suggest the presence of amyloid, consisted of young, cognitively normal subjects – a distinctly different population from the end-of-life autopsy cohort.

Another limitation of the study was that amyloid scans were interpreted by three trained nuclear medicine physicians and the median of the three results was used in the analysis. The authors acknowledgement that this was "...a process not likely to be replicated in clinical settings" highlights the issue of external validity and the study's generalizability to the community setting. There was intentional selection bias as subjects chosen were those most likely to provide the shortest possible interval between imaging and histopathological evaluation (e.g., they were likely to die soon). Also, there were no standardized criteria for determining AD or MCI. An additional limitation not stated by the authors is that the use of a semi-quantitative categorical (0 - 4) ranking of florbetapir images, rather than a binary interpretation, limited evaluation of sensitivity and specificity.

Clark C, Pontecorvo M, Bench T, Bedell B, Coleman R, Doraiswamy P. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic Aβ plaques: a prospective cohort study. Lancet Neural 2012;11:669-78.

In the Clark 2011 study, 35 patients had postmortem exams. To this group an additional 24 new subjects with postmortem exam were added for the Clark 2012 study, yielding a total of 59 subjects, whose cognitive status during life ranged from normal to advanced dementia. The authors concluded that florbetapir PET could be used to distinguish patients with no or sparse amyloid plaques from those with moderate to frequent plaques.

Unlike in the 2011 study, all subjects in the 2012 study were end-of-life and underwent a postmortem examination, thus eliminating age cohort as a limitation. Although this issue was addressed, the authors noted several other limitations of the 2012 study. Subjects represented an end-of-life population that is generally older and sicker than those who would seek diagnosis for cognitive impairment in a community setting.

Also, the Clark 2011 study used the median interpretation of three trained nuclear medicine readers, while the Clark 2012 study used the majority interpretation of five trained nuclear medicine readers. This discrepancy (the change in measurement) is a potential violation of internal validity.

Another limitation pointed out by the authors was that both imaging and histopathological results were distributed bimodally, with few "borderline" cases. This raises the question of whether a lower sensitivity might have been obtained if more participants who had intermediate results had been involved. The authors suggested that additional studies would be needed to assess the frequency of such borderline scans, and their implications for performance characteristics of the test, in community settings and with more typical patients. Finally, the authors noted that the "clinical significance of amyloid burden as measured with florbetapir PET must be interpreted in the context of other relevant diagnostic information."

Fleisher AS, Chen K, Liu X, Roontiva A, Thiyyagura P, Ayutyanont N. Using Positron Emission Tomography and Florbetapir F 18 to Image Cortical Amyloid in Patients With Mild Cognitive Impairment or Dementia Due to Alzheimer Disease. Arch Neurol. 2011;68(11):1404-1411.

Fleischer and associates felt that their study demonstrated that florbetapir PET SUVRs were able to characterize Aβ levels in clinically probable AD, MCI, and older health control groups using continuous and binary measures of fibrillar Aβ burden. But the authors commented on a number of limitations of the study. First, they noted that although mean cortical SUVRs were higher in ApoE4 carriers compared with non-carriers, the proportion of florbetapir PET positivity between carriers and non-carriers did not reach statistical significance. They felt that the small sample size of ApoE4 carriers was probably the reason. Second, there were a lack of standardization for image acquisition, cerebral and reference ROIs, and cut-off thresholds. Third, there was cohort selection bias. Additionally, we note that this study does not use the postmortem gold standard for diagnosing AD; rather, SUVR data from the scans (with a certain cut-off value derived from a small sample in a prior autopsy study) are compared to presence of AD as diagnosed clinically.

Doraiswamy P, Sperling R, Coleman R, Johnson K, Reiman E, Davis, M. Amyloid-β_ assessed by florbetapir F 18 PET and 18-month cognitive decline: A multicenter study. Neurology 2012;79:1636–1644.

The goal of the study performed by Doraiswamy and associates was to evaluate the prognostic use of detecting Aβ pathology using florbetapir PET in subjects at risk for progressive cognitive decline. The authors concluded that florbetapir PET may help identify individuals at increased risk for progressive cognitive decline, but identified a number of limitations of the study. They noted that the lower-than-expected conversion rates among the Aβ positive patients (compared to prior PIB studies) could have been due to the low sample size as well as the short duration of the study. They also noted that subjects with MCI in this study were less impaired at baseline compared to subjects with MCI in the Alzheimer's Disease Neuroimaging Initiative (ADNI; another study assessing neuroimaging in patients with AD). This was felt likely due to differing entry criteria as well as selection bias. This study did not collect other biomarker data (e.g., ApoE4) and could not assess the relative utility of PET versus other biomarkers. Also, the reference standard for AD was clinical diagnosis, not the postmortem gold standard.

In this study a positive scan was determined by the majority read of three nuclear medicine physicians. As has been noted before, this may not be replicated in clinical settings. Finally, the authors believe that larger, "longitudinal PET and cognitive data may help clarify its prognostic role in the clinical setting, its ability to improve [diagnostic] confidence . . . and for subject enrichment of therapeutic trials in the early clinical and preclinical stages of AD."

Grundman M. Pontecorvo M, Salloway S, Doraiswamy P, Fleisher A, Sadowsky C, et al. Potential Impact of Amyloid Imaging on Diagnosis and Intended Management in Patients With Progressive Cognitive Decline. Alzheimer Dis Assoc Disord 2012;00:000–000.

Grundman and associates sought to demonstrate that the use of florbetapir PET scans altered self-reported physician diagnosis and increased their diagnostic confidence. The researchers felt that the study showed that treatment plans were modified after florbetapir imaging both for patients who were in the midst of their workup and for those with a complete workup. But the study had a number of limitations, many noted by the authors. First, the study recorded intended change in management, but it did not evaluate actual change in management.

Second, there was intentional selection bias. Patients were subjectively selected for "specific attributes," and while they likely overlap populations of diagnostic interest, these populations were not defined, limiting the study's generalizability. Third, no postmortem gold standard was used. Finally, because expert nuclear medicine specialists over-read the scans, and the study was carried out in a clinical trial setting, where participating physicians were largely experts experienced in the diagnosis and/or care of AD patients, it may be difficult to duplicate the study's findings in a general setting.

Landau S, Mintun MD, Joshi A, Koeppe R, Petersen R, Aisen P, et al. Amyloid Deposition, Hypometabolism, and Longitudinal Cognitive Decline. Ann Neurol 2012;72:578–586.

Landau and associates concluded that a positive PET Aβ test in both the normal and late MCI patients (LMCI) groups was associated with ongoing decline, though in normal subjects, decline was more closely linked to amyloid status, whereas in LMCI, decline was more closely linked to hypometabolism. The researchers also acknowledge some limitations of the study. First, the associations with longitudinal cognitive decline are retrospective rather than predictive, as the florbetapir and FDG measurements were collected at the end of the follow-up period. Second, the distributions of FDG PET and florbetapir differ: florbetapir was more bimodal than FDG PET. Thus the use of dichotomous predictor variables may more accurately reflect the underlying characteristics of the florbetapir distribution. Additionally, we note that the reference standard for AD was clinical diagnosis, not the postmortem gold standard. Finally, cross-sectional data was used to show the relationships between Aβ (measured with florbetapir), hypometabolism (measured with FDG PET), and cognitive performance – and such cross-sectional designs are prone to ecological fallacy.

Three additional studies were submitted during the second comment period (Johnson et al. 2012, Zannas et al. 2013, Choi et al. 2012). Though Johnson and colleagues were able to demonstrate a lower frequency of amyloid burden as they compared AD patients to MCI patient and healthy controls, their results were consistently lower when compared to previous studies using other PET amyloid tracers. The authors listed a number of possible explanations including selection criteria as well as image reader variability. These factors could negate the findings of the study, thus calling into question its validity.

Zannas and associates' objective was to determine if clinical management changed based on the results of the florbetapir PET image. Though this was a small case series study, when results were obtained, it revealed that there were inconsistencies in management based on results: some patients who tested negative for beta amyloid were kept on Alzheimer's medications, while some patients who were thought to have depression or MCI who tested positive for beta amyloid plaque, were never treated with Alzheimer's medications.

Choi and colleagues evaluated the ability of florbetapir F18 to identify and quantify amyloid aggregates in autopsied brain tissue. Their study involved the use of brain specimens taken from a range of patients including those with AD, vascular dementia, progressive supranuclear palsy, and normal subjects. Though they were able to demonstrate a strong correlation between the density of invitro florbetapir F18 in patients with late-life cognitive impairment, they provided little information on the degree of correlation in patients with conditions other than AD.

While the addition of these articles served to round out the currently available evidence base for beta amyloid PET imaging in dementia and neurodegenerative disease, it did not change our final decision. The evidence is insufficient to conclude that beta amyloid PET is reasonable and necessary; it is sufficient for coverage under CED.

A. Discussion

The clinical usefulness of AD testing, including PET Aβ imaging, is limited by the current absence of therapies that meaningfully prevent, stabilize or reverse the progressive course of the condition. This leads to a corresponding limitation in the evidence that might be brought to bear on the impact of testing on meaningful clinical outcomes. Thus we have no evidence that PET Aβ imaging leads through informed physician management to the prevention, stabilization or reversal of AD.

That said, we recognize that there are other incurable conditions, for example, some cancers, where the prudent use of diagnostic testing can meaningfully inform physician decision-making and patient management. In the case of cancer, a positive imaging test that leads to a definitive diagnosis by biopsy could reasonably guide physician management toward palliative goals that are acceptable to the patient and consistent with scientific evidence. Thus we are open to reasoned, evidence-based arguments that would identify benefit that may be achieved by the avoidance of burdensome or hazardous interventions that will not ultimately help the beneficiary.

The expectation that a medical test inform physician management is well established. It is also consistent with federal regulation at 42 C.F.R. §410.32(a), which requires that:

". . . diagnostic tests must be ordered by the physician who. . . treats a beneficiary for a specific medical problem and who uses the results in the management of the beneficiary's specific medical problem."

Accordingly, we ask: Does the test lead the physician to reconsider the pre-test treatment plan and make appropriate modifications in light of the test result? What evidence is available to support assertions of benefit from testing?

We recognize that the medical literature often describes test characteristics and has not consistently considered the impact of testing on physician decision making and patient health outcomes, such as mortality, morbidity or reduction of invasive testing. However, we believe that evidence of improved health outcomes is more persuasive than descriptions of test characteristics. (Please see Appendix A: General Methodological Principles of Study Design).

In evaluating diagnostic tests, Mol (2003) states: "Whether or not patients are better off from undergoing a diagnostic test will depend on how test information is used to guide subsequent decisions on starting, stopping or modifying treatment. Consequently, the practical value of a diagnostic test can only be assessed by taking into account subsequent health outcomes." For example, we recognize that if a particular diagnostic test result can be shown to change patient management, and if other evidence has confidently demonstrated that those patient management changes improve health outcomes, then a combination of such sources of evidence may be sufficient to demonstrate positive health outcomes from the diagnostic test. We also note for completeness that we are unaware of any claims that florbetapir administration itself exerts any direct therapeutic effect.

Prior to the posting of the proposed decision memo, the industry-sponsored Grundman (2012) study was the sole prospective study attempting to measure the impact of scan results on intended clinical decision making and management. Since that time, other studies (e.g., Zannas 2013) have tried to explore this relationship but have been met with mixed results (see above response to public comments).

The Grundman 2012 authors opine (and we agree), that "[a] remaining question is whether clinical care that includes amyloid imaging will translate into better outcomes." Grundman also states that "[a]dditional longitudinal studies would be required, however, to explicitly quantify the relationship between amyloid imaging and patient outcomes." Our overall assessment of Grundman 2012 is that it is a good hypothesis-generating study. It raises the possibility that PET Aβ scans could improve medication management, and reduce other testing, but does not establish these conclusively. Also, its lack of objective criteria, both for patient selection and for changes in decision-making and management, markedly limit its ability to inform community practice outside of a clinical study. We now discuss this assessment in more detail.

We mentioned earlier that virtually all subjects in Grundman 2012 who had a positive amyloid scan ended up being given a clinical diagnosis of AD by the physicians (112/113), while virtually all patients who had a negative amyloid scan ended up being given a clinical diagnosis other than AD (115/116). While we know that these diagnostic decisions were made, we have no information on whether they were ultimately appropriate, because there was no longitudinal follow-up to a postmortem gold standard diagnosis.

The diagnostic conclusions the physicians reached, based on their own unexplained judgment, would be consistent with very high negative and positive predictive values of the test. This could stem from a combination of factors: (1) the physicians' acceptance of the high sensitivity and specificity for detecting amyloid in human brain, reported for the end-of-life population in Clark 2012; (2) an assumption that these performance characteristics apply to their current patients, who represent various (but undefined) subpopulations with cognitive impairment, but certainly not an end-of-life population as in Clark; and (3) that cognitive impairment plus a positive amyloid scan equals AD (e.g., a clear preference for one of at least two plausible hypothesis about the role of amyloid in AD development).

There is no empirical evidence internal to this study (or in prior studies) to support or explain the phenomenon of clinical decision making observed. This study appears to assume – but does not prove – such high negative and positive predictive values of the test (nor are these demonstrated in other studies, to our knowledge). This

assumption may be implied by the authors themselves: "as AD is responsible for the large majority of cases of dementia with amyloid pathology (Barker 2002) physicians [in the study] may also be using their knowledge of the known clinical-pathologic correlations in making their diagnostic determinations" (Grundman 2012). These "correlations" have to do with the role of amyloid in AD; however, competing hypotheses of this role are vigorously debated in the literature.

An additional question about the decision making of the study physicians arises because their intended management does not always align with their revised, post-scan diagnosis. For instance, while 99% of subjects with a negative scan were given a final diagnosis of something other than AD, as pointed out by a MEDCAC panel member, approximately half of patients with a negative scan who were planned to get AD medications were still to receive them despite the negative scan (Grundman 2012, Table 5). Patients in the other half in this pool were no longer planned to get such medications as a result of the negative scan. The study did not explicitly discuss the reasons for these decisions, let alone quantitatively assess the likely harms supposedly avoided. As we discuss in more detail later, harm potentially exists if patients with FTD are mistakenly diagnosed with AD and placed on such medications.

The underlying design of the study produces an apparent circular logic: the scan is meaningful because its results alter diagnosis and management; but it does so appropriately only if one assumes its results are meaningful. This logic appears in other parts of the paper's discussion section, for example:

"Changes in diagnosis occurred almost equally for subjects who had already undergone extensive evaluations (group A) and those in the middle of an ongoing diagnostic work-up (group B), arguing that in these patients, florbetapir PET scans provided potentially valuable information that seemed independent of other commonly performed diagnostic tests."

In other words, because changes in diagnosis were (subjectively) made based on the scans, the scans must have provided valuable information.

As some MEDCAC panel members commented, the study "…raises more questions than it answers." But this gets to its real value: it is a good hypothesis-generating study. It is possible that amyloid scans will someday meaningfully alter "the pattern of medication use, additional diagnostic testing, referral to AD resources, and clinical trial consideration." We address the logic of, and evidence for, many of these possibilities when discussing "the value of a negative scan" later in this DM.

With respect specifically to the Grundman 2012 finding of decreased utilization of other tests, such as MRI and/or CT, we view this as a plausible hypothesis but one that has yet to be demonstrated. It is equally plausible that, even if PET Aβ imaging were widely available, most patients in the real world would continue to get MRIs and/or CTs anyway (to rule out other causes of, or contributors to, cognitive impairment, such as cerebrovascular disease, intracranial hemorrhage, and normal pressure hydrocephalus), ordered by other physicians, before the patient is evaluated by a dementia specialist. Perhaps more importantly, at least from a beneficiary's perspective,

given that radiation exposure in the elderly is less harmful than in younger populations, inappropriate imaging likely represents a much lower direct harm than being inappropriately placed on toxic medications.

Finally, there was no evaluation in Grundman 2012 of when amyloid imaging might be used instead of, or in combination with, other studies – or if it should be used at all – for particular patients. This foreshadows issues we will explore in detail later: what are the risk pools, how are they defined, what is the prevalence of disease in them, and what combination of tests are most appropriate for diagnosing patients in those pools? Answers to these questions are what are needed to define evidence-based coverage criteria for any given test – including PET Aβ imaging.

The meaning of a negative and positive scan

The Grundman 2012 study aside, there are other arguments and supporting evidence, presented by experts writing in the medical literature, speaking at the MEDCAC meeting, or in the NCD request itself, that are germane to the central questions of this NCD.

The core argument from many commenters is that although the gold standard for diagnosis of AD remains postmortem, and there is no cure or effective treatment for AD, there is value nonetheless to patient outcomes, directly or indirectly, in a negative scan. A negative study is "inconsistent with the diagnosis of AD," as stated in the FDA-approved label, and this information could be useful to:

- effectively exclude AD in most patients, and therefore avoid potentially harmful and burdensome treatments for those who, if not for the scan, might be mistakenly diagnosed with AD;
- hasten clinical work up for a correct diagnosis that perhaps could be effectively treated; and
- improve the quality and efficiency of research to develop better treatments for AD, by selecting patients for clinical trials based on biological, rather than just clinical and epidemiological, factors.

Additionally, it is argued, there is a "value of knowing" that is not only intrinsic, but also directly linked to access to health care services and support which materially and substantially impact the patient's quality of life. As discussed above, we consider both avoidance of harm and quality of life to be legitimate health outcomes, hence germane to national coverage decisions.

We examine the logic of, and evidence for, these arguments, as they connect to key sub-questions generated in part by MEDCAC panel discussions:

1. What is the meaning of a negative and positive amyloid scan for a patient? Does this depend on what risk pool, or subpopulation, a patient falls into? Have these been identified, and do they include Medicare beneficiaries?

2. In what specific scenarios might the test meaningfully change patient management to improve health outcomes? Would such outcomes likely be sustainable outside the expert clinical trial setting, in general community practice?

3. Do evidence gaps exist, and if so, what clinical studies could be done to confidently close those gaps?

Assessing performance characteristics of the scan

Fundamentally, a physician orders a test in an attempt to identify the "true state" of the patient. Does the patient have the disease or not? If the true state is known, there is no clinical need for testing on the same question. Since the physician here is trying to determine whether or not the patient has AD, predictive values are more clinically relevant than sensitivity and specificity.

Both sensitivity and specificity are based on prior knowledge of the patient's true state, diseased or non-diseased. Sensitivity asks what portion of diseased persons will be identified as positive. Specificity asks what portion of non-diseased persons will be identified as negative. Sensitivity and specificity are test characteristics that vary depending on the chosen cut-off between positive and negative. One can set the test cut-off point according to the desires of the user since there are inherent methodological tradeoffs between high sensitivity and high specificity, and thus one must consider the risks of having more false positive or false negative results. A receiver operating characteristic (ROC) curve is customarily used to illustrate this tradeoff.

Data on the sensitivity and specificity of PET Aβ imaging are prominent results in virtually all relevant clinical trials. Yet when clinical trials use different reference standards for determining these values, they mean different things and so the studies are not comparable. For instance, a study could compare (1) an F18 imaging agent to PIB in detecting amyloid plaque burden in living brain; (2) a given imaging agent to autopsy findings of amyloid burden; (3) results of an imaging agent to the clinical diagnosis of AD, or of MCI; or (4) results of an imaging agent to the gold-standard diagnosis of AD, which requires both (a) the presence of moderate to frequent Aβ plaques and neurofibrillary tangles on autopsy, and (b) clinical documentation of progressive dementia during life.

While the last comparison would be most informative, it has not to our knowledge ever actually been studied. The apparent purpose of studies undertaken for the FDA, which led to the publications of Clark 2011 and 2012, was never to diagnosis AD per se, but to assess the ability of florbetapir to identify amyloid plaque in human brain. Of interest, an initial plan to simply compare florbetapir to PIB PET Aβ imaging was rejected (by an FDA advisory committee) in favor of using autopsy findings as the appropriate reference standard – again not for diagnosis of AD, but for presence of amyloid in the brain.

Other studies that use clinical diagnosis as the reference standard are less useful as the reason amyloid imaging

is being investigated in the first place is precisely because of known, systematic inaccuracies in the clinical diagnosis of AD.

On reviewing Clark 2012, along with the prior studies that led up to it, we do not doubt that amyloid imaging is safe in humans, and "efficacious" for detecting amyloid burden in the end-of-life population in which it was tested (consistent with FDA findings). However, the critique by Laforce and Rabinovici of PIB PET amyloid imaging is apposite to florbetapir PET imaging: "Technical and patient factors that could lead to false positives and false negatives are not clear. PIB binds to both diffuse and neuritic plaques (Lockhart 2007) (the latter being more common in normal aging), and the relative contribution of each to the in vivo signal has not been determined" (Laforce 2011).

Finally, there are a total of 59 subjects (with specificity determined by a subset of 20 subjects) imaged with a PET amyloid imaging agent that is both clinically-relevant and FDA-approved (florbetapir), who have autopsy correlation, representing an end-of-life population only. This is not enough to confidently determine sensitivity and specificity (and test and patient factors that could alter these) let alone, as discussed next, the positive and negative predictive values of the test, in different patient subpopulations.

Lack of positive and negative predictive values for the scan

In comparison to sensitivity and specificity, positive and negative predictive values (PPVs and NPVs) address a more clinically relevant question. In patients whose true states are unknown, what portion of those with a positive test actually have the disease? What portion of those with a negative test do not have disease? These predictive values depend on the prevalence of the disease in the tested population (with prevalence being the proportion of persons in a defined population at a given point in time who have the disease). If a test is applied to both a high risk and a low risk population, a positive result is more likely to be a true positive in the high risk population. Conversely, a negative result is more likely to be a true negative in a low risk population (Coulthard 2007). Further discussion and examples are available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2083733/.

When referencing the sensitivity and specificity from Clark 2012, the follow up study by Grundman 2012 (discussed at length above) said "florbetapir PET has been shown to be > 90% sensitive and specific for identifying subjects with moderate to frequent neuritic plaques, as assessed at autopsy within 1 year of scan." Grundman does not quote the "100% specificity" reported by Clark.

Consider for the sake of illustration that – although this has never been demonstrated – the impact of the test in wider community practice (not just in the expert clinical trial setting) has an impressive 90% sensitivity and 90% specificity (using the postmortem gold standard as the reference). What does this mean for a particular patient who gets the test? As discussed above, this depends on the PPVs and NPVs of the test. But these values vary, depending on what defined risk pool the patient falls into, and what prevalence of AD exists in that pool.

Now consider that pool to be the general older American population, which has a prevalence of AD of approximately 12.5% (NIA 2013). The above 90% values for sensitivity and specificity would generate a > 98% NPV (the chance the patient does not have the disease if the test is negative) but a PPV of only about 56% (the chance the patient has the disease if the test is positive). In this case, a negative scan virtually excludes AD, echoing the FDA-approved label that "a negative scan is inconsistent with the diagnosis of AD." But the meaning of a positive scan is unclear (also consistent with the FDA-approved label that a positive scan does not confirm the diagnosis of AD or other disease).

There has been extensive research for other diseases to define patient subpopulations at risk (risk pools) and their associated prevalence of disease (e.g., for thromboembolic disease, to evaluate the usefulness of diagnostic tests, such as a D-dimer). We note a similar path is emerging in AD-related research on the subtypes of MCI (discussed later). Other factors (e.g., age, genetic predisposition, comorbidities, cognitive reserve) complicate any subtyping schemata. As Laforce and Rabinovici argue: "not yet established is whether the threshold of [amyloid]-positivity should be adjusted based on demographic factors such as age (as is done when scoring plaques at autopsy) (Braak 1999) or genetic variables such as the ApoE4 genotype. Significantly, the relationship between amyloid and dementia is weaker in older versus younger individuals (Savva 2009). The positive predictive value of a positive amyloid scan in determining the cause of the dementia will therefore be lower in older individuals [e.g., the Medicare population]. In general, amyloid PET will be more useful in ruling out (given the high sensitivity to pathology) than in ruling in AD as the cause of dementia, since the detection of amyloid may be incidental or secondary to a primary, non-Aβ pathology in some cases . . ." (Laforce 2011).

Laforce's last point brings up another issue: throughout our above discussion of statistical prediction we have been regarding performance characteristics of the test with respect to the presence of AD itself. However, these performance values apply only to the presence of amyloid in human brain, and that may not equate to AD per se. While there are competing views of what the presence of a given threshold of amyloid in human brain means, a leading hypothesis acknowledges that while amyloid plaques may be virtually necessary, they may not be sufficient, as either a trigger for or marker of the progressive dementia of AD.

The implications of a negative scan

The first part of that equation – that presence of amyloid plaques is virtually necessary – reflects the FDA-approved label that "a negative scan is inconsistent with the diagnosis of AD," and is not the question that is before us in this NCD. (Note however that if the scan is performed too early, and is negative, this does not exclude subsequent amyloid plaque formation that later does reach a threshold for positivity – although this is unlikely to apply to those aged 65 and older, who comprise the vast majority (83%) of Medicare beneficiaries

(http://www.statehealthfacts.org/comparetable.jsp?ind=294&cat=6&sort=431, accessed April 22, 2013)).

A question that *is* before us in this NCD is, given that a negative PET amyloid scan could virtually exclude AD in many patients, what is its clinical utility? We now turn to the arguments that "the value is in a negative scan."

First, ***could a negative scan excluding AD avoid harm that would have otherwise occurred if patients were misdiagnosed with AD and given medications for symptoms that were in fact caused by other disease(s)?*** We have already discussed that we consider avoidance of harm to be an informative health outcome. Medications typically given to AD patients, such as memantine and cholinesterase inhibitors, are not AD medications per se. They do not prevent, cure or modify the disease process of AD or, for that matter, any known disease. They may offer moderate, temporary improvement to patients with cognitive and/or neuropsychiatric symptoms stemming from a variety of etiologies (TEC 2013). For instance, they have demonstrated efficacy in dementia with Lewy bodies (DLB) (Graff-Radford 2012); and are perhaps even more effective for DLB than for AD (Samuel 2000). Cholinesterase inhibitors may improve symptoms in Huntington's disease and possibly vascular dementia (de Tommaso 2007, Kavirajan 2007, TEC 2013). In these particular cases, no additional harm appears to result from a misdiagnosis that places patients on such dementia medications.

It is primarily in differentiating frontotemporal dementia (FTD) and AD that potential for harm appears to exist (and this indeed was the example presented in the NCD request). Cholinesterase inhibitors have been shown to exacerbate symptoms in some patients with FTD, and use of memantine has correlated with greater functional and cognitive decline (TEC 2013, Kertesz 2008, Mendez 2007, Moretti 2004, Boxer 2012).

The differential of FTD and AD can be clinically challenging. Both are characterized by progressive dementia. AD typically begins with memory loss; FTD, with behavioral and language disturbances. AD is more likely in older persons; FTD, in younger. However, there is significant overlap such that patients with histopathology of FTD have often met the diagnostic criteria for AD during life (Varma 1999), and 10%-40% of patients diagnosed clinically with FTD are found to have AD by postmortem gold standard (Rabinovici 2011). Complicating the issue is that some individuals can have co-morbid disease.

CMS covered FDG PET in 2004 for use specifically in the differential of FTD and AD. The two diseases have relatively distinct patterns of hypometabolism on PET (predominantly temporoparietal in AD, and frontal and anterotemporal in FTD).

In a study of 45 subjects, Foster (2007) demonstrated that use of FDG PET in clinical assessment was more reliable and accurate in distinguishing FTD from AD than clinical assessment alone. Rabinovici 2011 was a head-to-head comparison of PIB amyloid versus FDG PET in the differential of AD and FTLD. Although there was a total of 110 subjects, only a small sample size (n = 22) had histopathology. For these 22 subjects, overall classification accuracy (using two visual and one quantitative techniques) was 97% for PIB (n = 12) and 87% for FDG (n = 10).

Second, ***could a negative amyloid scan improve the quality and efficiency of clinical trials to develop effective treatments for AD?*** The argument, articulated here by Laforce and Rabinovici but made by many, is that amyloid imaging could "improve clinical trial design by enrolling patients based on biological, rather than clinical, phenotype. This is a necessary first step for the development and testing of disease-specific therapies" (Laforce 2011). Laforce continues that "initial studies have found that requiring a positive molecular biomarker for inclusion will render AD clinical trials more efficient . . . ." Although some evidence suggests otherwise, most evidence, including similar use of diagnostics in trials for other diseases, and a recent European decision approving amyloid imaging for enrichment of clinical trials, suggests a promising role for amyloid imaging for this purpose (EMA 2011, Pearson 2012).

Third, ***could a negative scan also hasten the work up for other, potentially treatable diseases?*** Plausible arguments are made either way, but all lack conclusive evidence. An argument for answering "No" to this question is this. If you had a convincing clinical picture of AD, many experts agree the scan would not be needed (e.g., Johnson 2013). How physician concerns about liability would impact real-world decisions whether to get the test, if it were available, is an open question however.

Conversely, if you did not have such a convincing clinical picture, work up to exclude other, diagnosable and potentially treatable diseases should proceed anyway (as it would if an amyloid scan were negative). The unavailability of an amyloid scan does not change that logic.

An argument for answering "Yes" to this question derives from examples such as this (raised by a speaker at the MEDCAC): A patient with progressive cognitive impairment and a differential diagnosis of normal pressure hydrocephalus (NPH) versus AD was referred to a surgeon for a possible shunt, but the surgeon declined because the patient did not fit the typical criteria for NPH. The patient was thus given a presumptive diagnosis of AD. His cognitive impairment persisted for twelve years, after which he finally received an Amyvid scan, which was negative. See this example in its entirety from *part 00109 -line 9* to *part 00110- line 10* of the MEDCAC transcript found at http://www.cms.gov/Regulations-and-Guidance/Guidance/FACA/Downloads/id66d.pdf.

The evidence for such arguments, either way, is of limited persuasiveness, based almost entirely on clinical vignettes and case studies, which carry unmitigated risk of methodological bias and confounding, rather than on clinical trials.

The implications of a positive scan

Perhaps a greater challenge is that while a negative scan might be helpful or even just reassuring for many patients, if the scan happens to be positive for those very same patients, the meaning of this result is unclear, certainly much less clear than that of a negative scan.

McEvoy and Brewer (2012) present the following clinical scenario and analysis:

Given the high prevalence of AD and its devastating effects, there is a lot of anxiety among older individuals about developing this disorder, especially among those with relatives with the disease. Thus, minor slips in memory function, including those that are normal in healthy aging, can become an obsession, generating a vicious cycle in which a patient notices a slip in memory, becomes attuned to additional slips, and develops increasing anxiety about memory function, which itself may interfere with memory and memory testing. It is not

uncommon to see cognitively unimpaired and, often, highly educated elderly patients presenting to the physician's office debilitated by fear that they are developing dementia . . .

Imagine, then, adding to this patient's clinical evaluation an assessment for amyloid pathology, with the hope that the patient will be one of the approximate 35-85% (dependent on age (Rowe 2010)) of cognitively healthy older individuals with a negative test. A negative test would relieve the patient's fear of AD, since an absence of amyloid is inconsistent with a diagnosis of AD. However, this would not rule out other neurodegenerative disorders. A positive test would be even harder to interpret, since 20-65% (dependent on age) of cognitively healthy individuals can be expected to test positive for amyloid (Rowe 2010).

Given that elevated amyloid deposition is thought to precede development of cognitive impairment by more than a decade, we believe that findings of amyloid positivity in the absence of objective cognitive impairment would be irrelevant, and possibly harmful to the well-being of the patient. Even if future research were to demonstrate that all healthy older individuals with elevated amyloid eventually develop AD, an amyloid test cannot yet tell whether the patient will decline in the coming year or even in the coming decade; a positive test gives no indication of the phase of this slowly developing disease. For elderly patients especially, a warning sign loses all relevance if it can only suggest that cognitive impairment is likely to develop sometime in the next 10-20 years.

We agree with the authors' reasoning, cited evidence, and concerns about real-world clinical impact. This concern is especially relevant given statements by some experts (including at the MEDCAC meeting) that they intend to use an amyloid scan in clinical practice to help make a positive diagnosis of AD (despite lack of empirical evidence of when and how to do this, and despite the inconsistency of such use with the FDA-approved label). However, we note that McEvoy and Brewer's argument is explicitly about "findings of amyloid positivity in the absence of objective cognitive impairment." Whether documentation of cognitive impairment opens a window for appropriate use is a topic we will return to later. McEvoy's discussion is a good segue into the next issue, on the "value of knowing."

The "value of knowing"

Expert speakers at the MEDCAC, public commentary, and numerous discussions in the literature have brought up the value to individuals and their families of definitively knowing they have AD. Patients were even described by clinicians as "being relieved" by knowing they had a diagnosis of AD. However, there are several limitations of this argument (including but not limited to the clinical meaning of a positive scan); we address these one by one.

First, the argument is clearly not generalizable. Given that there is no cure or effective treatment for AD, many do not "want to know." In an international poll, the question (number 26) was asked: "In the future, a medical test might become available that would tell people before they had symptoms whether they will get Alzheimer's disease in the future. If such a test became available, how likely do you think it is that you would get the test—very likely, somewhat likely, not too likely, or not at all likely?" In the U.S., only 29.5% responded "very likely," while an additional 34.6% responded "somewhat likely." Other, more recent polls have also made clear that answers to various related questions are variable.

We recognize that conclusions drawn from public opinion polls, even those done with statistically robust polling methodologies, are of questionable evidentiary value for fundamental questions about disease. At most we can conclude from them that patient and family responses to a diagnosis of AD are likely to vary, and we will need to rely on future empiric evidence to know this with greater certainty.

More importantly, implicit to the question is the assumption that the test is definitive. These poll responses cannot apply to PET amyloid scans as, again, the meaning of a positive scan is unclear. The complexity and uncertainty surrounding the science renders polling difficult. There are no polls, to our knowledge, where subjects were asked: "Would you want this scan if there is an X-Y% chance that you will be misdiagnosed with AD, based on the risk pool you fall into – which is itself unknowable as the criteria for such pools have yet to be clearly demonstrated – and by the way, here is the potential impact of being misdiagnosed with AD . . ."

Ultimately, we recognize that patients and families may make different decisions when a hypothetical scenario of disease becomes a real one. We can anticipate that these decisions will reflect the diversity of personal and cultural values in the population, including some, e.g., religious beliefs and prior family experience with illness, that are not readily studied in randomized clinical trials. Indeed we can envision the possibility that five different families will arrive at five different decisions, and that by some measure all five might be judged "appropriate" by various persons. Seeking answers to such questions will extend beyond the traditional boundaries of evidence based health insurance coverage. That does not diminish their ultimate importance to our beneficiaries, but it does alert us of the challenges of applying an evidence based review paradigm in this context.

Prognosis versus diagnosis

Doraiswamy 2012 connects to this "value of knowing" argument. As discussed previously, a key finding of this study was that, in the MCI population, 29% of those with positive scans, compared to 10% of those with negative scans, converted to clinically diagnosed AD. Some experts, including at the MEDCAC meeting, pointing to these data (and prior supporting studies), argue that patients with a positive scan and symptoms of MCI have AD, and it is just a matter of time before this manifests (Aisen MEDCAC presentation, Sperling 2011, Hardy 1991, 1992). So, along this line of thinking, why do roughly 33% of cognitively normal older individuals have significant amounts of amyloid in their brain? Because it is an indolent process. As with prostate cancer, many of these individuals will die with, rather than of, the disease.

A competing hypothesis is that "Aβ accumulation is necessary but not sufficient to produce the clinical manifestations of AD. It is likely that the cognitive decline would occur only in the setting of Aβ accumulation plus synaptic dysfunction and/or neurodegeneration" (Sperling 2011). Amyloid accumulation appears to plateau, and downstream neuronal lesions are required, and indeed better correlate with clinical severity of disease than does amyloid. In this competing view, while some of the infamous 33% with high Aβ and normal cognition may actually have AD but have just never manifested symptoms – and maybe never will in their lifetime – some, perhaps even the majority, may have simply not been "tipped" by other, distinct, downstream lesions that are necessary for AD, and perhaps never would be even if they lived longer. That is, they do not, and never will, have the disease.

In this light, the NIA-AA guideline authors conclude (and we agree) that "at this point, it remains unclear whether it is meaningful or feasible to make the distinction between Aβ as a risk factor for developing the clinical syndrome of AD versus Aβ accumulation as an early detectable stage of AD because current evidence suggests that both concepts are plausible" (Sperling 2011).

Some experts have even suggested that amyloid plaque formation could be the body's protective mechanism to the (unknown) underlying disease process (Selko 2002, Lee 2004, Shankar 2008).

Returning to the Doraiswamy study, what this study demonstrates is the progression of symptoms to the clinical state of dementia, not the etiology(ies) driving that progression, because the endpoint is not autopsy, essential for the gold standard diagnosis of AD. Prognosis and diagnosis can be different things, and this study is really about the former.

So armed with this study, *what do we really know?* Not which individuals have AD. Thus an amyloid scan here would not inform the use of effective disease-specific treatments – again, if these existed. And if they did exist, and merely had mild adverse effects, such treatments would be tried on a host of symptomatic patients, and there might well emerge classifications of cognitive impairment and dementia based on whether individuals were susceptible or resistant to a given treatment. If so, and these treatments were efficacious for more than one etiology of cognitive impairment and/or dementia, the diagnosis of AD in itself would become less relevant.

Leaving diagnosis aside, and returning to the strong hand of the Doraiswamy study, prognosis, how might prognosis alone, as predicted by a positive amyloid scan, change one's decision-making and management?

The study was not designed to test this (no one study can do everything), but even theoretically this is unclear – at least at 18 months, the limit of the study follow-up. The study reports a 29% chance of progression from MCI to dementia if the amyloid scan is positive, compared to a 10% chance of the same if it is negative. Say you are one of these patients who get a scan, your result is negative, and therefore you are in the 10% group. How would this change your (or your physician-advisor's) decision to do or not do something? Put another way, if you knew you had a 29% chance of a very bad thing happening to you, and you could take some meaningful actions as a result for you and/or your family, would you now not take those actions because you had only a 10% chance of that fate? If there were a 29% chance the airplane you were about to board would crash, would you now board it because there was only a 10% chance? More longitudinal data could certainly alter these numbers, and provide clearer implications for rational decision making and management.

Mild cognitive impairment (MCI)

In reviewing the Grundman 2012 study we noted that it did not identify a potentially high-yield, objectively defined, target population. Fortunately, multiple other studies do: it is the MCI population. This was a key insight shared by MEDCAC panel members and expert presenters alike during the meeting. Deriving from research beginning in the 1990s, with the term coined in 1999, MCI lies between the cognitive changes of normal aging and dementia. Individuals with MCI experience memory loss (amnestic MCI) or loss of thinking skills other than memory loss (nonamnestic MCI), to a greater extent than expected for age, but without impairment of day-to-day functioning. Individuals with MCI are at increased risk for developing dementia (whether from AD or another etiology), but many do not progress to dementia, and some get better (Petersen 1999 and 2009, Wolk 2009, Hughes 2011, Ward 2012, Landau 2012, Sachdev 2012).

Both amnestic and nonamnestic MCI have subtypes of "single" and "multiple" domain. For example, a person without memory loss but with documented impairment in attention and concentration, and subtle impairment in visuospatial skills, would have multi-domain, non-amnestic MCI (Petersen 2009).

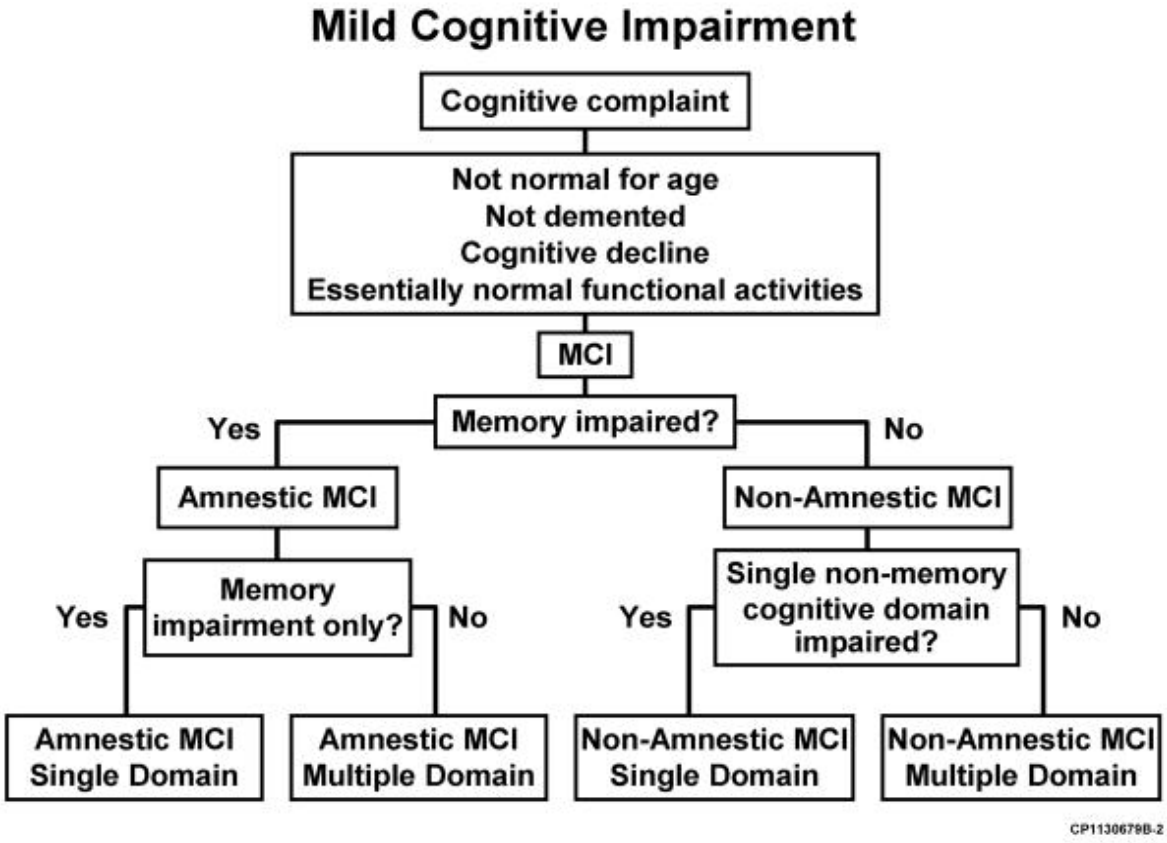

Figure obtained from Peterson, R. Early Diagnosis of Alzheimer's Disease: Is MCI Too Late? *Current Alzheimer Research*. 2009; 6(4):329.

More recent subtypes (under investigation in the Alzheimer's Disease Neuroimaging Initiative (ADNI) Go and ADNI 2 trials) include "early" and "late" MCI. Early MCI represents subtle memory impairment that is intermediate between normal subjects and late MCI, as determined by say, education-adjusted scores on the Wechsler Memory Scale Logical Memory II (Landau 2012).

The field of MCI research is overlapping that of PET amyloid imaging, using various tracers including first PIB and increasingly florbetapir as well as other agents (Wolk 2009, Hughes 2011, Ward 2012, Landau 2012, Sachdev 2012, Cordell 2011). The ADNI family of prospective, longitudinal studies involve well over 1,000 participants at over 50 medical centers across the US and Canada, incorporate clinical classifications of MCI, AD and healthy

controls, have regular, standardized clinical, imaging and CSF biomarker testing, and have autopsy as their endpoint. Other large, prospective, longitudinal studies of interest are underway at Mayo (Roberts 2008), in Australia (Sachdev 2012) and in Italy (Di Carlo 2007), although the degree of standardization that would enable meta-analysis across studies is not known to us at this time.

MCI subtypes, and associated objective scores on "bedside" mental status exams and neuropsychiatric testing, could, when combined with other patient characteristics (e.g., age, genetics, cognitive reserve, comorbidities) and biomarkers (for hypometabolism, plaque accumulation, synaptic dysfunction and neuronal loss), serve as the foundation for the development of objectively defined "risk pools," or subpopulations of individuals who are at risk of progressing from MCI to AD. Ideally, risk stratification would eventually be able to identify persons at high risk for developing AD before symptoms occur. This may be especially important as a chain of evidence from multiple studies (animal and human) suggest that future therapies might be most (or only) effective if they begin early in or prior to the process of abnormal amyloid accumulation – perhaps10 to as much as 25 years prior to the onset of symptoms. Lifestyle changes, whether as a complimentary or an essential effort, may be a lifelong requirement (Gandy 2012, Goate 1991, Nicoll 2003, Bateman 2012, Jonsson 2012, Pollack 2012).

Generalizability

Generalizability – evidence that beneficial outcomes would be sustainable outside the clinical trial setting, in broad community use – is also a well-established factor that we consider in CMS coverage decisions. It is through this lens that we examine the questions of who should order, and who should interpret, PET Aβ imaging scans. We agree with the AIT that the ordering of PET Aβ imaging tests should be done by dementia specialists within the fields of neurology, neuropsychiatry and geriatric medicine who are actively managing the patient's care (Johnson 2013).

As to the qualifications and training of physicians who would interpret (or "read") the scans, we believe there is not enough evidence to support that the limited on-line training that currently exists suffices to ensure quality of reads in broader community practice. There are no experts we are aware of who do not acknowledge that this issue was a major problem with the initial launch of FDG PET, and we have learned from that experience as well as from the emergence of other new imaging technologies since then. A training and certificate model that may have some applicability for PET Aβ imaging is that for cardiac CT (Pelberg 2011). We believe that the training requirements included in the labeling should be viewed as absolute minimums and we encourage the development and maintenance of professional society standards. These might, for example, require formal mentoring of real cases and create facilitated pathways for "expert panel" interpretations of equivocal images.

Additionally, important questions remain about scan interpretation techniques themselves. Could quantitative measurements and visual interpretation be integrated by the reader (as done in say, CT brain perfusion imaging) to improve performance characteristics of the test? Should the anatomical distribution, as well as overall burden, of amyloid be considered in scan interpretation, especially given the discrepancies in frontal and medial temporal lobe findings between imaging and histopathology (Moghbel 2012, Kepe 2013). As mentioned earlier, PET amyloid tracers bind to both neuritic and diffuse plaques (Lockhart 2007), the latter being more common in normal aging, and the relative contribution of each to imaging results remains unclear. Also, it is unclear to what other substrates (Aβ structures, brain structures or receptors) these agents bind (Kepe 2013, EMA 2013 Annex 1). Finally, how could standardization – of PET generally (e.g., Wahl 2009) but also in amyloid imaging specifically – be improved to allow more meaningful comparisons across centers and trials?

In summary, we find that use of PET Aβ imaging is promising: (1) for excluding AD in narrowly defined and clinically difficult differentials, such as AD versus FTD, to prevent the harm of inappropriate use of potentially toxic medications; and (2) to improve the quality and efficiency of trials seeking to develop better interventions for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors. PET Aβ imaging may someday prove useful in limiting other testing, and, along with other biomarkers, in establishing a positive diagnosis of AD in certain subpopulations (to be defined), but the evidence to date is less substantial here. We also believe that further studies could be embedded into existing longitudinal, clinical research infrastructure, to potentially provide the building blocks for evidence-based appropriate use criteria. Finally, improvements in reading techniques, training and standardization of PET imaging protocols are needed.

B. CED

There are many outstanding questions about the diagnosis and management of AD and other dementias and the potential roles of PET Aβ imaging in that context. The goal of therapeutic trials may be to prevent, modify or cure the disease process, or to improve or slow the decline of patient cognition and functioning. Here the potential power of a negative scan to virtually exclude significant brain beta amyloid deposition could benefit Medicare beneficiaries, by helping them avoid potentially harmful, experimental therapies, and directing them to trials or treatments more likely to benefit them. Better patient selection could in turn improve the quality and efficiency of the therapeutic trials themselves. Due to the immense burden AD poses to Medicare beneficiaries (without considering burdens to their families and the Medicare system itself – which go beyond the scope of this NCD), the importance of developing effective therapies for AD rivals the difficulty of doing so.

While important questions on some ultimate outcomes may require comparison to autopsy findings that may not be available for years, other questions lend themselves to shorter time frames. For example, do community based physicians, relying on the result of scans interpreted by community based readers, consistently modify drug therapy to avoid certain adverse events? Are these adverse events actually avoided, or are the predictive values of imaging in these settings different or less reliable?

Some commenters suggest that the experience of CED for FDG PET for dementia and neurodegenerative diseases is relevant to the current consideration of CED for PET Aβ. They note specifically that a planned large trial of FDG PET has been minimally enrolled despite the passage of many years. While we believe there are lessons to be learned from that experience, we do not agree that the conclusion of those lessons is that CED should be abandoned in this important clinical area. We have, since that FDG PET NCD, formally articulated the CED paradigm in guidance convened the MEDCAC on CED. We have described our relationship with AHRQ, and AHRQ's role in supporting CED.

As we noted above in the response to public comment, CED is not limited to a single trial that addresses every aspect of the CED question(s). We acknowledge that approvable CED protocols may address one or more aspects of the CED questions, and that nontraditional study designs, e.g. practical observational studies and registries, may be methodologically appropriate or even favored for some aspects.

Ongoing research initiatives such as the ADNI could provide much of the infrastructure for generating the evidence we seek. As stated at the outset of this discussion section, to date, no prospective, longitudinal data have emerged to provide sufficient evidence to conclude that the use of PET Aβ imaging would meaningfully improve health outcomes, directly or indirectly, for Medicare beneficiaries who have or are at risk for developing AD. However, it may be possible to embed within such infrastructure the studies needed to close evidence gaps identified in this DM, at the MEDCAC meeting, and in the literature. Indeed, some are underway. These would include prospective, controlled, longitudinal studies, with, where appropriate, randomization and autopsy as an endpoint. Hopefully, surrogate markers could be eventually identified to render unnecessary the longitudinal follow up to autopsy; what these surrogates might be remains unclear at this time however. These studies should focus not on what clinicians intend to do, but on actual management following objective protocols.

Risk pools might be objectively determined combining clinical MCI subtypes, for instance, with other clinical, imaging and laboratory biomarker testing (as described above). The prevalence of AD could then be determined for each risk pool (by gold standard), and this in turn, combined with more data points for estimating sensitivity and specificity, could generate quantitative negative and predictive values for biomarker tests, alone or in combination, for each pool. These predictive values would determine the meaning of a test result – and if the test should even be obtained in the first place – for a particular patient. Establishing the clinically utility of that test – its meaningful impact on patient management that can be linked to downstream processes that improve health outcomes – is also of course important.

It is possible that different combinations of biomarkers (again, of plaque accumulation, synaptic dysfunction, neuronal loss, hypometabolism, etc.) may be appropriate for patients in different pools. Further research could give weights to the partial and combined contributions of these various biomarker and clinical tests for specific risk pools. Identifying such pools, and the predictive values of diagnostic tests for each, has been essential for determining which individuals need what test, when, in clinical research of other diseases (such as thromboembolic disease, the example given earlier), where they have informed the development of evidence-based appropriate use criteria for diagnostic tests.

It is in this light that we assess the first iteration of the appropriate use criteria recently published by the joint Amyloid Imaging Taskforce (AIT) of the AA and SNMMI (Johnson 2013). It is a consensus statement. It does not delve into specifics about risk pools, their associated prevalence of disease, and the predictive values for various biomarker tests, alone or in combination, for each pool. It does not use these building blocks of evidenced-based appropriate use criteria, because these blocks themselves do not yet exist for amyloid imaging in AD.

With respect specifically to biomarkers, the AIT "did not consider other proposed diagnostic biomarkers for AD and therefore did not draw any conclusions as to the relative value of amyloid PET compared to CSF, MRI and FDG PET." Yet the AIT acknowledges that "the appropriate use of amyloid PET requires knowledge of all relevant findings of clinical evaluations, laboratory tests and imaging relating how each component of the accumulated evidence should be weighed." Our assessment of the current literature is that there is insufficient data to empirically determine the relative weights of those components. This conclusion echoes that of the authors of the NIA-AA guideline workgroups:

"There was a broad consensus within all three workgroups that much additional work is needed to validate the application of biomarkers for diagnostic purposes . . . additional biomarker comparison studies are needed, as is more thorough validation with postmortem studies, and the use of combinations of biomarkers in studies has been limited. Extensive work on biomarker standardization is needed before wide-spread adoption of these recommendations at any stage of the disease" (Jack 2011).

Knowing all this, the AIT's approach seems to reflect the acceptance of certain premises: assuming the test will be used given FDA approval, and given the evidence that currently exists (as limited as it may be), what is the best guidance we can give to clinicians on how, and how not, to use this new technology? NCDs are inherently not guidance documents and thus reflect different premises. That said, we believe the AIT approach is informative and can help guide physician approaches to dementia management amid the challenges of an immature evidence base.

In its introduction, the AIT states that while "promising . . . experience with clinical amyloid PET imaging is limited. Most published studies to date have been designed to validate this technology and understand disease mechanisms rather than to evaluate applications in clinical practice. As a result, published data are available primarily from highly selected populations with prototypical findings rather than from patients with comorbidities, complex histories, and atypical features often seen in clinical practice. . . Empirical evidence for the value of added certainty resulting from amyloid PET has not yet been reported" (Johnson 2013).

This is consistent with CMS' historic use of CED. We note in particular that the last sentence quoted above (with which we agree, based on our independent assessment of the literature) means it would be difficult for clinicians to be able to meet clause (iii) of the Preamble of the AIT's appropriate use criteria:

"Amyloid imaging is appropriate in the situations listed here for individuals with all of the following characteristics: . . . (iii) when knowledge of the presence or absence of Aβ pathology is expected to increase diagnostic certainty and alter management" (Johnson 2013).

We believe emerging and future investigations, some of which are described in this DM, could no doubt better inform future iterations of the AIT's guidelines.

We thus have finalized this decision as coverage with evidence development (CED). Many Medicare beneficiaries are potential candidates for AD-related therapeutic trials. Some therapies may prove successful in preventing or slowing the downstream cascade of neurodegeneration that correlates with severity of disease. However, we temper our enthusiasm as it also possible that future therapies, if they are effective at all, might be so only if used prior to or early in the process of amyloid accumulation. If the latter is the case, most patients who would benefit would be younger than age 65. We acknowledge that this would in turn create a healthier pool entering Medicare's ranks; however, such dynamic, temporal analysis is outside the scope of our inquiry, which focuses solely on the Medicare population of today.

We have concluded that PET beta amyloid imaging is not reasonable and necessary under 1862(a)(1)(A) of the Act. However, CMS remains aware of significant evidence gaps that, if narrowed or closed, could further inform clinical decision making and future coverage policy. We believe Medicare could support this endeavor with CED. We have concluded that PET beta amyloid imaging is reasonable and necessary under 1862(a)(1)(E) of the Act.

Health Disparities

Subjects in key clinical trials on PET Aβ imaging (e.g., Clark 2011 and 2012, Grundman 2012) are generally > 90% white, despite data that older African-Americans are twice as likely, and older Hispanics 1.5 times as likely, to have AD (and other dementias) as older whites (see the Background section of this DM). This lack of evidence about racial and ethnic factors represents in our view an evidence gap that we encourage trial designers to consider when proposing clinical trial designs under this NCD. While recognizing that this consideration may complicate the design of appropriate clinical studies, we will nevertheless prefer clinical study proposals in which data on racial and ethnic factors are specifically collected and analyzed.

Summary

We have carefully and deliberately reviewed the available evidence, including published clinical studies, the MEDCAC recommendations, public comment and expert opinion, and we have reached the following answers to our analytic questions, which are repeated below for the convenience of the reader.

Question a:

Is the evidence adequate to conclude that PET Aβ imaging improves meaningful health outcomes in beneficiaries who display signs and symptoms of AD?

Answer a:

We cannot confidently conclude that PET Aβ imaging improves health outcomes in beneficiaries who display signs or symptoms of AD. We believe that additional clinical studies are needed to address these important issues, and that CED can facilitate this effort.

Question b:

Is the evidence adequate to conclude that PET Aβ imaging results inform the treating physician's management of the beneficiary to improve meaningful health outcomes? Those outcomes may include reasonably considered beneficial therapeutic management or the avoidance of unnecessary, burdensome interventions.

Answer b.

We have concluded from the available evidence that it is promising but not conclusive that PET Aβ imaging could, in community care settings, inform the identification of a specific population of beneficiaries in whom the harms of mismanagement with anticholinesterase therapy may be reduced if certain medications are in fact avoided.

CMS remains aware of significant evidence gaps that, if narrowed or closed, could further inform clinical decision making and future coverage policy. We believe Medicare could support this endeavor with CED.

IX. Conclusion

A. The Centers for Medicare & Medicaid Services (CMS) has determined that the evidence is insufficient to conclude that the use of positron emission tomography (PET) amyloid-beta (Aβ) imaging is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member for Medicare beneficiaries with dementia or neurodegenerative disease, and thus PET Aβ imaging is not covered under §1862(a)(1)(A) of the Social Security Act ("the Act").

B. However, there is sufficient evidence that the use of PET Aβ imaging is promising in two scenarios: (1) to exclude Alzheimer's disease (AD) in narrowly defined and clinically difficult differential diagnoses, such as AD versus frontotemporal dementia (FTD); and (2) to enrich clinical trials seeking better treatments or prevention strategies for AD, by allowing for selection of patients on the basis of biological as well as clinical and epidemiological factors.

Therefore, we will cover one PET Aβ scan per patient through coverage with evidence development (CED), under §1862(a)(1)(E) of the Act, in clinical studies that meet the criteria in each of the paragraphs below.

Clinical study objectives must be to (1) develop better treatments or prevention strategies for AD, or, as a strategy to identify subpopulations at risk for developing AD, or (2) resolve clinically difficult differential

diagnoses (e.g., frontotemporal dementia (FTD) versus AD) where the use of PET Aβ imaging appears to improve health outcomes. These may include short term outcomes related to changes in management as well as longer term dementia outcomes.

Clinical studies must be approved by CMS, involve subjects from appropriate populations, and be comparative and longitudinal. Where appropriate, studies should be prospective, randomized, and use postmortem diagnosis as the endpoint. Radiopharmaceuticals used in the PET Aβ scans must be FDA approved. Approved studies must address one or more aspects of the following questions. For Medicare beneficiaries with cognitive impairment suspicious for AD, or who may be at risk for developing AD:

1. Do the results of PET Aβ imaging lead to improved health outcomes? Meaningful health outcomes of interest include: avoidance of futile treatment or tests; improving, or slowing the decline of, quality of life; and survival.

2. Are there specific subpopulations, patient characteristics or differential diagnoses that are predictive of improved health outcomes in patients whose management is guided by the PET Aβ imaging?

3. Does using PET Aβ imaging in guiding patient management, to enrich clinical trials seeking better treatments or prevention strategies for AD, by selecting patients on the basis of biological as well as clinical and epidemiological factors, lead to improved health outcomes?

Any clinical study undertaken pursuant to this national coverage determination (NCD) must adhere to the timeframe designated in the approved clinical study protocol. Any approved clinical study must also adhere to the following standards of scientific integrity and relevance to the Medicare population.

a. The principal purpose of the research study is to test whether a particular intervention potentially improves the participants' health outcomes.

b. The research study is well supported by available scientific and medical information or it is intended to clarify or establish the health outcomes of interventions already in common clinical use.

c. The research study does not unjustifiably duplicate existing studies.

d. The research study design is appropriate to answer the research question being asked in the study.

e. The research study is sponsored by an organization or individual capable of executing the proposed study successfully.

f. The research study is in compliance with all applicable Federal regulations concerning the protection of human subjects found at 45 CFR Part 46. If a study is regulated by the Food and Drug Administration (FDA), it must be in compliance with 21 CFR parts 50 and 56.

g. All aspects of the research study are conducted according to appropriate standards of scientific integrity (see http://www.icmje.org).

h. The research study has a written protocol that clearly addresses, or incorporates by reference, the standards listed here as Medicare requirements.

i. The clinical research study is not designed to exclusively test toxicity or disease pathophysiology in healthy individuals. Trials of all medical technologies measuring therapeutic outcomes as one of the objectives meet this standard only if the disease or condition being studied is life threatening as defined in 21 CFR §312.81(a) and the patient has no other viable treatment options.

j. The clinical research study is registered on the ClinicalTrials.gov website by the principal sponsor/investigator prior to the enrollment of the first study subject.

k. The research study protocol specifies the method and timing of public release of all pre-specified outcomes to be measured including release of outcomes if outcomes are negative or study is terminated early. The results must be made public within 24 months of the end of data collection. If a report is planned to be published in a peer reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors

(http://www.icmje.org). However a full report of the outcomes must be made public no later than three (3) years after the end of data collection.

l. The research study protocol must explicitly discuss subpopulations affected by the treatment under investigation, particularly traditionally underrepresented groups in clinical studies, how the inclusion and exclusion criteria effect enrollment of these populations, and a plan for the retention and reporting of said populations on the trial. If the inclusion and exclusion criteria are expected to have a negative effect on the recruitment or retention of underrepresented populations, the protocol must discuss why these criteria are necessary.

m. The research study protocol explicitly discusses how the results are or are not expected to be generalizable to the Medicare population to infer whether Medicare patients may benefit from the intervention. Separate discussions in the protocol may be necessary for populations eligible for Medicare due to age, disability or Medicaid eligibility.

Consistent with §1142 of the Act, the Agency for Healthcare Research and Quality (AHRQ) supports clinical research studies that CMS determines meet the above-listed standards and address the above-listed research questions.

All other uses are noncovered.

APPENDIX A

General Methodological Principles of Study Design

(Section VI of the Decision Memorandum)

When making national coverage determinations, CMS evaluates relevant clinical evidence to determine whether or not the evidence is of sufficient quality to support a finding that an item or service is reasonable and necessary. The overall objective for the critical appraisal of the evidence is to determine to what degree we are confident that: 1) the specific assessment questions can be answered conclusively; and 2) the intervention will improve health outcomes for patients.

We divide the assessment of clinical evidence into three stages: 1) the quality of the individual studies; 2) the generalizability of findings from individual studies to the Medicare population; and 3) overarching conclusions that can be drawn from the body of the evidence on the direction and magnitude of the intervention's potential risks and benefits.

The methodological principles described below represent a broad discussion of the issues we consider when reviewing clinical evidence. However, it should be noted that each coverage determination has its unique methodological aspects.

Assessing Individual Studies

Methodologists have developed criteria to determine weaknesses and strengths of clinical research. Strength of evidence generally refers to: 1) the scientific validity underlying study findings regarding causal relationships between health care interventions and health outcomes; and 2) the reduction of bias. In general, some of the methodological attributes associated with stronger evidence include those listed below:

- Use of randomization (allocation of patients to either intervention or control group) in order to minimize bias.
- Use of contemporaneous control groups (rather than historical controls) in order to ensure comparability between the intervention and control groups.
- Prospective (rather than retrospective) studies to ensure a more thorough and systematical assessment of factors related to outcomes.

- Larger sample sizes, to demonstrate both statistically significant as well as clinically significant outcomes that can be extrapolated to the Medicare population. Sample size should be large enough to make chance an unlikely explanation for what was found.
- Masking (blinding) to ensure patients and investigators do not know to that group patients were assigned (intervention or control). This is important especially in subjective outcomes, such as pain or quality of life, where enthusiasm and psychological factors may lead to an improved perceived outcome by either the patient or assessor.

Regardless of whether the design of a study is a randomized controlled trial, a non-randomized controlled trial, a cohort study or a case-control study, the primary criterion for methodological strength or quality is to the extent that differences between intervention and control groups can be attributed to the intervention studied. This is known as internal validity. Various types of bias can undermine internal validity. These include:

- Different characteristics between patients participating and those theoretically eligible for study but not participating (selection bias).
- Co-interventions or provision of care apart from the intervention under evaluation (performance bias).
- Differential assessment of outcome (detection bias).
- Occurrence and reporting of patients who do not complete the study (attrition bias).

In principle, rankings of research design have been based on the ability of each study design category to minimize these biases. A randomized controlled trial minimizes systematic bias (in theory) by selecting a sample of participants from a particular population and allocating them randomly to the intervention and control groups. Thus, in general, randomized controlled studies have been typically assigned the greatest strength, followed by non-randomized clinical trials and controlled observational studies. The design, conduct and analysis of trials are important factors as well. For example, a welldesigned and conducted observational study with a large sample size may provide stronger evidence than a poorly designed and conducted randomized controlled trial with a small sample size. The following is a representative list of study designs (some of that have alternative names) ranked from most to least methodologically rigorous in their potential ability to minimize systematic bias:

Randomized controlled trials
Non-randomized controlled trials
Prospective cohort studies
Retrospective case control studies
Cross-sectional studies
Surveillance studies (e. g., using registries or surveys)
Consecutive case series
Single case reports

When there are merely associations but not causal relationships between a study's variables and outcomes, it is important not to draw causal inferences. Confounding refers to independent variables that systematically vary with the causal variable. This distorts measurement of the outcome of interest because its effect size is mixed with the effects of other extraneous factors. For observational, and in some cases randomized controlled trials, the method in that confounding factors are handled (either through stratification or appropriate statistical modeling) are of particular concern. For example, in order to interpret and generalize conclusions to our population of Medicare patients, it may be necessary for studies to match or stratify their intervention and control groups by patient age or co-morbidities.

Methodological strength is, therefore, a multidimensional concept that relates to the design, implementation and analysis of a clinical study. In addition, thorough documentation of the conduct of the research, particularly study selection criteria, rate of attrition and process for data collection, is essential for CMS to adequately assess and consider the evidence.

Generalizability of Clinical Evidence to the Medicare Population

The applicability of the results of a study to other populations, settings, treatment regimens and outcomes assessed is known as external validity. Even well-designed and well-conducted trials may not supply the evidence needed if the results of a study are not applicable to the Medicare population. Evidence that provides accurate information about a population or setting not well represented in the Medicare program would be considered but would suffer from limited generalizability.

The extent to that the results of a trial are applicable to other circumstances is often a matter of judgment that depends on specific study characteristics, primarily the patient population studied (age, sex, severity of disease and presence of co-morbidities) and the care setting (primary to tertiary level of care, as well as the experience and specialization of the care provider). Additional relevant variables are treatment regimens (dosage, timing and route of administration), co-interventions or concomitant therapies, and type of outcome and length of follow-up.

The level of care and the experience of the providers in the study are other crucial elements in assessing a study's external validity. Trial participants in an academic medical center may receive more or different attention than is typically available in on-tertiary settings. For example, an investigator's lengthy and detailed explanations of the potential benefits of the intervention and/or the use of new equipment provided to the academic center by the study sponsor may raise doubts about the applicability of study findings to community practice.

Given the evidence available in the research literature, some degree of generalization about an intervention's potential benefits and harms is invariably required in making coverage determinations for the Medicare population. Conditions that assist us in making reasonable generalizations are biologic plausibility, similarities between the populations studied and Medicare patients (age, sex, ethnicity and clinical presentation) and similarities of the intervention studied to those that would be routinely available in community practice.

A study's selected outcomes are an important consideration in generalizing available clinical evidence to Medicare coverage determinations. One of the goals of our determination process is to assess health outcomes. These outcomes include resultant risks and benefits such as increased or decreased morbidity and mortality. In order to make this determination, it is often necessary to evaluate whether the strength of the evidence is adequate to draw conclusions about the direction and magnitude of each individual outcome relevant to the intervention under study. In addition, it is important that an intervention's benefits are clinically significant and durable, rather than marginal or short-lived. Generally, an intervention is not reasonable and necessary if its risks outweigh its benefits.

If key health outcomes have not been studied or the direction of clinical effect is inconclusive, we may also evaluate the strength and adequacy of indirect evidence linking intermediate or surrogate outcomes to our outcomes of interest.

Assessing the Relative Magnitude of Risks and Benefits

Generally, an intervention is not reasonable and necessary if its risks outweigh its benefits. Health outcomes are one of several considerations in determining whether an item or service is reasonable and necessary. CMS places greater emphasis on health outcomes actually experienced by patients, such as quality of life, functional status, duration of disability, morbidity and mortality, and less emphasis on outcomes that patients do not directly experience, such as intermediate outcomes, surrogate outcomes, and laboratory or radiographic responses. The direction, magnitude, and consistency of the risks and benefits across studies are also important considerations. Based on the analysis of the strength of the evidence, CMS assesses the relative magnitude of an intervention or technology's benefits and risk of harm to Medicare beneficiaries.

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Review Article

A Survey of FDG- and Amyloid-PET Imaging in Dementia and GRADE Analysis

Perani Daniela,[1] Schillaci Orazio,[2] Padovani Alessandro,[3] Nobili Flavio Mariano,[4] Iaccarino Leonardo,[1] Della Rosa Pasquale Anthony,[5] Frisoni Giovanni,[6] and Caltagirone Carlo[7]

[1] *Nuclear Medicine Department, Vita-Salute San Raffaele University, San Raffaele Hospital and Division of Neuroscience, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy*
[2] *Nuclear Medicine Department, University of Rome "Tor Vergata" and IRCCS Neuromed, 86077 Pozzilli, Italy*
[3] *Department of Medical and Experimental Sciences, Unit of Neurology, Brescia University, 25123 Brescia, Italy*
[4] *Department of Neuroscience Ophthalmology and Genetics, University of Genoa, 16132 Genoa, Italy*
[5] *IBFM-CNR, Via F.lli Cervi 93, Segrate, 20090 Milan, Italy*
[6] *IRCCS Centro San Giovanni di Dio Fatebenefratelli, and Memory Clinic and LANVIE, Laboratory of Neuroimaging of Aging, University Hospitals and University of Geneva, 1225 Geneva, Switzerland*
[7] *University of Rome Tor Vergata and IRCSS S. Lucia, 00142 Rome, Italy*

Correspondence should be addressed to Perani Daniela; perani.daniela@hsr.it

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PET based tools can improve the early diagnosis of Alzheimer's disease (AD) and differential diagnosis of dementia. The importance of identifying individuals at risk of developing dementia among people with subjective cognitive complaints or mild cognitive impairment has clinical, social, and therapeutic implications. Within the two major classes of AD biomarkers currently identified, that is, markers of pathology and neurodegeneration, amyloid- and FDG-PET imaging represent decisive tools for their measurement. As a consequence, the PET tools have been recognized to be of crucial value in the recent guidelines for the early diagnosis of AD and other dementia conditions. The references based recommendations, however, include large PET imaging literature based on visual methods that greatly reduces sensitivity and specificity and lacks a clear cut-off between normal and pathological findings. PET imaging can be assessed using parametric or voxel-wise analyses by comparing the subject's scan with a normative data set, significantly increasing the diagnostic accuracy. This paper is a survey of the relevant literature on FDG and amyloid-PET imaging aimed at providing the value of quantification for the early and differential diagnosis of AD. This allowed a meta-analysis and GRADE analysis revealing high values for PET imaging that might be useful in considering recommendations.

1. Introduction

In Western countries, during the last century, the elderly population (over 65) has almost triplicated and in the next fifty years it will represent almost 35% of the total population. Along with ageing, dementia will become not only a dramatic clinical entity, but also a serious socio-economic issue, given that patients diagnosed with this devastating disease will likely increase by 50% by 2030.

However, the 2011 World Alzheimer Report (http://www.alz.co.uk/research/world-report) has underlined that only a percentage ranging between 20 and 50% of dementia cases are identified and recognized in the early stages, that is, at least half of the population of dementia patients suffering do not receive a complete diagnostic workup since disease onset.

This diagnostic delay gives rise to a so-called "treatment gap" between early stages of the disease and a formal

diagnosis which can then trigger necessary care and organized support ameliorating the patient's quality of life along with that of caregivers and family members. Clinical diagnosis *per se* has limited accuracy and requires the presence of cognitive symptoms, while biomarkers that are specific for AD-related pathologic phenomena would allow more accurate diagnosis when patients are in the prodromal or even preclinical stage of the disease, a period that is generally held to be the best intervention time for AD, at least at present days. PET allows the investigation of both the measurements of cerebral glucose metabolism by ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) and the Aβ amyloid deposition through specific molecular imaging techniques involving radiopharmaceuticals binding to amyloid. In the last decades, PET evidence for functional and molecular changes in neurodegenerative diseases has been largely shown [1–4]. In Alzheimer's disease (AD), within the two major classes of biomarkers now identified, biomarkers of disease state (i.e., biomarkers of amyloid β [Aβ] accumulation) and biomarkers of disease stage (i.e., biomarkers of neuronal injury), amyloid-PET, and FDG-PET imaging represent critical and decisive tools. PET imaging is now recognized of value to the early diagnosis and to clearly support the final diagnosis of AD [5–8]. Revisions of the NINCDS-ADRDA diagnostic criteria of AD [5, 9], as well as the new National Institute of Aging-Alzheimer Association criteria of MCI due to AD [6] have been proposed, positing that individuals with memory impairment who are positive for AD biomarkers have a high likelihood of having AD pathology. The corollary is that biomarker positive MCI patients frequently progress to dementia. Crucially, when both Abeta and neuronal injury biomarkers are negative, the dementia is unlikely to be attributable to AD pathology [1, 10–12].

The references based recommendations rely on sensitivity and specificity of the PET methods derived by the imaging literature that is based either on parametric approaches or on visual method that greatly depends on the observer's experience and lacks a clear cut-off between normal and pathological findings.

On the other hand, PET neuroimaging research has focused on the development of tools improving either detection of people at higher risk of dementia or early diagnosis of Alzheimer disease (AD) [13–16]. These methods improve the accuracy for the diagnosis of AD and prediction of progression from mild cognitive impairment to AD dementia [17–23]. Noteworthy, markers of amyloidosis and neurodegeneration are currently being used as outcomes in proof-of-concept drug studies [24].

The sensitivity and specificity of the PET methods indeed greatly depends on the use of quantification methods [15, 25, 26]. For example, FDG-PET can be assessed using software that analyses the pattern of tracer uptake voxel-wise by comparing the subject's scan with a reference data set of normal ageing, allowing a better recognition of the patterns of hypometabolism compared with visual interpretation [15, 17, 27].

The same is true for measurements of amyloid load using PET [25, 28, 29]. In AD, it has been shown that quantification or parametric measurements of amyloid load are fundamental since they allow cut off scores for a better differentiation between normal subjects, preclinical AD, and AD individuals [21, 22, 30]. In addition, due to the demonstration between group and intersubject variability, quantification of amyloid load would be crucial for multicentre studies and therapy monitoring. A real problem exists, whether a dichotomous readout such as that of amyloid-PET scans will be used (or misused) in the diagnostic procedures. It needs to be prevented a positive amyloid scan to become a *de facto* diagnosis of AD. Semiautomated (such as standardized uptake value ratio (SUVR)) or automated semiquantitative measures (such as using SPM-based protocols) will have the advantage of being operator independent. Semiquantitative or quantitative measures require thresholds for positivity/negativity. Thresholds include information on risk to develop dementia for subthreshold degrees of amyloid positivity. Semiquantitative or quantitative measures might in the future discriminate "accumulators" from "nonaccumulators," distinction that in normal persons could predict the development of MCI as a prodromal step to full blown AD [31]. Finally, it has to be highlighted that, today, the rationale for the use of PET biomarkers in prodromal AD diagnosis is that biomarkers change over decades before full-blown AD dementia develops [32].

Aim of this paper was to provide a survey of the specific PET literature based on the above considerations, with a meta-analysis and a GRADE analysis on FDG- and amyloid-PET imaging in the early and differential diagnosis of Alzheimer disease.

This survey was based indeed on restricted inclusion criteria of the relevant literature, namely,

(1) only articles published since 2001 which retain high quality 3D PET scans and control to an optimal degree any methodological shortcoming;

(2) for FDG-PET, only studies employing voxel-based analysis techniques (such as SPM, Neurostat, and AD t-sum) with statistical parametric mapping procedures that can provide unbiased, statistically defined measures of brain abnormality in the individual brain toward a reference control population throughout the whole brain;

(3) specifically to amyloid-PET, only articles reporting quantification or parameterization of β-amyloid deposition (in AD, MCI subjects, and normal controls) either with short half-life ^{11}C-labeled ligands (^{11}C PIB) and ^{18}F-labeled tracers (^{18}F-AV-45 Florbetapir, ^{18}F-BAY94-9172 Florbetaben, and the ^{11}C-PiB derivative ^{18}F-GE-067 Flutemetamol).

In addition, we included a descriptive analysis of the related literature reporting differences in the levels of sensitivity and specificity for the standard visual FDG-PET scan or dichotomous readout based amyloid-PET with respect to parametric or semiquantitative analysis [33–35].

1.1. Premises on FDG-PET Imaging Studies. ^{18}F-Fluorodeoxyglucose-PET (^{18}F-FDG) is used to measure cerebral

metabolic rate of glucose that is considered an index of synaptic functionality and density [36]. It has been widely used for various purposes, ranging from early diagnosis to differential diagnosis of dementias [3, 4]. There is substantial agreement about its effectiveness for diagnosis of dementia mainly for the typical hypometabolism patterns associated with the different neurodegenerative conditions (see [16]). Hypometabolism in AD has showed a very peculiar pattern since the emergence of early PET evidences [37, 38] recently defined in detail as involving parietal and temporal regions, precuneus, posterior cingulate cortex, medial temporal cortex, and structures (like hippocampus) [10, 14, 39–41]. Cerebral map of glucose metabolism can be visually inspected by experienced raters to evaluate possible neurodegenerative patterns. Despite the potential of visual inspection, modern techniques for quantification of FDG uptake are now widely used, and have been demonstrated to improve diagnosis accuracy and readability of hypometabolism patterns [33]. Statistical parametric mapping (SPM) produces unbiased smoothed and regularized images that allow a comparison between a single patient and a control group to define functionally abnormal regions. ^{18}F-FDG has been otherwise widely used to differentiate AD from non-AD dementias like DLB or FTLD spectrum. In a landmark study, Minoshima and coworkers [42] reported that relying on occipital cortex metabolism produced a sensitivity of 90% and a specificity of 80% in discriminating AD versus DLB, using autopsy pathology as reference. Similarly, Foster et al. [33] showed that ^{18}F-FDG can help discriminate between AD versus FTLD spectrum with 97% sensitivity and 86% specificity (93% accuracy). Importantly, studies have been also underlying that an absence of peculiar hypometabolism patterns may exclude a diagnosis of dementia [1].

As a matter of fact, hippocampal hypometabolism, a crucial marker of AD, is often missed, particularly in voxel-based analysis using smoothing procedure. As suggested in literature [41], by using manual region-of-interest-based (ROI) analytical methods and MRI/PET coregistration methods, the temporal medial dysfunction should be highlighted. In addition, even if has to be clarified, the method-related nature of this MRI/PET inconsistency, using coronal and/or sagittal dimensions (anterior-posterior) instead of axial orientation (inferior-superior) may at least partially overcome this "hippocampal issue," as this formation is smaller in axial view rather than in coronal or sagittal [41].

It appears that the normalization and smoothing procedures of SPM package tool that is necessary to minimize between individual inhomogeneity in brain shape and dimension may mask reduced uptake in small structures, such as the hippocampus. Moreover, spatial resolution of PET systems is best in superficial cortical areas close to the detectors while it is worst in midline and medial structures far from the detectors. Lastly, a pathophysiological explanation admits that the high synaptic density at posterior temporal-parietal association cortex and limbic cortex makes it easier to detect glucose hypometabolism in these regions as compared to the MTL structures which are rich in cell bodies but relatively poorer in synaptic density [43].

Furthermore, another florid field of research regards longitudinal studies to predict MCI-AD conversion and therefore early diagnosis of AD. Different techniques (MRI, PET, CSF, and clinical evaluation) have been extensively compared, and even though combined predictors are now considered the best solution, it has widely reported a major role (namely, in sensitivities, specificities, and prediction accuracy) of the PET [44–47].

1.2. Premises on Amyloid-PET Imaging Studies. β-amyloid plaques are a hallmark of AD and can be found in moderate to high number in cortical gray matter in all cases of AD and develop many years before the onset of dementia. The amyloid theory postulates that amyloid accumulation is the main causative event leading to synaptic and neuronal degeneration and subsequent gray matter atrophy [31]. This hypothesis is supported by the evidence that the soluble form of β-amyloid in equilibrium with the soluble β-amyloid found in plaques is potentially neurotoxic though the time interval between the deposition of β-amyloid and the beginning of a neurodegenerative process that still remains unclear [48].

In contrast, Aβ plaques are not found in frontotemporal dementia (FTD) or pure vascular dementia [12]. The amyloid hypothesis is still debated and several arguments point against amyloid as a main pathogenic factor in AD pathology [49]. Whatever the role of amyloid is, whether causative or merely an epiphenomenon, all patients with AD have an increased brain amyloid load. Therefore, the development of imaging tools for the detection and quantification of amyloid deposition is of particular relevance for the confirmation or exclusion of AD, the distinction of AD from other dementias, and its early diagnosis [50].

The first tracer for amyloid was developed at the University of Pittsburgh through modification of thioflavin T; a fluorescent dye used to identify plaques in brain tissue specimen [51] that was given the name Pittsburgh compound B (^{11}C-PiB). ^{11}C-PiB was found to bind to the amyloid in the classic (i.e., neuritic) plaques of AD, which are distributed around the degenerating neuritis. ^{11}C-PiB could label β-amyloid in living brains, and it was used in patients suffering from AD since the earliest investigations [52]. It lacks specificity to these classic plaques, as it also binds to diffuse amyloid plaques that can be found in a substantial proportion of healthy elderly and are not specific for AD [53]. Further, PiB binds to cerebrovascular amyloid in cerebral amyloid angiopathy (CAA), mainly in posterior parietal and occipital cortex. As such, PiB cannot be regarded as a specific marker of AD-amyloidosis but rather of brain amyloidosis more in general.

Leinonen et al. [54] evaluated ^{11}C-PiB uptake findings in AD patients with and without typical AD neuropathological lesions in frontal cortical biopsy specimens. The authors found a significantly higher PiB uptake in the frontal, parietal, and lateral temporal cortices and striatum in patients with Aβ aggregates in the frontal cortex compared with those without notable Aβ aggregates in the brain biopsy specimen. Moreover, the patients with the highest Aβ load in

the biopsy specimen had also the highest ^{11}C-PiB uptake in PET imaging.

Several authors investigated the diagnostic accuracy of AD by means of ^{11}C-PiB PET as unique imaging method or in combination with other measures (usually FDG-PET or volumetric MRI) and mainly using clinical criteria as reference test. For example, by comparing ^{18}F-FDG to ^{11}C-PiB PET scan, Lowe et al. [55] obtained a similar diagnostic accuracy in early cognitive impairment, but ^{11}C-PiB PET scan allowed a better discrimination between amnestic MCI and nonamnestic MCI, thus demonstrating that amyloid deposition occurs before cerebral metabolic dysfunction.

Devanand et al. [56] found that ^{11}C-PiB binding potential (BP) analysis slightly outperformed regional cerebral metabolic rate for cerebral glucose analysis of FDGPET images in discriminating AD patients from healthy controls (HC).

Similarly, [34] demonstrated the higher sensitivity of ^{11}C-PiB BP analysis in discriminating AD from FTD patients. Other two studies, comparing ^{18}F-FDG-PET and ^{11}C-PiB PET, have concluded that they give complementary information for the early diagnosis and followup of patients with dementia [57, 58]. This is a central issue, since dissociation between metabolic reduction and amyloid deposition has been also shown. In particular, in a 3 and 5 years of follow-up study on MCI and AD patients, Kadir and coworkers found that fibrillar amyloid load progressively increased in MCI patients and was followed by more stable level in clinical AD patients, whereas glucose metabolism started to decline early in MCI patients and became more pronounced in advanced clinical stage [59]. Also, the mismatch between the two imaging modalities was shown in a study investigating the effects of phenserine treatment on glucose metabolism and amyloid load in 20 AD patients [60].

A number of longitudinal studies have argued for the role of ^{11}C-PiB tracer in predicting conversion from MCI to AD. For example, it has been shown that, compared to nonconverting MCI patients and healthy controls (HC), MCI patients that converted to AD at clinical followup displayed significantly higher ^{11}C-PiB retention, at levels comparable to that of AD patients [61]. Okello et al. [21] found that the 50% of MCI patients showing a positive ^{11}C-PiB uptake at baseline converted to overt AD at 1-year followup and had greater ^{11}C-PiB retention than nonconverter patients. Similarly, in a 2-year follow-up study, Koivunen and colleagues [62], measuring ^{11}C-PiB retention in MCI and control subjects, showed that MCI patients who converted to AD had greater ^{11}C-PiB retention in several brain areas, including cingulum, frontal and temporal cortices, putamen, and caudate.

Now, it is widely accepted that ^{11}C-PiB PET can provide a quantitative representation of fibrillar deposition amyloid-beta deposition in the brain. Therefore, it is of the utmost importance to develop quantitative methods of amyloid-PET data analysis and that such methods can be standardized and applied across centers.

Analyses of PET images for the quantification of Aβ deposition have been done both qualitatively (e.g., visual analysis of tracer uptake) and quantitatively. In this latter case, analysis of tracer retention requires normalization of the uptake values, to allow inter- and intrasubject comparisons. The standard uptake value ratio (SUVR) normalizes the uptake values to the mean uptake value within a region containing nonspecific binding, usually the cerebellar grey matter. Another method, for example, based on distribution volume ratios (DVRs) and their combination with arterial plasma input, metabolite correction, or references tissue models may yield different results [63].

The interrater reliability of manual and automated ROI delineation for ^{11}C-PiB PET imaging was recently assessed for the detection of early amyloid deposition in human brain [64]. Despite methodological differences in the manual and automated approaches, the analysis revealed good agreement in primary cortical areas and the cerebellar reference region for SUV and SUVR outcomes. These data are important because a reliable methodology is needed for the detection of low levels of amyloid deposition on a cross-sectional basis and small changes in amyloid deposition on a longitudinal basis and also to enable valid definition of amyloid positivity thresholds and determination of relationships between *in vivo* PET imaging and postmortem assessments of amyloid-beta load.

A new noninvasive efficient graphical approach, called the relative equilibrium-based (RE) graphical plot, has been developed for tracer kinetics analysis, with equilibrium relative to input function; this method has been recently used to improve and simplify two of the most common approaches for ^{11}C-PiB PET quantification [65]. In this paper, results from theoretical analysis were confirmed by 78 PET studies of nondemented older adults, indicating that the RE plot could improve pixel wise quantification of amyloid-beta burden when compared with 2 frequently used methods like the Logan plot and the SUVR.

In the majority of ^{11}C-PiB PET studies, the cerebellum has been chosen as a reference region. However, because cerebellar amyloid may be present in genetic AD, cerebral amyloid angiopathy and prion diseases, whether the pons could be used as an alternative reference region for the analysis of ^{11}C-PIB binding in AD has been evaluated [66]. The findings of the study in 12 sporadic AD patients, 10 age-matched controls, and 3 other subjects (2 with presymptomatic presenilin-1 mutation carriers and one probable familial AD) suggest that that the target-to-pons ratio for the analysis of ^{11}C PIB images has low test-retest variability and high reproducibility and can be used as a simplified method of quantification when the cerebellum as a reference is not appropriate.

The definition of a cutoff that separates individuals with no significant amyloid-beta deposition from those in which deposition has begun is crucial for the clinical acceptance of ^{11}C-PiB PET. In a cohort of older subjects in which the separation between PiB positive and PiB negative subjects was not so distinct, the application of visual read and quantitative approaches optimized the identification of early amyloid-beta deposition [26].

In addition to ^{11}C-PiB, other ^{18}F-labeled tracers have been developed and investigated. Flutemetamol (GE-067) is

the 3′-fluoro-derivative of PiB, whereas florbetaben (BAY-94-9172, AV-1) and florbetapir (AV-45) are stilbene and styrylpyridine derivatives, which exhibit high affinity binding for fibrillary amyloid. Flutemetamol kinetic analysis of tracer binding showed reliable quantification by use of relative standardized uptake value ratios with the cerebellar cortex as a reference region, and data acquisition for this analysis requires only 20 min scanning and is feasible in a standard clinical setting [67]. Florbetaben and florbetapir are chemically closely related compounds but the former has slower kinetics, resulting in a longer imaging acquisition time (for stable uptake up to 130 min after injection), in comparison with Flutemetamol (90 min) and Florbetapir (60 min) [68].

In a recent PET study using ^{18}F-Florbetapir with 74 HC and 29 AD patients with terminal disease, demonstrated a high correlation between *in-vivo* tracer uptake and the presence of β-amyloid at autopsy, as well as 96% sensitivity and 100% specificity in distinguishing HC from AD, thus suggesting that ^{18}F-Florbetapir PET provides an accurate and reliable assessment of amyloid burden [69]. A large study pooling data from the 4 registered phases I and II trials of florbetapir PET imaging, confirmed the ability of florbetapir uptake analysis to characterize amyloid levels in clinically probable AD, MCI, and HC groups using both continuous and binary quantitative measures of amyloid burden [70].

2. Methods

2.1. Study Inclusion Criteria. The general inclusion criteria for relevant research studies were the following:

(i) articles had to be published in a peer-review scientific journal;

(ii) studies reporting sensitivity and specificity measures in relation to a histopathological or clinical diagnosis of neurodegenerative diseases;

(iii) studies including large cohorts of subjects (see Table 1: early diagnosis FDG: range 20–395; Table 2: differential diagnosis FDG: range 45–297; Table 3: early diagnosis amyloid: range 13–107);

(iv) studies investigating the prediction of mild cognitive impairment (MCI) to Alzheimer's disease (AD) conversion that retrospectively analyzed the initial characteristics of those who were progressive and those who remained stable.

2.1.1. Specifically to FDG-PET. (i) Only articles published since 2001 were considered, which retain high quality by controlling to an optimal degree both clinical and methodological shortcomings.

(ii) Only studies employing voxel-based analysis techniques (such as SPM, Neurostat, and AD t-sum) with statistical parametric mapping procedures can provide unbiased, statistically defined measures of brain abnormality throughout the whole brain on a voxel-by-voxel basis; the basic procedure in voxel-based analysis involves the spatial normalization and smoothing of each individual's PET scan to an anatomically defined standard brain reference volume (the template or atlas volume) in the stereotactic space. This enables voxel-by-voxel statistical comparison of the ^{18}F-FDG pattern in the individual brain toward a reference control population. FDG uptake in each voxel must be previously normalized to the average uptake of a reference region, since without arterial blood sampling or other validated quantification methods, the standard PET procedure does not allow true quantitative measurements of glucose consumption. The reference region can change; the "default" reference region in SPM is the whole brain while Neurostat allows choosing among the whole brain, the cerebellum, and the thalamus. By changing the reference region, the results of parametric mapping may change as well. Final agreement on the region to be used is still lacking; the choice of whole brain tends to reduce sensitivity because the hypometabolic voxels are included in the average, while the cerebellum tends to increase sensitivity because it is less affected by neurodegeneration in AD. Taking in mind these limitations and that they do not allow true quantitative estimation of glucose metabolism but rather of glucose metabolism distribution, all these procedures result in an observer-independent mapping of regional abnormalities of glucose metabolism.

2.1.2. Specifically to Amyloid-PET. (i) Only articles reporting parameterization of β-amyloid deposition in patients with AD, MCI and normal controls either with short half-life ^{11}C-labeled ligands ^{11}C PIB and ^{18}F-labeled tracers (^{18}F-AV-45 Florbetapir, ^{18}F-BAY94-9172 Florbetaben, and ^{18}F-GE-067 Flutemetamol). Articles reporting quantification with other β-amyloid compounds have been excluded when (a) there was uncertainty about the selectiveness of the binding to amyloid plaques (e.g., ^{11}C BF-227) or (b) utilization of recently released compounds still needing for a systematic evaluation (e.g., ^{18}F-AZ4694, namely, NAV4694).

(ii) Furthermore, only articles using quantification methods such as distribution volume ratio (DVR) or standardized volume uptake ratio (SUVR) were included in the analysis. Similar to FDG-PET, to calculate the uptake without blood sampling, results are shown as ratios with a reference region, usually cerebellum (even though utilization of pons is currently debated [66] see also *Pet Amyloid Imaging studies paragraph*). Obviously the change of reference region can affect the results, but as a final agreement is lacking, this is up to the authors to rely on the affinity of the different compounds for multiple reference regions. As regards SUVR, to discriminate between "amyloid positive" and "amyloid negative" burdens (as well as between "low" and "high" retention), authors have been applying cut-off scores, usually obtained by control groups (like in [71] or using values reported in literature i.e., [72] for ^{11}C-PIB PET or [73] for ^{18}F-Florbetapir). Therefore, manipulating cut-off scores can heavily affect results, leading to radically different groups' characterization. Despite these variations in the methodology of amyloid quantification, automated algorithms can fairly discriminate between different patterns of retention, in an observer-independent fashion, leading to important advantages in clinical practice and diagnosis.

Table 1: Summary of included 18F-FDG-PET for early diagnosis and conversion prediction, with LHR, increase in LHR+, GRADE, and population.

Authors	Population	Method	Cohort investigated	Follow-up (months)	Sensitivity	Specificity	LHR+	Increase in the LHR+	Quality of evidence (GRADE)
Arnáiz et al., 2001 [79]	20MCI	ROI	20	36	0.67	0.82	3.72	Small	L
*Herholz et al., 2002 [27]	110 HC; 395 pAD	AD t-sum	395	—	0.93	0.93	13.29	Large	M
Mosconi et al., 2004 [80]	37 MCI	SPM	37	12	1	0.9	10.00	Moderate	M
Drzezga et al., 2005 [82]	30 MCI	SPM + Minoshima	30	16	0.92	0.89	8.36	Moderate	M
Anchisi et al., 2005 [17]	48 aMCI	SPM	48	12	0.929	0.824	5.28	Moderate	M
*Haense et al., 2009a [84]	89 AD; 102 HC	AD t-sum	89	—	0.83	0.78	3.77	Small	L
*Haense et al., 2009b [84]	237 AD; 37 HC	AD t-sum	237	—	0.78	0.94	13.00	Large	M
Yuan et al., 2009 [20]	280 MCI	Meta-analysis	280	14.25	0.888	0.849	5.88	Moderate	M
*Landau et al., 2010 [85]	85 MCI; 97 AD; 102 HC	SPM + ROI	97	—	0.82	0.7	2.73	Small	L
Brück et al., 2013 [86]	22 MCI	SPM + ROI	22	24	0.87	0.78	3.95	Small	L
*Arbizu et al., 2013 [87]	80 HC; 36 MCIc; 85 MCIs; 67 AD	Automated voxel-based analytical method	67	—	0.818	0.86	5.84	Moderate	M

Total number of patients and healthy controls considered in the study. Method: quantitative method applied in the study. Cohort investigated: number of patients considered for sensitivity and specificity estimations. Followup: duration of observational period (for early diagnosis study). Sensitivity and specificity: results of the study. LHR+: likelihood ratio. Increase in the LHR+: increase in the probability of the likelihood of the disease. GRADE: results of GRADE evaluation. Quality of evidence was evaluated based on LHR+ values, LHR+ increase probability, and size of the sample included.

Abbreviations: pAD: probable Alzheimer's disease; MCI: mild cognitive impairment; aMCI: amnestic mild cognitive impairment; MCIc: MCI converters; MCIs: MCI stable; HC: healthy controls.

*Studies including early diagnosis of AD.

Table 2: Summary of the included PET studies for differential diagnosis, with LHR+, increase of the LHR+, and GRADE.

Authors	Population	Method	Cohort investigated	Sensitivity	Specificity	LHR+	Increase in the LHR+	Quality of evidence (GRADE)
Minoshima et al., 2001 [42]	AD + LBD	Minoshima	74	0.9	0.8	4.50	Small	L
Gilman et al., 2005 [88]	AD + LBD	VOI lCMRglc	45	0.643	0.652	1.85	Minimal	VL
Foster et al., 2007 [33]	AD + FTD	Minoshima	45	0.732	0.976	30.50	Large	M
Mosconi et al., 2008a [89]	AD + FTD	Minoshima	297	0.99	0.65	2.83	Small	L
Mosconi et al., 2008b [89]	AD + LBD	Minoshima	226	0.99	0.71	3.41	Small	L
Mosconi et al., 2008c [89]	AD + HC	Minoshima	199	0.99	0.98	49.50	Large	M
Mosconi et al., 2008d [89]	FTD + LBD	Minoshima	125	0.71	0.65	2.03	Small	L

Population: different dementias considered in the diagnosis. Method: quantitative method applied in the study. Cohort investigated: number of patients considered for sensitivity and specificity estimations in the discrimination. Sensitivity and specificity: results of the study data show potential of discrimination. LHR+: likelihood ratio. Increase in the LHR+: increase in the probability of the likelihood of the disease. GRADE: results of GRADE evaluation. Quality of evidence was evaluated based on LHR+ values, LHR+ increase probability, and size of the sample included.
Abbreviations: AD: Alzheimer's disease; LBD: Lewy body dementia; FTD: frontotemporal dementia; HC: healthy controls.

Table 3: Summary of the included amyloid-PET studies included with LHR and GRADE analysis.

Authors	Population	Method	Cohort investigated	Follow-up months	Sens.	Spec.	LHR+	Increase in the LHR+	Quality of evidence (GRADE)
Barthel et al., 2011 [96, 97]	81 AD; 69 HC	ROI SUVR analysis	81	—	0.85	0.91	9.44	Moderate	M
Rabinovici et al., 2011 [34]	62 AD; 45 FTD	ROI DVR analysis	107	12	0.89	0.83	5.24	Moderate	M
Rostomian et al., 2011 [58]	42 AD; 31 FTD	ROI DVR analysis	73	16	0.905	0.84	5.66	Moderate	M
Rowe et al., 2008 [93]	15 AD; 5 FTD; 15 HC	SUVR analysis	20	12	1	0.9	10.00	Moderate	L
Villemagne et al., 2011 [12]	30 AD; 20 MCI; 32 HC; 11 FTD; 7 LBD; 5 PD; 4 VaD	SUVR analysis	30	—	0.97	0.84	6.06	Moderate	M
Clark et al., 2012 [102]	5 MCI; 29 AD; 12 HC; 13 ODD	SUVR analysis	47	24	0.97	0.99	97.00	Large	M
Camus et al., 2012 [29]	13 AD; 12 MCI; 21 HC	SUVR + Visual	13	—	0.923	0.905	9.72	Moderate	VL
Koivunen et al., 2011 [62]	29 MCI; 13 HC	PiB retention analysis	29	24	0.94	0.42	1.62	Minimal	VL
Mosconi et al., 2009 [19]	31 MCI	ROI ratio SPM	31	32.16	0.93	0.76	3.88	Small	L
Forsberg et al., 2010 [100]	37 mild AD; 21 MCI	ROI ratio SPM	58	33	1	0.71	3.45	Small	L
Jack et al., 2010 [46]	53 MCI	DVR	53	20.4	0.83	0.46	1.54	Minimal	VL
Wolk et al., 2009 [101]	23 MCI	DVR SPM	23	21	1	0.56	2.27	Small	L

Population: total number of patients and healthy controls considered in the study. Method: quantitative method applied in the study. Cohort investigated: number of patients considered for sensitivity and specificity estimations. Followup: duration of observational period (for early diagnosis study). Sensitivity and specificity: results of the study. LHR+: likelihood ratio. Increase in the LHR+: increase in the probability of the likelihood of the disease. GRADE: results of GRADE evaluation. Quality of evidence was evaluated based on LHR+ values, LHR+ increase probability, and size of the sample included.
Abbreviations: AD: Alzheimer's disease; FTD: frontotemporal dementia; MCI: mild cognitive impairment; ODD: other dementia; LBD: Lewy body dementia; VaD: vascular dementia; HC: healthy controls.

2.2. Meta-Analysis and GRADE Analysis

2.2.1. GRADE Evaluation. Scientific evidences available regarding each of the tests (^{18}F-FDG-PET or amyloid-PET) for the early and differential diagnosis of AD, as well as for MCI conversion prediction, are graded in terms of Level of Confidence (LoC: VL = very low, L = low, M = moderate, and H = high), as reported by GRADE system [74–76]. Tables 1, 2, and 3 show the level of confidence ratings assigned to the studies reviewed in this paper, indicating that none of the studies was rated high whereas most studies were rated moderate to low.

It is to be mentioned that according to the GRADE system, the best way to assess any "diagnostic strategy" is randomized controlled trials in which investigators randomize patients to experimental or control diagnostic approaches in order to provide high quality evidence of test accuracy for the development of recommendations about diagnostic testing.

Both the clinical context and complex implementation of brain FDG or amyloid-PET protocols, however, paralleled with ethical issues raised by the degree of invasiveness of both procedures, are not comparable to randomized trials or many observational studies in which the alternative diagnostic test has been carried out in order to establish high quality of evidence or clear differences in patient important outcomes based on GRADE framework.

Furthermore, it must be acknowledged that the results of FDG- or amyloid-PET diagnostic approaches do not have nothing to do with effective treatments (as the usual GRADE evaluative study set); however, they may have a significant positive impact in terms of patient outcomes, such as reducing the treatment gap between AD pathological onset and diagnosis of the disease, thus improving ability to plan which can be considered analogous to an effective patient treatment [77]; the correct diagnostic inclusion of patients in pharmacological trials [78], the appropriate family context, and behavior induced by the diagnosis are very useful in supporting pharmacological and cognitive remediation approaches.

Notwithstanding the here selected criteria for investigations employing FDG- or amyloid-PET brain imaging have been rated only as "low" or "moderate" quality evidence for recommendations about diagnostic procedures in a GRADE system, we have to consider that there will be great indirect benefits for their "patient-outcome" (i.e., test accuracy in terms of sensitivity and specificity). Assessing the directness of evidence supporting the use of a diagnostic test requires judgments about the relationship between test results and patient-important consequences, therefore in this paper a severe challenge arose in the attempt to apply GRADE to two crucial questions about FDG- or amyloid-PET as accurate, valid and powerful diagnostic tests, for (1) the early diagnosis and (2) the differential diagnosis of AD.

Guyatt et al. [76] stated that "GRADE will disappoint those who hope for a framework that eliminates disagreements in interpreting evidence and in deciding on the best among alternative courses of action. Although the GRADE system makes judgments about quality of evidence and strength of recommendations in a more systematic and transparent manner, it does not eliminate the need for judgments."

That is, applying a GRADE system in a PET functional and molecular imaging evaluation for diagnosis can be accepted due to the high value for low and moderate results in such a setting.

In this survey, we performed three different meta-analyses for evaluating the accuracy and effectiveness of diagnostic tests (i.e., FDG or amyloid), in order to make a judgment about quality of evidence (GRADE) on the early or differential diagnosis and for conversion prediction of dementia in our population. Given that the sensitivity of a test shows the proportion of patients with the disease (i.e., AD) whom the test classifies as positive while the specificity shows the proportion without the disease (i.e., no neurodegenerative disease) whom the test classifies as negative, we computed the positive likelihood ratio for each study included in the three meta-analyses, (i.e., FDG-PET or amyloid-PET imaging in the early diagnosis of Alzheimer disease and FDG-PET in the differential diagnosis of Alzheimer disease) which combines information from sensitivity and specificity and gives an indication of how much the odds of disease change based on a positive or a negative result (i.e., accuracy). For example, a positive likelihood ratio of 10 means that a positive test result is ten times more likely in a diseased subject than in a healthy person. The resulting positive likelihood ratio (LR+) for each study was interpreted according to general guidelines for evaluating the probability increase of detecting the disease through a test (i.e., LR+ > 10 = large; 5 > LR+ > 10 = moderate; 2 > LR+ > 5 = small; 1 > LR+ > 2 = minimal; 0 > LR+ > 1 = no increase). Available scientific evidence regarding each of the topics was graded in terms of level of confidence (LoC: VL = very low, L = low, M = moderate, and H = high), as reported by the GRADE collaboration [74, 75]. In the GRADE system, valid diagnostic accuracy studies can provide high quality evidence of test accuracy. Quality of evidence (GRADE) for each study was evaluated based on LR+ values, LR+ probability increase, and the size of the sample included for each study (i.e., e.g., a study with a moderate LR+ probability increase but with a relatively small sample ($n = 20$) would be rated as low in terms of quality of evidence) (see Tables 1, 2, and 3).

In addition, we obtained a summary measure of effectiveness in each meta-analysis by weighting individual study effect measures according to their variance and by adopting a general inverse-variance weighted fixed-effects model to summarize individual effect measures (i.e., sensitivity analysis) and a Q test was performed to measure heterogeneity among studies. Sensitivity measures for each study were then arranged in a forest plot together with their 95% confidence intervals In order to represent the position of each study included over the central tendency, represented by the calculated summary fixed-effect sensitivity measure (see Figures 1(a), 1(b), and 1(c)).

2.3. Qualitative versus Quantitative Assessment. A description of differences in the levels of sensitivity and specificity for the standard visual FDG-PET scan or dichotomous

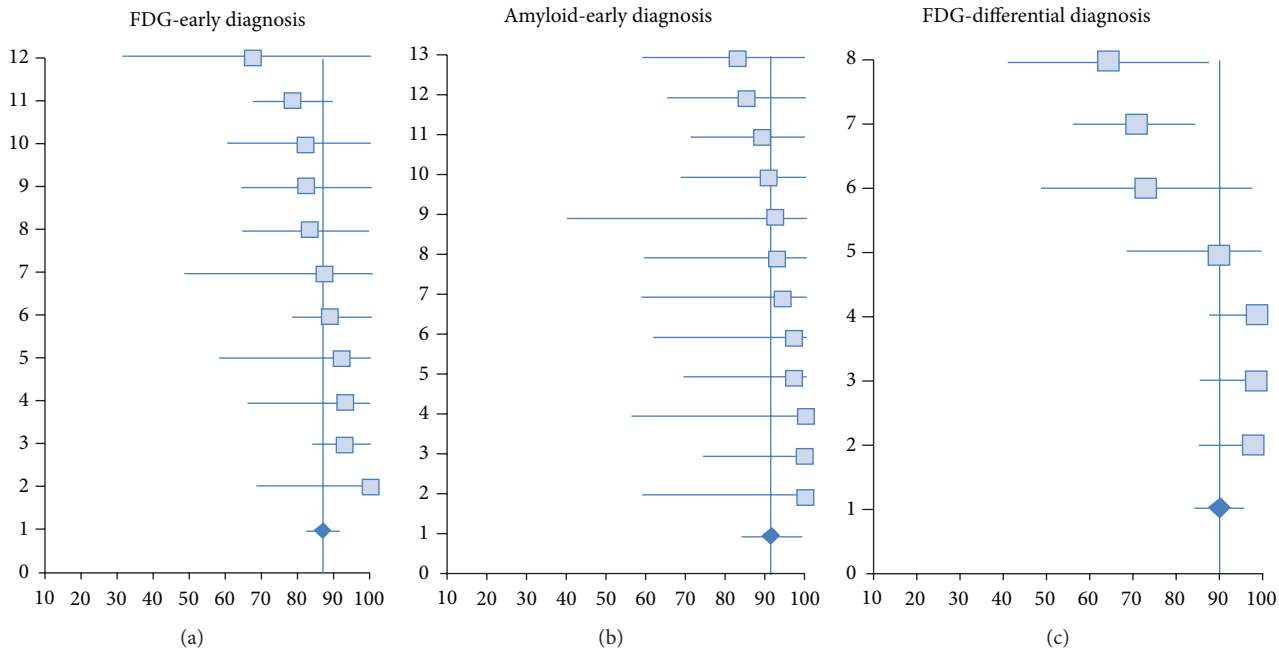

Figure 1: Forest plots of sensitivity measures and 95% confidence intervals for individual studies included in each meta-analysis.

readout based amyloid- PET with respect to parametric or semiquantitative analysis was performed on the basis of the data in literature reporting sensitivity and specificity of both the visual and the parametric methods in the same population.

3. Results

3.1. ^{18}F-FDG PET in the Early Diagnosis of AD. The systematic review identified a total of 10 studies that met our inclusion criteria (see Table 1); the most relevant findings were as follows.

Arnáiz et al. [79] showed that, in a cohort of $N = 20$ MCI followed for a mean observational period of 36 months, reduced glucose metabolism from left temporoparietal area could predict conversion with a 75% percentage of correct classification, resulting in 67% sensitivity and 82% specificity. Authors conclude that these measures of temporoparietal metabolism may aid (together also with neuropsychological data) in predicting evolution of MCI patients to AD.

In a landmark study, Herholz and colleagues [42] investigated metabolic abnormalities with ^{18}F-FDG-PET in a cohort of $N = 110$ HC and $N = 395$ probable AD. Despite the cross-sectional nature of the study, useful information was provided about an early diagnosis of AD because of the fragmentation of the pAD group in different subgroups related to probable disease severity (e.g., very mild probable AD group, MMSE ≥24). Authors calculated an AD t-sum score for each individual, and this score was applied to discriminate between various subgroups and controls. This method yielded 93% sensitivity and 93% specificity in classification of pAD versus HC, acting as a very useful tool to early diagnosis of AD.

Similarly, Mosconi and colleagues [80] followed a group of $N = 37$ MCI patients for a 12-month period. At the followup, $N = 8$ MCI converted while $N = 29$ remained stable. Authors analyzed, with a voxel-based method and analysis of variance, regional differences in cerebral glucose metabolism, using conversion (y/n) as outcome and APOE genotype (E4+/E4−) as grouping factor. Results show that for the whole MCI sample, inferior parietal cortex hypometabolism could predict conversion to AD with 84% diagnostic accuracy, 100% sensitivity, and 95% specificity. Furthermore, E4 carriers (E4+) converters ($N = 5$) presented significantly decreased metabolism in frontal areas, such as anterior cingulate cortex (ACC) and inferior frontal cortex (IFC). The authors' conclusion is that ^{18}F-FDG-PET may improve prediction of the MCI-AD conversion especially when combined with APOE genotype information.

Anchisi and coworkers [17] investigated in a longitudinal study a cohort of $N = 67$ amnestic-MCI patients of which $N = 48$ underwent follow-up examination at a (at least) 12-month interval. The ROC curve calculated for the glucose metabolism measured in two voxel ROIs (posterior cingulate and temporoparietal) showed an area under the curve (AUC) of 0.0863. With a cut-off at 1.138, authors reported 92.9% and 82.4% as, respectively, sensitivity and specificity in discriminating converters versus nonconverters. In addition, negative predictive value of 96.55% and a positive predictive value of 68.4% were reported. Furthermore, authors combined functional metabolism impairment with memory test score (Long free delay recall part of the California verbal learning test, CVLT-LFDR) [81] showing an inverse pattern: lower sensitivity (85.7%), higher specificity (97.1%), lower negative predictive value (94.3%), and a higher positive predictive value (92.3%). Authors claim that using

^{18}F-FDG-PET may help in predicting short-term conversion to AD, particularly combined with memory scores and also to account for the functional heterogeneity among subjects with aMCI.

Drzezga and coworkers [82] in a longitudinal prospective study on 30 MCI patients (mean observation period, 16 months) assessed the value of FDG-PET in detecting brain metabolic abnormalities in early AD, by using Neurostat [83] to perform an observer-independent statistical comparison with an age-matched reference database. The authors reported that the sensitivity and specificity of FDG-PET with regard to early diagnosis of AD in MCI patients were 92% and 89%, respectively.

Haense et al. [84] also investigated performance of ^{18}F-FDG-PET for detection of AD within two different samples, from ADNI and Network for Standardisation of Dementia Diagnosis (NEST-DD). The cohort from ADNI consisted in $N = 102$ HC and $N = 89$ AD, while the sample from NEST-DD comprised $N = 36$ HC and $N = 237$ AD. The authors generated AD t-sum maps and used a preset cut point for discrimination. Results were twofold: (1) AD presented much higher AD t-sum maps than HC in both samples and (2) early onset-AD presented higher AD t-sum maps than late-onset AD. The cut-off threshold yielded sensitivity and specificity of 83% and 78%, respectively, in ADNI; in NEST-DD, results showed 78% sensitivity and 94% specificity. Authors conclude that this automated procedure to analyze ^{18}F-FDG-PET scans is useful for the discrimination and is also more accurate for early onset AD.

Yuan and colleagues [20] performed a meta-analysis to evaluate and compare the ability of FDG-PET, single-photon emission tomography (SPECT), and structural MR imaging to predict conversion to AD in patients with MCI. Relevant studies were identified with MEDLINE from January 1990 to April 2008 and a meta-regression was carried out from eligible studies on the diagnostic performance data for each technique. This study included data from 1112 MCI patients (of which $N = 280$ investigated by FDG-PET) and showed that FDG-PET had better concordance with follow-up results for the prediction of conversion to AD dementia. Approximately 88.9% of the patients with progressive MCI were detected as positive by FDG-PET, whereas 84.9% of stable patients had negative FDG-PET at first scanning time (sensitivity 88.9%, specificity 84.9%). Further, FDG-PET was found to perform better than SPECT and structural MR imaging in the prediction of conversion to AD in patients with MCI.

Recently, Landau and coworkers [85] compared different biomarkers of conversion and decline in MCI investigating a fairly large cohort throughout the different predictors (FDG-PET, MRI/hippocampal volume, CSF biomarkers, Memory Score/Rey Auditory Verbal Learning Test). As regards ^{18}F-FDG-PET, $N = 85$ MCI were followed for a period of (mean) 21 months. During the observation period, $N = 28$ converted (MCIc) while $N = 57$ remained stable (MCIs). To evaluate the power of the prediction with ^{18}F-FDG-PET measured metabolism (parametrically analyzed with SPM, metaROI global index), the authors obtained cut-off scores from an independent sample rather than using cut-off scores present in literature. To do so, $N = 102$ Healthy controls and $N = 97$ AD were screened, resulting in a cut-off set at 1.21 to discriminate between "AD(+)" and "AD(−)". ROC curve at this score showed 82% sensitivity, 70% specificity and an overall accuracy of 76% in discriminating between AD and controls. Thereafter, the derived cut-off was used to calculate predictive values of conversion for the MCI group, resulting in a positive predictive value of 41% and a negative predictive value of 78%. To say, the 78% of MCI classified as "AD(−)" at baseline remained stable, whereas MCI classified as "AD(+)" had a 2.72 greater risk of conversion. Then authors concluded that the FDG-PET was the most informative biomarker, especially when combined with RAVLT episodic memory score.

In a longitudinal study comparing ^{11}C-PIB-PET, ^{18}F-FDG-PET and MRI, Brück and coworkers [86] investigated MCI conversion in a sample of $N = 29$ MCI (of which, only $N = 22$ underwent also ^{18}F-FDG). Clinical follow-up was carried on at a 24-months interval. During the observation period $N = 13$ MCI converted to AD while $N = 9$ MCI remained stable. All the ^{18}F-FDG-PET were optimized and analyzed with region of interest approach and SPM methodology, deriving a cut-off of 1.16 in left lateral temporal cortex (internally derived). This cut point was used to classify patients in "High" and "Low" ^{18}F-FDG, resulting in a sensitivity of 87% and a specificity of 78% in predicting conversion to AD. Similarly, patients were divided in "High" and "Low" ^{11}C-PIB depending on PiB uptake in lateral frontal cortex (internally derived cut-off: 1.57), providing 65% sensitivity and 75% specificity. When combined, ^{18}F-FDG and ^{11}C-PIB (e.g., Low FDG-High PiB) resulted in 87.5% sensitivity and 71.4% specificity. The authors' claim is that ^{18}F-FDG and ^{11}C-PIB are better than hippocampal volume in predicting conversion.

Arbizu and colleagues very recently [87] proposed a new score for automated analysis of ^{18}F-FDG-PET, called AD-Conv score, as a tool for single-subject prediction of conversion to AD. Their cohort comprised $N = 80$ HC, $N = 121$ MCI of which $N = 36$ MCIc (at 18-months interval) and $N = 85$ MCIs (at 24-months interval) and $N = 67$ AD. Briefly, their method consisted in generating an "AD-PET-pattern" from an external reference population and based on z-score map obtained with SPM. This map was then compared with individual hypometabolism voxel-by-voxel resulting in an AD-PET-index, that combined with age and gender generated the AD-Score. Starting from this score, meant to discriminate between AD and HC, authors generated a score to discriminate between MCIc and MCIs applying several modifications. First, instead of using a whole brain z-map (the AD-PET-pattern), AD-PET index was segmented in five volumes-of-interest (VOIs), namely left parietal, right parietal, left temporal, right temporal and posterior cingulate, and then compared with individual hypometabolism resulting in the MCI-PET-Index. Furthermore, to compute the score, APOE genotype (E4+/E4−), years of education and MMSE were combined with age obtaining the AD-Conv-Score. Further statistical analysis

showed that only hypometabolism in posterior cingulate area was significant in differentiating MCIc from MCIs and, together with APOE4 genotype and MMSE, yielded the AD-Conv-Score parameter. With an AD-Conv cut-off score at 0.28, the method classified MCIc and MCIs with 91.7% sensitivity and 62.4% specificity. As regards predictive values, a positive predictive value of 51% and a negative predictive value of 95% were shown.

3.2. ^{18}F-FDG PET in Differential Diagnosis between Forms of Dementia. A total of 4 papers addressing the discrimination power of FDG-PET between different neurodegenerative forms met the criteria outlined above (see Table 2). Among the studies pinpointed in Table 2, three studies included patients with a clinical diagnosis of probable AD, three studies included patients diagnosed with Lewy-Body Dementia (LBD), and two studies included patients with a diagnosis of Frontotemporal lobar degeneration (FTLD).

Minoshima et al. [42] examined brain glucose metabolism of DLB and AD and showed that FDG-PET discriminates DLB from AD with 90% sensitivity and 80% specificity using autopsy confirmation. They also concluded that the presence of occipital hypometabolism preceded some clinical features of DLB and that FDG-PET sensitivity was superior in differentiating DLB from AD with respect to medical charts exclusively based on clinical diagnostic criteria.

Similarly, Gilman and coworkers [88] investigated metabolism differences between AD and DLB measured with ^{18}F-FDG-PET in a sample of $N = 25$ AD, $N = 20$ DLB and $N = 19$ elderly HC. ^{18}F-FDG scans were analyzed with Minoshima method on selected VOIs (global cortex and occipital cortex, known to discriminate between DLB and AD in terms of CMRglc). Furthermore discrimination power was estimated also for neuropsychological scores such as MMSE, confrontation naming test and verbal fluencies. Logistic regression showed that glucose metabolism in BA17 (visual cortex) presented 64.3% sensitivity and 65.2% specificity for diagnosis of DLB. To say, the hypometabolism patterns of these two diseases were similar except for the metabolic rate in visual cortex.

In the widely cited study by Foster et al. [33] the utility of ^{18}F-FDG statistical parametric maps rather than simple transaxial FDG-PET scans for dementia diagnosis was evaluated. Six experienced raters were forced to make a diagnosis about a cohort of $N = 45$ patients, all pathologically confirmed, of which $N = 31$ AD and $N = 14$ FTD. Results showed that the utilization of ^{18}F-FDG statistical maps (stereotactic surface projection maps SSP) yielded high diagnostic accuracy (89.6%), showing 73% sensitivity and 97.6% specificity. Authors conclude that also after a brief training in visual interpretation of ^{18}F-FDG statistical maps this method is more reliable and accurate than clinical methods alone.

Mosconi and colleagues [89], in a large multicenter study, examined FDG-PET measures in the differentiation of AD, FTD, and DLB from normal aging and from each other ($N = 548$ subjects, including 111 healthy individuals). Each PET scan was Z-transformed by using automated voxel-based comparison resulting in statistical maps of disease-specific patterns of brain ^{18}F-FDG uptake. The differentiation and classification of patients in independent groups between patients and controls and among dementia forms yielded 99% sensitivity, 65% specificity (97% accuracy) for AD compared with FTD; 99% sensitivity, 71% specificity (97% accuracy) for AD compared to DLB; 99% sensitivity, 98% specificity (98% accuracy) for differentiating between AD and healthy controls; 71% sensitivity, 65% specificity (68% accuracy) for DLB with respect to FTD. Thus, this study strongly supported the validity and diagnostic accuracy of FDG-PET in the differential diagnosis of the three major neurodegenerative disorders.

3.3. FDG-PET Summary. These data provide strong evidence for FDG-PET parametric imaging to detect pathological changes occurring in the brain. FDG-PET holds great promise for diagnostic assessment of patients with Alzheimer disease (AD) and the other two major neurodegenerative diseases (i.e., DLB and FTLD) to the point that the recently revised diagnostic criteria of AD [5, 9] as well as the new National Institute of Aging-Alzheimer Association criteria of MCI due to AD [6] for the first time recognize the specific role of FDG-PET as a topographical functional biomarker in Alzheimer disease definition. What is especially relevant in this context is that FDG-PET as a neurodegeneration biomarker has been placed before brain atrophy in specific regions, as shown by means of MRI, in the hypothetical cascade model of AD biomarkers [46]. In fact, FDG-PET maps distribution of glucose metabolism occurring mainly at synaptic level [90]. Thus, pathologic phenomena leading to neuritic dysfunction affects synaptic glucose consumption prior of causing cell death and detectable atrophy [91, 92]. As such, FDG-PET is a proxy of reduced glucose utilization at synaptic level of still alive neurons.

It must be acknowledged that voxel-based procedures for objective image analysis can now be easily applied clearly providing evidence for a role of FDG-PET in assessment of dementia through the identification of disease-specific hypometabolic patterns. The main advantages of automatic methods consist in the fact that images can be interpreted even by intermediate-skilled readers and that false positive results are virtually eliminated, thus increasing specificity.

The primary objective of both tabulated surveys was to select studies on the basis of the mandatory need for the evaluation of the FDG-PET scans based on an automatic, unbiased voxel-based analysis in order to achieve higher confidence in diagnostic accuracy to significantly reduce the gap with post-mortem gold standard confirmatory diagnosis. The evidence provided in the tabulated surveys supports the role of FDG-PET as an effective tool aiding in the early diagnosis and differential diagnosis of dementia. The diagnostic accuracy of FDG-PET resulted to be high also in subjects with prodromal disease, for whom the clinical diagnosis and differential diagnosis are especially challenging. In fact, [1] claimed that "the sensitivity and specificity available with

FDG-PET near the time of initial diagnosis of AD is similar to longitudinal clinical diagnosis over 3-4 years".

3.4. Amyloid-PET in the Diagnosis of AD. The systematic review identified a total of 12 studies that met our inclusion criteria (see Table 3); the most relevant findings were as follows.

In their study, Rowe and coworkers [93], investigated the reliability of the ^{18}F-BAY94-9172 (Florbetaben) in a relatively small cohort (N = 15 AD, N = 15 HC and N = 5 FTD) in discriminating between the three conditions. Authors analyzed quantitatively the neocortex uptake with SUVR measure, using the cerebellum as reference region. Experienced raters then visually inspected the maps of SUVR distributions. Visual inspection of SUVR maps yielded 100% sensitivity and 90% specificity in discriminating AD versus HC or FTLD. Authors conclude that florbetaben imaging can be included successfully in clinical use.

Using ^{18}F-Flutemetamol PET scan in 25 HC, 20 MCI and 37 AD patients, Vandenberghe et al. [94] using SUVR distributions showed 93.1% sensitivity and 93.3% specificity and a very high correlation with ^{11}C-PIB uptake (r = .89) for visual inspection. It is noteworthy that sensitivities and specificities did not differ significantly between qualitative (visual) and quantitative methods (SUVR cutoff automated classification in raised uptake category). Further, it has been shown that the tracer uptake highly correlated with percentage of brain area of amyloid measured by cortical biopsy [95].

Barthel and colleagues [96, 97] investigated the use of ^{18}F-Florbetaben (^{18}F-BAY94-9172) PET analysis in two contiguous studies (phase 0 and 2) involving 69 HC and 81 AD patients and found that visual assessment of PET images allowed 80% sensitivity and 91% specificity. On the other side, linear discriminant analysis of regional SUVR yielded an 85% sensitivity and 91% specificity. The same tracer has been demonstrated to be useful in discriminating different forms of dementia as well as patients from controls [12, 93]. The first results on florbetaben indicate that this radiopharmaceutical, while having a narrower dynamic range than ^{11}C-PiB PET, is able to clearly differentiate HC from AD patients with a comparable effect size [98]. Moreover, quantification of β-amyloid binding from florbetaben PET data is feasible and all β-amyloid binding parameters including SUVR are excellent in discriminating between β-amyloid positive and negative scans [99].

In the study by Rostomian et al. [58], ^{18}F-FDG and ^{11}C-PIB were compared to evaluate the power of diagnosis of the *in vivo* imaging of fibrillar beta-amyloid versus metabolism or CSF. The authors tried first in a test cohort composed by N = 10 patients with various clinical diagnosis and, when identified the correct iterative algorithm, analyzed a sample of N = 42 AD and N = 31 FTLD with both FDG-PET and C-PIB PET (these map were obtained from t-test with reference regions, such as cerebellar for PiB). Results showed that with PIB PET had 90.5% sensitivity and 83.9% specificity (for AD), versus the, respectively, 88.1% and 83.9% with FDG-PET. Temporal pole and neocortex was significant for both the compounds, whereas the frontal lobe was particularly significant for PIB-PET. Authors conclude that the combined use of these two compounds can be very useful for early diagnosis of AD.

Other amyloid-PET studies addressing AD and MCI cases in large series came out in the literature reporting high sensitivity and intermediate/low values of specificity [21, 46, 62, 100, 101].

In the study by Villemagne et al. [12] authors still evaluated ^{18}F-Florbetaben in imaging AD versus other dementia types. Their cohort consisted in N = 32 HC, N = 20 MCI, N = 30 AD, N = 11 FTD, N = 5 LBD, N = 5 Parkinson's Disease (PD) and N = 4 Vascular Dementia (VaD). SUVR values for whole brain neocortical retention were calculated using cerebellar cortex as reference region. Results showed that almost all of the AD group (96%) and more than half of the MCI group (60%) presented diffuse cortical retention whereas the other groups presented far minor cortical retention (FTLD = 9%, VaD = 25%, DLB = 29%, PD = 0%, HC = 16%). Semiquantitative SUVR analysis yielded a 97% sensitivity and 84% specificity in discriminating AD versus Healthy Controls. Authors conclude that ^{18}F-Florbetaben can be useful in distinguishing AD from other dementias (e.g., FTLD) and that its effectiveness is comparable with the results obtained by ^{11}C-PiB compound.

In a prospective cohort study by Clark et al. aimed to compare florbetapir PET with neuropathology at autopsy for detecting neuritic amiloid-β plaques, also the relation between SUVR and neuritic plaque density was assessed [102]. Based on values from a series of young participants who were cognitively normal, Joshi et al. [73] had previously proposed a cutoff value of 1,10 to distinguish normal from abnormal scans. In the paper of Clark et al., all the cases with no or sparse plaques at autopsy had SUVR values of less than 1,10, and all but one with moderate or frequent plaques at autopsy had SUVR values greater than 1,10. SUVR analysis showed a 97% sensitivity and 99% specificity in detecting high or low burden of amyloid plaques with a 24-months autopsy reference.

Using PET with florbetapir to quantify brain amyloid load in a routine clinical environment to differentiate between patients with mild to moderate AD and MCI from HC, the quantitative assessment of the global cortex SUVR reached a sensitivity of 92.3% and specificity of 90.5% with a cut-off value of 1.12 [29].

3.5. Amyloid-PET Summary. Up to date, the literature demonstrates that ^{11}C-PiB PET allows reliable detection and in particular quantification of β-amyloid deposition in patients with AD.

However, because of the short half-life of ^{11}Carbon, which requires an on-site cyclotron and radiochemistry laboratory, ^{11}C-PiB has been compared with ^{18}F-labeled tracers like ^{18}F-Florbetapir, ^{18}F-Flutemetamol or ^{18}F-Florbetaben, which can be produced at central cyclotron and then delivered to clinical PET centers.

^{18}F-Florbetapir and ^{18}F-Flutemetamol are FDA approved in the US for clinical use, now also ^{18}F-Florbetapir by the EMA, whereas ^{18}F-florbetaben has not yet been approved in USA and Europe. These tracers could be largely used in detecting β-amyloid deposition and in distinguishing patients with AD from Frontotemporal dementia. As a limit, lipophilic plasma metabolites, which have been partially reported for ^{18}F-labeled tracers, could increase non-specific background activity.

The results of these included studies show a promising role of those ^{18}F-labeled tracers, but further data on larger number of patients also evaluated longitudinally are needed to clarify their diagnostic and prognostic potential roles in AD.

A central issue in PET estimation of amyloid load regards the use of semiquantitative analyses of images. In this view, a consensus regarding categorization of positive and negative subjects has not been established so far. For example, some groups have treated SUVR as a continuous variable whereas other groups have dichotomized subjects into positive and negative groups using a cut-off score, since the distribution of this variable is usually skewed. Further, there is variability in categorization approaches amongst studies that dichotomize into positive and negative groups. Some authors considered positive those subjects showing SUVR values that are 1, 1.5 or 2 standard deviations higher than normal controls [34, 56, 103–105], while others used more complex approaches such as cluster analyses [12, 48, 106, 107], iterative outlier removal [108] or complex functions [94]. SUVR cut-off values separating negative from positive subjects vary in the literature from 1.1 to 1.6, with a mean value around 1.3. The limit of classifying into positive and negative subjects relies on the fact that the threshold is often dependent on the distribution of SUVR values present in the control group under investigation rather than on a group of subjects lacking Aβ deposition.

In a recent study, ^{11}C-PiB and florbetapir PET were compared in a retrospective sample of cognitively normal older controls, patients with MCI, and patients with AD. ^{11}C-PiB and florbetapir retention ratios were strongly associated in the same individuals, and the relationship was consistent across several data analysis methods, despite scan-rescan intervals of more than a year. The findings of this study indicate that cutoff thresholds for determining positive or negative amyloid-β status can be reliably transformed from PIB to florbetapir units or vice versa using a population scanned with both radiopharmaceuticals [71].

Nordberg et al. [22] in a European multicentre PET study of fibrillar amyloid in AD based on very large datasets demonstrated the robustness of [^{11}C]-PIB PET as a marker of neocortical fibrillar amyloid deposition in brain when assessed in a multicentre setting. The variance of [^{11}C]PIB retention between different participating centers was low compared to the large differences between diagnostic groups, suggesting that results obtained from [^{11}C]PIB PET are highly consistent and reproducible. MCI PIB-positive patients showed more severe memory impairment than MCI PIB-negative patients and progressed to AD at an estimated rate of 25% per year. None of the MCI PIB-negative patients converted to AD, and thus PIB negativity had a 100% negative predictive value for progression to AD. This supports the notion that PIB-positive scans in MCI patients are an indicator of prodromal AD and that amyloid imaging is both a highly useful tool for diagnosis of AD in its earliest symptomatic stages and is suitable for identifying patients for antiamyloid therapy in multicentre clinical trials. The paper reports also the vast majority of healthy controls (46 out of 51) and showed neocortical [^{11}C]PIB retention ratios in the very narrow range of 1.13 to 1.39 (mean 1.26 ± 0.07). The upper 95% confidence limit in the normally distributed control population was 1.41, thus defining the normal limit.

One of the main issues since the advent of amyloid tracers remains and is represented by a percentage of HC showing an amyloid load in the range of patients with AD [22, 107, 109]. One of the future challenges in PET studies with ^{18}F amyloid tracers is to reach standardize quantitative measures (especially by means of longitudinal approaches) in order to establish reliable quantitative cut-offs that can be helpful in separating HC and AD subjects, in differential diagnosis of dementia and in providing prognostic indices for those subjects showing early signs of cognitive loss.

3.6. Qualitative versus Quantitative Assessment. Few papers in literature systematically investigated improvements in diagnostic accuracy and/or in differential diagnosis obtained by using quantified (or semiquantified) and qualitative analysis of FDG-PET scans. The results showed that the qualitative interpretation by visual reading of brain ^{18}F-FDG-PET scans and amyloid-PET scans clearly lacks clear-cut milestones to distinguish between a normal and a pathological scan. Indeed, in the already cited study by Foster and coworkers [33], authors compared five separate methods (clinical summaries, diagnostic checklist alone, summary and checklist, transaxial ^{18}F-FDG-PET scans and ^{18}F-FDG-PET stereotactic surface projection metabolic and statistical maps-SSP) for distinguishing AD from FTD in an autopsy-referenced cohort of $N = 31$ AD and M=14 FTD, adopted by six dementia experts. Data showed that the transaxial FDG-PET scans method yielded 96% sensitivity, 59% specificity and a mean accuracy of 84.8% in distinguishing AD versus FTD. On the other hand, the ^{18}F-FDG-PET SSP method improved sensitivity (97.6%), specificity (73.2%) and overall accuracy (89.2%). Authors conclude that ^{18}F-FDG-PET improves dementia diagnosis accuracy, especially when metabolism was quantitatively analyzed prior to visual expert rating and interpretation.

Recently, Rabinovici et al. [34] compared ^{11}C-PiB and ^{18}F-FDG in differential diagnosis of AD and FTLD in a cohort of $N = 62$ AD and $N = 45$ FTLD. It is noteworthy that the authors compared also qualitative (visual) and quantitative (DVR for ^{11}C-PiB, cut-off at 1.2 and regional ROI Z-score for ^{18}F-FDG) methods in their diagnostic efficacy. As regards qualitative evaluation of PET scans, ^{11}C-PiB PET yielded higher sensitivity for AD (89.5% versus 77.5%) and slightly lower specificity (83% versus 84%). Quantitative thresholds

for automated classification of scans provided interesting results. As a matter of fact, while ^{11}C-PiB PET DVRs yielded very similar results (89% sensitivity 83% specificity versus 89.5% sensitivity and 83% specificity), quantitative analysis of ^{18}F-FDG-PET increased specificity (98% versus. 84%). Authors conclude that with both methods ^{11}C-PiB PET was more sensitive, while ^{18}F-FDG-PET was more specific only when scans were interpreted quantitatively. Furthermore, a recent longitudinal study by Patterson et al. [35] showed that detection by Statistical Parametric Mapping (SPM) was more accurate ($N = 18$ subjects detected) than clinical evaluation of FDG-PET scans ($N = 10$ detected) in a cohort of $N = 31$ MCI followed for a 3-years period. Specifically, SPM detected correctly $N = 9$ MCI converters (versus $N = 5$ detected by subjective visual interpretation) and $N = 4$ subjects not meeting criteria for MCI (one of them was detected also visually), therefore highlighting a possible role for SPM in revealing metabolic defects anticipating clinical manifestations. Preliminary results in a study comparing inspection of visual FDG-uptake distribution maps and visual SPM hypometabolism maps in discrimination in a total cohort of $N = 95$ patients ($N = 45$ AD, $N = 30$ MCI, $N = 25$ FTLD) show higher sensitivity (96% versus 78%) and specificity (84% versus 50%) [110].

Other studies, even though not aiming as a primary endpoint to compare qualitative and quantitative analysis, provided results coherent with our claim. One of the most relevant findings is provided in the already cited study by Camus et al. [29] that investigated potential of ^{18}F-Florbetapir in discriminating AD versus HC. Their results showed that while visual assessment yielded 84.6% sensitivity and a 38.1% specificity, a quantitative global cortex SUVR analysis yielded 92.3% sensitivity and 90.5% specificity, with a cutoff point set at 1.122.

3.7. Meta-Analysis and GRADE Analysis. Tables 1, 2, and 3 show the characteristics of each study included in each meta-analysis, namely population sample, method employed, follow-up in months (i.e., only for early diagnosis), sensitivity and specificity measures, LR+, LR+ probability of increase, and GRADE evaluation [76, 77]. The total number of patients summed across all studies for each meta-analysis was computed and included 1322 patients for FDG-early diagnosis, 647 for amyloid-early diagnosis, and 1011 for FDG-differential diagnosis. Summary sensitivity effect measures were .86 for FDG-early diagnosis, .91 for amyloid-early diagnosis, and .90 for FDG-differential diagnosis. Q-test values for FDG-early diagnosis ($Q = 6{,}83$) and for amyloid-early diagnosis ($Q = 1, 94$) were below critical values assessed at $P < 0.05$, revealing low heterogeneity between studies included in each. The Q-value for studies included in the FDG-differential Diagnosis meta-analysis ($Q = 18.61$) was above critical values assessed at $P < 0.05$, indicating moderate heterogeneity. Forest plots for each meta-analysis show that the central tendency for the effectiveness of FDG-PET or amyloid-PET for the early or differential diagnosis of dementia is above 85%, however the 95% confidence intervals for studies FDG-early diagnosis reveal a lower degree of uncertainty with respect to amyloid-early diagnosis (see Figures 1(a) and 1(b)).

4. Discussion

Clinical, pathologic, and genetic evidence indicate that the primary dementias have different underlying aetiologies and pathogenetic mechanisms. Treatment approaches of these conditions are different and hopefully will be even more so in the future. Thus, accurate diagnosis is critical in order to maximize the efficacy and appropriateness of specific regimes. At present, best differential diagnosis of dementia relies on histopathological observations, usually available only at autopsy. When faced with a patient carrying a neurodegenerative disease possibly causing dementia, current guidelines suggest that the clinician must establish a probable etiopathogenic diagnosis based on evidence available from neurological and cognitive evaluation, blood tests, structural MRI neuroimaging, and PET imaging [5–8]. Attempts to differentiate between neurodegenerative diseases causing dementia based in the early prodromal phase can be hard, particularly when patients present with subtle prodromal symptoms or with clinical-neuropsychological characteristics that overlap between primary dementias or with an atypical profile of symptoms. Therefore, establishing valid and reliable markers of the main neurodegenerative diseases causing dementia which are capable to identify specific changes during the early clinical stages, or even in preclinical stages as it happens in genetic forms of AD, is a pivotal and strategical issue.

A decade ago, the American Academy of Neurology regarded CT and MR imaging as "optional" examinations for the diagnosis and evaluation of dementia [111]. This view was counterbalanced by a Consensus of the European Alzheimer Disease Consortium (EADC) in 2003, highlighting the changing philosophy on the role of neuroimaging in the dementia workup [112]. However, structural neuroimaging techniques, even if widely accepted and of high-value in the diagnosis and management, have no clear cut role in the very early stage of the diseases and at individual level. Attempts in measuring volumes of specific structures, such as the hippocampal formation, have been undertaken mainly in AD, with interesting results in group analysis, but still with lack of consistent and validated cut-off scores for individual analysis. In some neurodegenerative diseases other than AD, such as diffuse Lewy-body disease, MRI might present with multiple pattern of atrophy or even with null results in early stages. Thus, in the temporal dynamics of biomarkers in the Alzheimer's pathological cascade, atrophy represents the last phenomenon in comparison to biomarkers of brain dysfunction, early neurodegeneration, and amyloid deposition [46].

Functional neuroimaging techniques may aid in the early diagnosis of neurodegenerative disorders and to clearly support the final diagnosis. Positron emission topography (PET) allows the investigation of both the measurement

of cerebral glucose metabolism by ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) and the quantification of Aβ amyloid deposition through specific molecular imaging techniques involving radiopharmaceuticals binding to amyloid.

FDG-PET started to be used in AD about 30 years ago [37] but its role in the diagnostic road map of Alzheimer disease and related dementias has not gained general consensus up to few years ago. In fact, both the "Dubois" [5, 9] and the NIA-AA [6, 8] new diagnostic criteria have included FDG-PET as a valid tool for biomarker measure of neurodegeneration, by showing specific metabolic changes that precede atrophy as detected with MRI. The basic concept is that FDG-PET estimates glucose consumption at the synaptic-astrocyte level [90] thus picking-up very early changes already detectable even in asymptomatic subjects at high risk for AD [113, 114]. In AD, the core of such changes is the precuneus and the posterior cingulate cortex [17, 19], the MTL structures that are mainly highlighted with ROI-based than with voxel-based automatic approach, and the association posterior lateral parietal and temporal cortex. The same glucose utilization defect can be detected in other regions in FTLD [115, 116]; primary progressive aphasia (PPA) [117]; dementia with Lewy bodies (DLB) [88]. FDG-PET studies are therefore increasingly being used as an adjunct in the initial clinical evaluation of patients with suspected dementia, particularly to aid in early detection [17] or when clinical diagnosis is doubtful. As shown by the here included studies, voxel-based FDG-PET as *in vivo* biomarker measure plays a key role in the identification of early functional brain derangements. In this view, a recently introduced term designed to define the spectrum of cognitive function between healthy aging and dementia is mild cognitive impairment (MCI). It was [118] who first set out formal criteria for a diagnosis of MCI (subjective complaint of memory loss; objective impairment of ability; preserved general cognitive function; intact activities of daily living; individual does not meet criteria for dementia). People meeting these criteria are considered at higher risk of developing AD compared to general population [119]; consequently, MCI is considered the optimal clinical stage for both early detection and intervention of AD. More recently, the position paper by the International Working Group for New Research Criteria for the Diagnosis of AD [5] further introduced new concepts and distinguished between (i) preclinical states of AD, in which individuals are free of symptoms, yet have either biomarker evidence of Alzheimer's pathology or a monogenic form of AD and (ii) prodromal or predementia AD, referring to those clinically affected individuals who do not have dementia yet but are diagnosed to have AD on the basis of evidence of Alzheimer's pathology from biomarkers.

With regard to degenerative diseases such as AD, physicians' confidence in diagnosing dementia can be undermined by several factors such as young age of onset, high education level (where neuropsychological tests can fail to reveal a subtle, despite substantial, cognitive decline), atypical presentation, and presence of psychiatric or cognitive comorbidities. The information provided by FDG-PET can therefore satisfy a fundamental need not only as a disease confirmatory test (high sensitivity) but also as an exclusion test (high specificity), especially in the early stage of the disease.

On this regard, an international consortium of investigators argued that, due to its high sensitivity, a negative (i.e., normal) FDG-PET scan strongly favors a normal outcome at followup [1, 10].

Two decades of ^{18}F-FDG-PET studies in neurodegenerative diseases provided evidence for specific metabolic patterns [3].

Teune and colleagues [2] in a large study focusing on patients who had an FDG-PET scan at an early disease stage (96 patients: 20 patients with Parkinson's disease (PD), 21 with multiple system atrophy (MSA), 17 with progressive supranuclear palsy (PSP), 10 with corticobasal degeneration (CBD), 6 with dementia with Lewy bodies (DLB), 15 with Alzheimer's disease (AD), and 7 with frontotemporal dementia (FTD)) summarized the typical metabolic dysfunction in the different diseases. Each patient received a retrospectively confirmed diagnosis according to strictly defined clinical research criteria. FDG-PET images of each patient group were analyzed and compared with healthy controls using statistical parametric mapping (SPM5). The authors concluded that a combined method, including clinical information and voxel-based analysis technique, can discriminate patient groups across a spectrum of various neurodegenerative brain diseases, also at early disease stages. This implies that an early and more accurate diagnosis in individual patients can be made by comparing each subject's statistical objective map of brain glucose metabolism with a validated disease-specific hypometabolic pattern arising in specific brain areas, naturally grounded in a detailed clinical frame.

In the context of initial diagnosis, the exclusionary role of FDG-PET is especially clear in younger subjects with a suspicion of neurodegenerative disease. The high specificity of FDG-PET in AD, FTLD, and DLB implies that a negative, or normal, scan in the presence of the suspicion of dementia makes a diagnosis of a neurodegenerative disease very unlikely.

Based on the specificity of functional imaging with ^{18}F-FDG-PET that measures synaptic dysfunction in different networks, depending on the underlying pathology, and on the sufficiently large body of evidence in the literature, we strongly claim that ^{18}F-FDG-PET should be considered an essential component of the diagnostic workup of early onset dementia.

With regard to amyloid-PET, its potential clinical usefulness is strictly based on the assumption that early cerebral amyloidosis is virtually always detected in subjects on the path of AD. Even if there are still controversies about the so-called "amyloid hypothesis" in the pathogenesis of AD [120], the fact remains that amyloidosis is practically a held prerequisite for the diagnosis of AD. Nowadays, probably no physician would be highly confident with the diagnosis of AD in a patient in whom cerebral amyloidosis has not been confirmed. According to the temporal biomarker cascade hypothesis [52], brain amyloidosis would be a very early phenomenon, already detectable many years before the onset of symptoms.

As for differential diagnosis, amyloid-PET is less useful for the identification of DLB because most patients with this disease show brain amyloidosis that cannot be distinguished from that of AD patients [120]. In clinical practice, when a subject is evaluated because of cognitive symptoms, even if subtle, the demonstration of high brain amyloid load should strongly suggest one of the two main forms of neurodegenerative disease with amyloidosis, that is, AD or DLB. The topographic pattern of amyloid deposition is similar in these two conditions, but the pattern of neurodegeneration harbors significant differences because glucose hypometabolism specifically and extensively affects the occipital lobes in DLB and just marginally in AD whereas MTL hypometabolism, which is the classical fingerprint of AD, is seldom found in DLB [121]. Still in doubtful cases, the demonstration of nigrostriatal dopamine transporter deficit leads to identifying DLB with high accuracy [122].

Further, at least in AD, brain amyloid deposition seems to be a very early phenomenon and rather rapidly reaches a "plateau" at the time cognitive deficits become detectable [123], thus mirroring Aβ 1–42 levels in cerebrospinal fluid [124]. As such, the amount of amyloid deposition, along with Aβ 1–42 levels in cerebrospinal fluid, should not be viewed as an accurate index of disease progression. As a matter of fact, there is evidence that cognitive decline is much more related to the markers of neurodegeneration rather than to severity of amyloidosis, thus arguing for a higher sensitivity of PET-FDG and CSF levels of Tau and Phospho-Tau.

In the literature, visual inspection of amyloid burden has been reported to parallel the accuracy by quantification of the uptake (e.g., SUVR; see [34]). Other results, however, reported different findings (see [29]). It is of note that this may be true when discriminating mild to moderate AD with conditions in which amyloid retentions are null or nonsignificant (e.g., FTLD spectrum). When comparing early stages of AD pathology (MCI versus AD or even preclinical AD conditions), the methods based on quantification or semiquantification acquire relevance and might become mandatory. Typically, when considering patterns of accumulations in MCI during a follow-up period, quantitative analysis shows their power to detect changes [125].

In addition, while the *in vivo* detection of Aβ amyloid is gaining ground in the diagnosis of AD especially in MCI patients, the meaning of a positive PET scan in nondemented patients remains yet unclear. In our opinion, quantitative amyloid-PET scans, better defining the amount of amyloid load in these individuals, can prevent a positive amyloid scan to become a *de facto* diagnosis of AD. A paper from Mintun and colleagues [126] focused on this aspect by using ^{11}C-PiB PET scan in 41 nondemented subjects and 10 AD patients. Results showed that, globally, patients had greater uptake ratios, although 4 of the controls had cortical binding values that were comparable to those of AD patients, thus supporting the hypothesis that amyloid imaging could be used to detect preclinical stages of AD. A similar result has been described more recently by Mormino and coworkers [108] who found that the 15% of a large cohort of elderly HC showed positive ^{11}C-PiB uptake ratios. The clinical significance of these observations is still unclear and only long-term follow-up studies can clarify it. On the basis of the data available to date, it appears that these apparently healthy subjects with high amyloid load are likely to be on the path of AD, although we still ignore the time span from amyloid deposition and onset of first cognitive symptoms [46]. There is strong debate about the fate of "healthy" controls who displayed a positive amyloid-PET scan as we still ignore the time needed for an asymptomatic subject with amyloidosis to develop cognitive signs/symptoms. The time span has been indicated in a modeling of AD in the order of 10 years [46], but how to predict this time on an a real individual basis is still unknown. Noteworthy, recent evidence in individuals at risk for developing AD showed significant amyloid burden in autosomal dominant familial AD, even 15-16 years prior expected/predicted symptoms onset [113, 127] or 17 years before in sporadic AD cases [128]. The "nun" study has demonstrated that at least some individuals die with high brain amyloid load, but without any cognitive symptom or sign [129]. The biological evidence of amyloid load in human brains extended to elderly health individuals. This also implies ethical issues regarding what to communicate to an healthy volunteer found to be amyloid positive during clinical trials [130].

But just in this context of brain amyloidosis without symptoms, the demonstration of early signs of neurodegeneration in specific sites using voxel-based FDG-PET would be of great value. Starting from the observation that FDG-PET can be positive several years before the onset of dementia [64, 65], it would be possible to narrow the time of uncertainty in asymptomatic subject with amyloidosis. In other words, cognitively normal subjects showing cerebral amyloidosis through PET amyloid tracers along with glucose hypometabolism at specific sites would be at very high risk of developing a dementia process within few years. On the other hand, in a symptomatic patient with a suspicion of early AD, it has been proposed that amyloid-PET should precede any other evaluation just after morphological MRI [131] as a positive scan would strongly support the diagnosis of AD, thus avoiding most of the other diagnostic procedures, while a negative amyloid-PET scan would lead to search for other causes. Of utmost importance is the possibility to scan with amyloid-PET subjects in the MCI stage which represents a significant step toward the selection of groups with earlier AD for clinical trials. This would avoid including patients with a misdiagnosis and give experimental drugs the chance to be tested at the very onset of symptoms instead of when the disease has been already too progressed. While the potential of amyloid-PET is not a matter of debate in research, its misuse in clinical sets needs a careful regulation in order to give a proper role and a specific clinical context to this technique. That is why, recently, the Society of Nuclear Medicine and Molecular Imaging and the Alzheimer's Association have jointly convened the Amyloid Imaging Task Force (AIT) and published the Appropriate Use Criteria for amyloid-PET [132, 133]. They provided the appropriate use criteria for Amy-PET in which the circumstances for executing Amy-PET are listed.

According to those, Amy-PET will be appropriate for patients with persistent or progressive unexplained MCI, or satisfying core clinical criteria for possible AD (i.e., atypical clinical course or etiologically mixed presentation; for patients with atypically young-onset dementia). Crucially, the AIT also define the inappropriate use of amyloid-PET in the following conditions: (1) in patients with core clinical criteria for probable AD and with typical age of onset; (2) determination of dementia severity; (3) positive family history of dementia or presence of apolipoprotein E (APOE) $\varepsilon 4$; (4) in patients with a subjective cognitive complaint that is unconfirmed on clinical evaluation; (5) as an alternative to genotyping for suspected autosomal mutation carriers; (6) in asymptomatic individuals; (7) nonmedical uses such as (legal, insurance coverage, or employment screening).

In conclusion, on the basis of the present survey and also on the meta-analyses and GRADE analysis, we showed that there is moderate quality evidence for the effects of both modalities of PET imaging (FDG and Amyloid) in the early diagnosis of AD and conversion prediction, and, equally, moderate quality evidence for the differential diagnosis of patients with AD and the other major neurodegenerative dementia (i.e., DLB and FTLD). The three meta-analyses conducted through the three categories of studies (early diagnosis, disease progression and differential diagnosis), as remarked in the Results section, yielded significant results. Summary sensitivity effect measures were 0.86 for ^{18}F-FDG-PET (1322 cases), 0.91 for amyloid-PET (797 cases), and 0.90 for differential Diagnosis (1011 cases). Therefore, on the basis of the studies included in the present survey, amyloid-PET seems to be more sensitive than ^{18}F-FDG-PET in early diagnosis of AD. It is of note that our analysis included a sample of patients investigated by ^{18}F-FDG-PET larger than the cohort investigated by amyloid-PET. Hence, even if the effect measure is lower, we can interpret that result as more robust. In addition, the grade analysis classified more ^{18}F-FDG-PET studies as M (moderate, $N = 7$) than for amyloid-PET ($N = 5$) that is coherent with the previous claim. Lastly, as anticipated in Results section, the 95% confidence intervals for ^{18}F-FDG-PET early diagnosis and disease progression reveal a lower degree of uncertainty with respect to amyloid-PET early diagnosis (see Foster plots, Figure 1). For these reasons, we can definitely conclude that both the topographical and pathological PET markers are very accurate and sensitive to early diagnosis of AD, as well as to differential diagnosis with other dementia (e.g., FTD or DLB) when appropriate data analysis at single subject level is performed.

This survey and GRADE analysis show a good overall quality of evidence for PET functional (FDG) and molecular (amyloid) imaging in early and differential diagnosis of AD, on the basis of voxel-based or parametric data quantifications. This approach will allow net benefits in terms of diagnostic and prognostic value of the information provided by PET imaging considering its sensitivity and accuracy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Molecular Imaging Insights into Neurodegeneration: Focus on Tau PET Radiotracers

Maliha Shah and Ana M. Catafau

Department of Clinical Research and Development–Neurosciences, Piramal Imaging GmbH, Berlin, Germany

Neurodegenerative diseases are characterized by progressive dysfunction and neuronal death, showing specific protein inclusions at autopsy. In vivo detection of these key proteins, namely amyloid-β, tau, α-synuclein, and trans-active response DNA-binding protein 43 kDa, is possible by means of molecular neuroimaging techniques, such as PET. The development of selective PET radiotracers targeting these proteins is critical for early and accurate diagnosis and for the successful development of disease-modifying therapies. Selective PET radiotracers for amyloid-β are already available, and potential tau tracers are emerging as new-generation biomarkers. An overview of the tau-PET radiotracer development scenario, focusing on tracers that are presently being examined in humans, is presented.

Key Words: PET; tau; radiotracer; neurodegeneration; Alzheimer's

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Tubulin-associated unit, or tau, is an intracellular protein playing a vital role by binding to and stabilizing axonal microtubules in neurons, thereby regulating intracellular transport. The microtubule-binding region of tau consists of 3 or 4 tandem repeats via which tau mediates additional functions (*1*). Disease-associated posttranslational modifications, such as hyperphosphorylation, cause tau to dissociate from microtubules and assemble into large fibrils, referred to as paired helical filaments. The normal functions of tau are thus altered. The paired helical filaments and similar tau filaments, in turn, associate to form neurofibrillary tangles, which are the pathologic hallmark of various neurodegenerative diseases. tau aggregates or inclusions depict different diseases depending on the structural basis of the aberration. For instance, accumulations of the 3 tandem repeats are typical of Pick disease and the 4 tandem repeats are characteristic of corticobasal degeneration, progressive supranuclear palsy, and argyrophilic dementias, whereas Alzheimer disease (AD) often presents a mixture of 3 and 4 tandem repeats. Thus, in ways presently not understood, structural differences within the tau protein lead to distinct histopathology and disease phenotypes (*1*).

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For correspondence or reprints contact: Ana M Catafau, Piramal Imaging GmbH, SC Trade Center 1, Avinguda Corts Catalanes 5-7, Office 14, 08173 Sant Cugat del Valles, Barcelona, Spain.
E-mail: ana.catafau@piramal.com
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CLINICAL RELEVANCE OF TAU PET IMAGING

Diseases characterized by the presence of pathologic tau, such as dementias and movement disorders as well as traumatic brain injury and chronic traumatic encephalopathy, could potentially benefit from tau PET imaging. The severity of tau pathology is closely related to neuronal loss and cognitive impairment in AD (*2*), supporting the use of tau as a biomarker of neurodegeneration. Tau, amyloid-β (Aβ), α-synuclein, and trans-active response DNA-binding protein 43 kDa (TDP-43) may coexist in different relative concentrations depending on the disease (e.g., Aβ predominance in AD; α-synuclein in dementia with Lewy bodies), its stage (higher concentrations in advanced disease), and its cerebral region (mesial temporal cortex tau predominance in AD). However, pure tau pathology in the absence of Aβ plaques is characteristic of a heterogeneous group of neurodegenerative disorders included under the term *frontotemporal lobar degeneration.* About half the frontotemporal lobar degenerations are ubiquitinopathies with TDP-43 (e.g., amyotrophic lateral sclerosis), and the other half are tauopathies, including Pick disease, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia and parkinsonism linked to chromosome 17. The familial tauopathies linked to tau gene mutations have provided compelling evidence that tau abnormalities alone could cause pathologic protein aggregates and massive neuronal loss leading to symptomatic onset (*3,4*). The last proposed clinical diagnosis criteria for frontotemporal lobar degeneration are based on recent advances in molecular genetics, biochemistry, and neuropathology (*4*). Specific therapies targeting the hallmark proteins of these pathologies are being investigated. However, it may be challenging to accurately classify these diseases in the clinic, particularly in the early stages, and to select appropriate patients for specific therapies in the absence of biomarkers. Accurate detection of pathologic hallmarks other than Aβ in vivo is therefore a current unmet need. In clinical practice, tau PET imaging could be potentially useful in the early detection and differential diagnosis of AD and non-AD tauopathies as well as in identifying subjects with traumatic brain injury–chronic traumatic encephalopathy who are at risk of developing dementia. As a marker of neurodegeneration, tau PET imaging could help in monitoring disease progression and severity. Clinical trials of disease-modifying therapies could also benefit from

tau PET imaging. Enriched populations of patients showing cerebral tau accumulations early in the disease process, and the measurement of changes in tau over time, may decrease risks inherent in the drug development process and contribute to the discovery of effective treatments. As opposed to nonimaging biomarkers for tau (e.g., cerebrospinal fluid), PET imaging offers the advantage of providing a topographic mapping of the amount and distribution of the tau protein in the human brain in vivo. Hence, intensive research is currently ongoing to develop PET radioligands that are capable of detecting deposits of tau in vivo in an accurate and diagnostically relevant manner.

IDEAL CHARACTERISTICS OF A TAU PET TRACER AND CHALLENGES IN ITS DEVELOPMENT

The ideal radioligand for tau must share the general characteristics required of any PET radiotracer for brain imaging, such as the ability to cross the blood–brain barrier, not being a P-glycoprotein substrate, having high affinity and selectivity for the target, having suitable pharmacokinetics for PET imaging, lacking radioactive metabolites that cross the blood–brain barrier, and having favorable dosimetry. However, because of the peculiarities of the tau protein, fulfilling all these requirements may be challenging (Fig. 1) (*5,6*). First, given the intracellular location of tau, the radiotracer must be able to cross not only the blood-brain-barrier but also the cell membrane and enter the neuron. Second, the ideal tau radiotracer should be able to recognize and bind to deposits of all 6 tau isoforms, allowing imaging of both AD and non-AD tauopathies. Third, tau aggregates may also be present in white matter; hence, an optimal tau tracer should not show nonspecific tracer retention in this region. Also, the frequent colocalization of various protein deposits in the brain at different relative concentrations requires stringent selectivity and affinity criteria. For this reason, results from human brain homogenates containing mixtures of proteins should be interpreted with caution. Lastly, a truly comprehensive tau tracer should be able to recognize and bind to tau deposits irrespective of the number or identity of posttranslational modifications seen in vivo. For this reason, experiments should ideally be performed on human brain slices rather than on recombinant tau fibrils, which lack various posttranslational modifications and other morphologic aspects of in vivo tau deposits. In conclusion, the hunt for a tau tracer that overcomes all these challenges is considerably more daunting than appears at first sight. Nevertheless, considerable progress has been made and several ligands have entered human imaging studies.

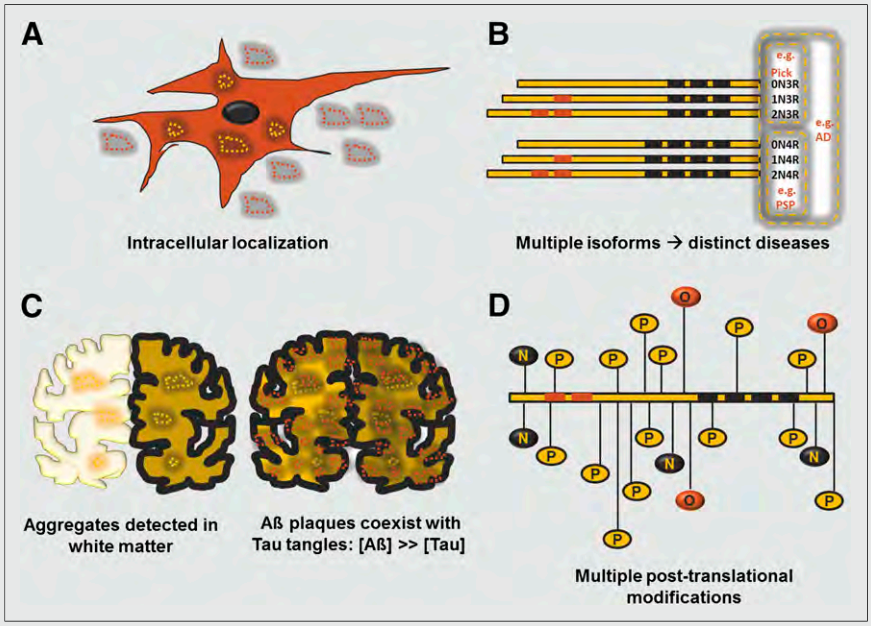
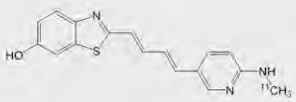
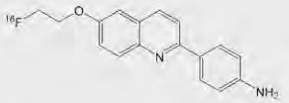
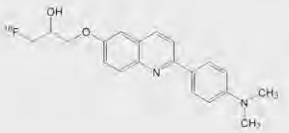
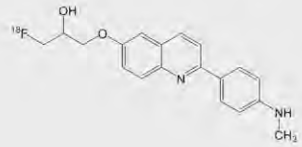
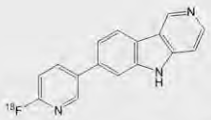
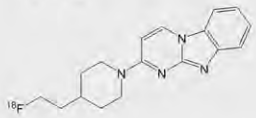


FIGURE 1. Challenges during development of tau radioligand. (A) tau aggregates (yellow punctata) are intracellularly localized as opposed to extracellular Aβ deposits (red punctata). (B) The 6 isoforms of tau are differentially represented in distinct diseases. (C) tau aggregates are present in white matter. Also, tau aggregates [Tau] colocalize with and are present in much lower concentrations than Aβ plaques [Aβ]. (D) Diverse posttranslational modifications of tau, for example, hyperphosphorylation (P), nitration (N), and O-GlcNAcylation (O) of multiple residues, give rise to various forms of protein.

OVERVIEW OF TAU PET TRACERS UNDER DEVELOPMENT

^{18}F-FDDNP was the first ^{18}F-labeled tracer aimed at tau PET imaging. However, binding to Aβ and α-synuclein besides tau has been demonstrated (*7*), complicating image interpretation. Several molecules are being validated for use as alternative tau tracers. These include, but are not restricted to, ^{11}C-*N*-methyl lansoprazole, ^{11}C-PBB3, ^{18}F-THK523, ^{18}F-THK5105, ^{18}F-THK5117, ^{18}F-T808, and ^{18}F-T807. A summary of the characteristics of each compound is provided in Figure 2.

^{11}C-*N*-methyl lansoprazole is the ^{11}C-labeled version of the Food and Drug Administration–approved drug lansoprazole and appears to have an exceptionally high affinity for tau (*8*). However, to the best of our knowledge no human data have been published with this radiotracer yet. ^{11}C-PBB3 shows high specificity for tau deposits over Aβ plaques (*9,10*). Initial human studies with this radiotracer show limited white matter background signal and minimal Aβ detection as compared with the Aβ ligand ^{11}C-Pittsburgh compound B (Fig. 3A). Preliminary results with ^{11}C-PBB3 have shown that tau accumulation correlates with clinical symptoms and that its localization in the brain follows the Braak and Braak stages, starting in the hippocampus and limbic system in a subset of nondemented elderly subjects and in subjects with mild cognitive impairment, and spreading to the neocortex in subjects with AD. In addition, ^{11}C-PBB3 appears to detect tau pathology in diseases other than AD, for example, corticobasal syndrome, suggesting an ability to recognize multiple isoforms of the tau

Ligand	Structure	Affinity for Tau [nM]	Specificity for Tau relative to Aß	Mouse brain uptake at 2 min [%ID/g]	Mouse brain washout (2min/ 30 min)
^{11}C-PBB3[9]		K_{d1}=2.5* K_{d2}=100	40-to-50-fold	NA	NA
^{18}F-THK523[13, 15]		K_{d1}=1.99† K_{d2}=50.7	15-fold	2.72	1.9
^{18}F-THK5105[15]		K_{d1}=1.45† K_{d2}=7.40	25-fold	9.2	2.6
^{18}F-THK5117[15]		(K_i=10.5)‡	(NA)	6.06	10.3
^{18}F-T807[18]		K_d=14.6*	>25-fold	4.16	6.7
^{18}F-T808[17]		K_d=22*	27-fold	6.7§	2.9‖

*K_d determined by ligand-binding to Tau-positive human brain sections
†K_d determined by ligand-binding to Tau-fibrils
‡K_i determined by competitive inhibition of ^{18}F-THK5105 to Tau-fibrils
§ 2.5 min; ‖ washout 2.5 min/20 min
NA= Not available; N.B. The affinity values for various ligands are generated from different types of experiments and may not always be directly comparable.

FIGURE 2. Summary of characteristics of tau PET radiotracers being tested in humans.

protein (Fig. 3B). However, ^{11}C-labeled tracers (half-life, 20 min) are not suitable for widespread use, particularly in clinical practice, where ^{18}F-labeled tracers (half-life, 110 min) are preferable.

From the family of arylquinolines, ^{18}F-THK523 shows a high affinity to and selectivity for tau fibrils in vitro (*11–13*). In humans, the pattern of cortical ^{18}F-THK523 retention does not correlate with Aβ distribution as assessed by ^{11}C-Pittsburgh compound B; instead, it follows the known distribution of tau deposits in the AD brain, thus suggesting that ^{18}F-THK523 selectively binds to tau in AD patients. Unfortunately, failure to label tau-containing lesions in non-AD tauopathies and high retention of ^{18}F-THK523 in white matter precludes its use in research or clinical settings (*14*). ^{18}F-THK5105 and ^{18}F-THK5117 have been tested in vitro both on recombinant tau fibrils and on brain homogenates from the mesial temporal cortex, yielding promising affinity values (*15*). However, as previously mentioned, these in vitro assays should be carefully interpreted. Although both compounds show encouraging results in mice, further evidence is required to confirm the real nature of the signal observed in preliminary human imaging data (Fig. 3C) (*16*).

^{18}F-T808 and ^{18}F-T807 show strong affinities and selectivity for tau versus Aβ in vitro (*17,18*). Brain uptake and washout values for both are favorable (Fig. 2). Brain PET images with ^{18}F-T808 in humans, however, show intense bone uptake in the skull due to defluorination, which may hamper visual image interpretation (*19*). Human ^{18}F-T807 PET results seem to be the most promising, although the reported data come from a limited sample of 6 subjects (*20*). The kinetics appear to be slower than those of amyloid tracers but still favorable, with clearance from white matter. Static images do not show defluorination, and a pattern of retention consistent with Braak staging has been reported (Fig. 3D). However, no data are currently available on the suitability of this radiotracer in non-AD tauopathies.

CHALLENGES AND FUTURE DIRECTIONS

Human data currently available on tau PET imaging are still limited. Although it looks promising, an ideal radiotracer meeting all the extensive criteria outlined above has not been identified yet. Hence, research aimed at identifying an optimal compound is still active. Additional data are required to further characterize the existing tau radioligands, including quantitative validation toward full kinetic modeling, test–retest studies, and human dosimetry. Ultimately, neuropathologic correlations with PET imaging findings will be necessary to confirm tau selectivity against colocalized key proteins besides Aβ, such as α-synuclein and TDP-43 (*21*). Head-to-head studies will allow direct comparisons between radiotracers. Finally, evidence on impact in patient management and outcomes should be generated. In the meanwhile, the inclusion of tau PET imaging in clinical trials for disease-modifying therapies could certainly contribute to a faster and more efficient development of efficacious drugs.

CONCLUSION

The development of tau radiotracers for PET imaging constitutes a step toward meeting the clinical needs of biomarkers for neurodegeneration. In clinical practice, such a molecular imaging tool could help in early and more accurate diagnosis, as well as in monitoring disease progression of AD and non-AD tauopathies, including movement disorders and traumatic brain injury–chronic traumatic encephalopathy. In re-

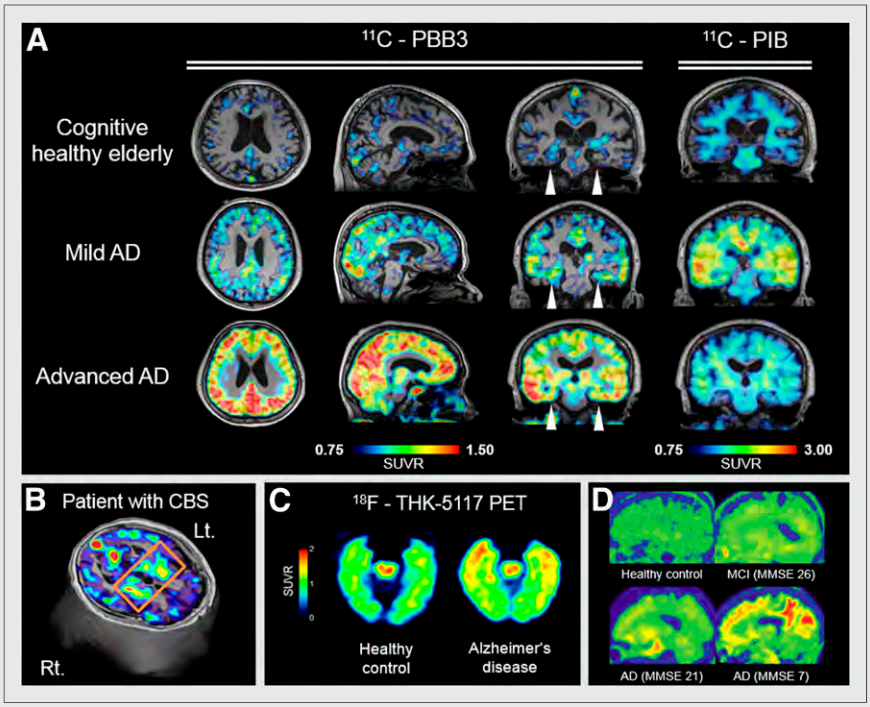


FIGURE 3. Tau PET scan images in humans. (A) Coregistered MR imaging and ^{11}C-PBB3 parametric standardized uptake value ratio (SUVR) images compared with ^{11}C-Pittsburgh compound B. No significant radiotracer retention is seen in cognitively healthy elderly subject. Note different uptake pattern in hippocampus between ^{11}C-PBB3 and ^{11}C-Pittsburgh compound B across subjects (arrowheads), and spread of ^{11}C-PBB3 accumulation to cortical areas in advanced AD patient, who shows some cortical uptake in ^{11}C-Pittsburgh compound B scan. (B) ^{11}C-PBB3 PET axial slice over 3-dimensional MR imaging of corticobasal syndrome patient with left-sided symptomatic predominance, showing ^{11}C-PBB3 accumulation in right basal ganglia (Courtesy of Drs. Hitoshi Shimada and Makoto Higuchi). (C) ^{18}F-THK-5117 SUVR images (50–60 min after injection). Higher tracer uptake in anterior pole and mesial aspect of temporal lobes in AD patient (87 y old, MMSE 25) compared with healthy control (80 y old, MMSE 28). Activity in midbrain may represent nonspecific binding (Courtesy of Dr. Nobuyuki Okamura). (D) ^{18}F-T807 sagittal images (80–100 min after injection). No significant radiotracer uptake is seen in healthy control (56 y old), whereas progressive radiotracer accumulation and spreading from mesial temporal to neocortex is seen in patients as MMSE scores decrease. (Reprinted with permission of (*20*).)

search, it could provide useful insights into the pathophysiology of these diseases and contribute to the development of potential new efficacious therapies. Several tau radiotracers are undergoing evaluation in human imaging studies and yielding encouraging data. Additional radioligands are also being intensely developed, and the future of tau imaging looks full of promise.

DISCLOSURE

Maliha Shah and Ana Catafau are Piramal Imaging GmbH employees. No other potential conflict of interest relevant to this article was reported.

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Molecular Imaging Insights into Neurodegeneration: Focus on Tau PET Radiotracers

Maliha Shah and Ana M. Catafau

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Review - Part of the Special Issue: Alzheimer's Disease – Amyloid, Tau and Beyond

Perspective on future role of biological markers in clinical therapy trials of Alzheimer's disease: A long-range point of view beyond 2020

Harald Hampel [a,*], Simone Lista [b,*], Stefan J. Teipel [c,d], Francesco Garaci [e,f], Robert Nisticò [g,h], Kaj Blennow [i], Henrik Zetterberg [i,j], Lars Bertram [k], Charles Duyckaerts [l], Hovagim Bakardjian [m,n], Alexander Drzezga [o], Olivier Colliot [p,q,r,s,t], Stéphane Epelbaum [u,v], Karl Broich [w], Stéphane Lehéricy [n,p,q,r,s], Alexis Brice [p,q,r,s,x], Zaven S. Khachaturian [y], Paul S. Aisen [z], Bruno Dubois [u,v]

[a] Université Pierre et Marie Curie, Département de Neurologie, Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Pavillon François Lhermitte, Hôpital de la Salpêtrière, Paris, France
[b] Department of Psychiatry, Psychotherapy and Psychosomatics, Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany
[c] Department of Psychosomatic Medicine, University of Rostock, Rostock, Germany
[d] German Center for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, Rostock, Germany
[e] Department of Diagnostic Imaging, Molecular Imaging, Interventional Radiology, and Radiotherapy, University of Rome "Tor Vergata", Rome, Italy
[f] IRCCS San Raffaele Pisana, Rome and San Raffaele Cassino, Cassino, Italy
[g] Department of Physiology and Pharmacology, University of Rome "La Sapienza", Rome, Italy
[h] IRCSS Santa Lucia Foundation, Rome, Italy
[i] Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
[j] University College London Institute of Neurology, Queen Square, London, UK
[k] Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany
[l] Laboratoire de Neuropathologie Raymond-Escourolle, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Paris, France
[m] IM2A – Institute of Memory and Alzheimer's Disease, Paris, France
[n] IHU-A-ICM – Paris Institute of Translational Neurosciences Pitié-Salpêtrière University Hospital, Paris, France
[o] Department of Nuclear Medicine, University Hospital of Cologne, Cologne, Germany
[p] Université Pierre et Marie Curie-Paris 6, Centre de Recherche de l'Institut du Cerveau et de la Moelle Épinière, UMR-S975 Paris, France
[q] Inserm, U975, Paris, France
[r] CNRS, UMR 7225, Paris, France
[s] ICM – Institut du Cerveau et de la Moelle Épinière, Paris, France
[t] INRIA, Aramis Team, Centre de Recherche Paris-Rocquencourt, France
[u] Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Département de Neurologie, Hôpital de la Pitié Salpêtrière, Paris, France
[v] Université Pierre et Marie Curie, Paris, France
[w] Federal Institute of Drugs and Medical Devices (BfArM), Bonn, Germany
[x] AP-HP, Hôpital de la Salpêtrière, Département de Génétique et Cytogénétique, Paris, France
[y] The Campaign to Prevent Alzheimer's Disease by 2020 (PAD2020), Potomac, MD, USA
[z] Department of Neurosciences, University of California, San Diego, San Diego, CA, USA

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ABSTRACT

Recent advances in understanding the molecular mechanisms underlying various paths toward the pathogenesis of Alzheimer's disease (AD) has begun to provide new insight for interventions to modify disease progression. The evolving knowledge gained from multidisciplinary basic research has begun to identify new concepts for treatments and distinct classes of therapeutic targets; as well as putative disease-modifying compounds that are now being tested in clinical trials.

There is a mounting consensus that such disease modifying compounds and/or interventions are more likely to be effectively administered as early as possible in the cascade of pathogenic processes preceding and underlying the clinical expression of AD. The budding sentiment is that "treatments" need to be applied before various molecular mechanisms converge into an irreversible pathway leading to morphological, metabolic and functional alterations that characterize the pathophysiology of AD. In light

* Corresponding authors at: Université Pierre et Marie Curie, Département de Neurologie, Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Pavillon François Lhermitte, Hôpital de la Salpêtrière, 47 Boulevard de l'Hôpital, 75013 Paris, France. Tel.: +33 01421 62128; fax: +33 09 81 123444.
E-mail addresses: harald.hampel@psl.aphp.fr (H. Hampel), slista@libero.it (S. Lista).

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of this, biological indicators of pathophysiological mechanisms are desired to chart and detect AD throughout the asymptomatic early molecular stages into the prodromal and early dementia phase.

A major conceptual development in the clinical AD research field was the recent proposal of new diagnostic criteria, which specifically incorporate the use of biomarkers as defining criteria for preclinical stages of AD. This paradigm shift in AD definition, conceptualization, operationalization, detection and diagnosis represents novel fundamental opportunities for the modification of interventional trial designs.

This perspective summarizes not only present knowledge regarding biological markers but also unresolved questions on the status of surrogate indicators for detection of the disease in asymptomatic people and diagnosis of AD.

Contents

1. Introduction

Sporadic Alzheimer's disease (AD) is currently conceptualized as a multifactorial neurodegenerative disease transitioning later through a prodromal cognitive stage into a late-stage dementia syndrome. This initially clinically "silent" multi-dimensional disease cascade chronically, non-linear progressively unfolds through the emergence and probably at some point convergence of a yet not fully understood and characterized parallelized and/or interrelated array of molecular mechanisms and signaling pathways. For many decades, the definite diagnosis of AD has relied on the *postmortem* detection of senile plaques (SPs) and neurofibrillary tangles (NFTs). There, these historic hallmark neuropathological lesions have been extensively studied. Their molecular constituents have been isolated (intracellular aggregation of tau protein and extracellular accumulation of amyloid beta (Aβ) peptide). The neuropathology is now better understood in terms of amyloid and tau pathology – as a consequence Aβ and tau assays having secondarily been developed and validated during the last two decades to provide first "core feasible" cerebrospinal fluid (CSF) biomarkers. The stereotyped progression of tau [1] and Aβ pathology [2] in the brain has been described and is the basis of the new National Institute on Aging and the Alzheimer's Association neuropathological criteria [3]. The amyloid cascade hypothesis, relying on the observation that all the mutations causing early-onset AD involve genes that alter Aβ production, has generated a theory emphasizing the central relevance of the amyloidogenic cascade and the Aβ peptide. As a consequence, many treatment trials in AD have been aimed at altering the abnormal production, accumulation and deposition Aβ. The optimism that reducing Aβ accumulation and/or

deposition would directly result into an improved clinical and functional patient status, however, has not yet been fulfilled. Recent evidence indicating that misfolding of Aβ and tau could be transmitted to normal proteins of the host through brain injections of affected samples is hypothesis generating and opens new translational research perspectives. A mono-linear amyloid cascade perspective, would seem reductionistic, since it fails to recognize the role of the many conformations that the proteins may adopt, explaining the progression of the disease through the connections and the transmissibility of the pathology in some experimental conditions. Therefore, in the advent of the worldwide AD epidemic, critical reassessment of the evidence-based significance and limitation of prevailing as well as of emerging fundamental concepts of AD pathophysiology seems to be necessary to foster breakthrough advances to effectively detect, treat or even prevent AD [4].

The search for biomarkers of preclinical AD is becoming increasingly important because pathogenesis-targeted neuroprotective strategies are being developed for future use in "at risk" populations. Advances in new neuroimaging probes and technologies, identification of new biochemical markers of AD in plasma, blood and CSF, and breakthroughs in molecular genetics and basic neuroscience are gradually translating into better understanding of predisposing and preclinical factors that lead to progressive neurodegeneration and finally cognitive and behavioral symptoms and dementia.

At present, the combination and integration of multimodal imaging methods, neurochemical markers, and genetic strategies are still in their infancy. However, significant indications on the existing state of the biomedicine on candidate markers of AD resulting from multiple analytical platforms – encompassing (I) structural/functional/metabolic neuroimaging modalities, (II) neurochemistry methods based on CSF and blood (plasma/serum) examination, (III) neurogenetic analyses, and (IV) procedures for cognitive and functional assessment – have been supplied [5–13].

The next-generation of studies is required to use multicenter data sets that exploit the large variety of affected systems to appraise the stability of multimodal diagnostic algorithms in a multinational multicenter setting. A growing number of national and international platforms are following this central line of research, among them the US Alzheimer's Disease Neuroimaging Initiative (US-ADNI) [14] and the European ADNI (E-ADNI) [15] that, in conjunction with other parallel projects around the globe, are collectively known as Worldwide ADNI (WW-ADNI) [16]. The ADNI has been designed to validate neuroimaging, CSF, and blood-plasma biomarker candidates for AD treatment trials, and therefore aid and speed drug development [16]. As a result, the approach of combining different source markers might be of help in the identification of those subjects who will develop AD and who are consequently potential targets for prevention as well as symptomatic pharmacological interventions.

When employed in AD clinical trials, biomarkers can be utilized: (I) to improve the diagnostic accuracy in trial participants, enabling patient cohorts to be enriched with characteristic molecular mechanisms of AD; (II) for stratification of AD patients; (III) for safety monitoring, *i.e.* to assess and predict tolerability and adverse side effects; (IV) as theragnostic markers, *i.e.* to identify and monitor the biochemical effects of drugs [5,6,17]. Notably, biomarkers provide the potential for characterization and validation of drug mechanisms of action, monitoring AD course and progression, and evaluating therapeutic response/outcome [18]. Furthermore, since biomarker profiles reflect different stages of the pathogenic process, they can be utilized to recruit optimal individuals for trials of different drugs and different clinical phenotypes at different stages of AD pathophysiology [19].

By using multimodal strategies, AD has been categorized into different stages according to the presence of biomarkers and the patterns of cognitive impairment. Following a pre-pathology stage characterized by normal biomarkers and absence of cognitive impairment, AD dimensionally (not categorically) emerges exhibiting through an asymptomatic stage (biomarkers abnormal, no cognitive impairment) subsequently to a symptomatic stage (biomarkers abnormal, cognitive impairment) that can be further differentiated into a subjective cognitive impairment (SCI) stage (AD-SCI), a prodromal, often categorized as a "mild cognitive impairment" (MCI) stage (AD-MCI), and finally a syndromal dementia stage (AD-dementia) [20]. Notably, these categories are mere restrictive research or practical clinical constructs and should not mask the true continuous dimensional character of AD.

The present review will summarize the current knowledge on the employment of biological markers in AD and provide perspectives as well as future directives on major areas of AD biomarker discovery and development emphasizing the role of such markers for use in clinical trials. Notably, since this manuscript is intended to raise evolving debate on the effective discovery, development, validation, and qualification process of biological markers resulting from all available technical modalities, it represents a major complement and extension to the antecedent perspective by Hampel et al. [7]. Current knowledge and perspectives/future directives on the employment of biological markers in AD are summarized in Tables 1 and 2, respectively.

2. International Work Group criteria

In 2007, an International Work Group (IWG) led by Dubois and colleagues has provided a novel description of AD as a clinico-biological syndrome that can be documented *in vivo*, prior to the onset of dementia, by a "core" clinical phenotype that includes an amnestic syndrome of the hippocampal type and indication from biomarkers reflecting the existence of Alzheimer-type pathology [21]. Such criteria may be used throughout any phase of the AD spectrum after the beginning of clinical signs [22]. Moreover, a specific terminology has been developed to resolve issues related to AD reconceptualization [23].

The IWG proposed two new sets of diagnostic criteria requiring the assessment of AD biomarkers. The first, covering asymptomatic AD individuals, is defined "preclinical AD". Preclinical AD has been then partitioned into the "asymptomatic at risk for AD" and the "presymptomatic AD" categories [23], the latter applying to asymptomatic individuals who carry familial autosomal dominant AD mutations. The second group, applying to symptomatic AD individuals, is designated as "AD". Individuals reflecting these criteria can be, in turn, categorized into "prodromal AD" (or "predementia AD") and "AD dementia" [23].

The most important progress inherent in the IWG criteria is the integration of biomarkers into a diagnostic scheme that allows a biology aided assessment of AD which is integrated with the clinical signs and symptoms, independent of disease severity. The use of biomarkers is integral to the diagnosis of AD in the IWG criteria; consequently, the presence of pathophysiologic or topographic aberrations representative of AD is strictly required. The pathophysiologic markers encompass the molecular signatures of AD in the CSF (low levels of the 42 amino acid-long form of the Aβ peptide ($A\beta_{1-42}$) plus increased concentrations of total-tau (t-tau) and/or hyperphosphorylated tau (phospho tau, p-tau) proteins) or significant binding of amyloid ligands using positron emission tomography (PET). The topographic markers consist of medial temporal/hippocampal atrophy on magnetic resonance imaging (MRI) or bilateral parieto-temporal hypometabolism on PET [22].

Importantly, the IWG criteria have abandoned the categorical concept of "MCI", which is heterogeneous in terms of AD progression and has many different underlying causes, in favor

Table 1
Current knowledge on the employment of biological markers in AD.

Area of markers	Key points
Genetics	
Familial AD	• "Featured genes" (causal genes): *APP*, *PSEN1*, *PSEN2* • Currently known mutations in *APP*, *PSEN1*, *PSEN2* genes do not account for all Mendelian AD cases, suggesting the existence of AD-causing mutations in other genes
Sporadic AD	• "Featured genes" (proposed susceptibility genes): *APOE*, *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *MS4A6A*, *MS4A4E*, *CD33*, *CD2AP*, *EPHA1*, *TREM2 and counting* • The advent of GWAS have led to the identification of novel loci linked to mostly LOAD risk • These genes appear to be mostly linked with three molecular pathways: (I) the amyloidogenic cascade, (II) cholesterol-lipid metabolism, and (III) immune-inflammatory mechanisms
Cerebrospinal fluid	• CSF biomarkers $A\beta_{1-42}$, t-tau, p-tau_{181}, and p-tau_{231} have a high diagnostic accuracy for AD, and for prodromal AD in patients with MCI • CSF levels of $A\beta_{1-42}$ start declining in the preclinical phase of sporadic AD, prior to any evident increase in t-tau or p-tau • CSF biomarkers, especially $A\beta_{1-42}$, convert to pathologic values several years before the first appearance of clinical signs, also in the familial form of AD • The diagnostic accuracy for the combination of CSF $A\beta_{1-42}$, t-tau, and p-tau has been reported to be higher than for any biomarker alone • CSF biomarkers are increasingly used in clinical trials, both for enrichment of patient populations with pure AD cases at the inclusion and to evaluate the biochemical effects of treatment (theragnostic markers) • CSF biomarker $A\beta_{1-42}$ is the central CSF biomarker for Aβ metabolism and deposition in clinical treatment trials. • CSF biomarkers t-tau and p-tau are the central CSF biomarkers to monitor the intensity of cortical axonal degeneration and tau phosphorylation state, respectively, in clinical treatment trials
Blood	• Definite data regarding the association of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations with incipient AD are presently lacking • The development of mass spectrometry-based technologies has elected proteomics as the chief platform to inspect the plasma/serum proteome for the discovery of next-generation biomarkers showing diagnostic, prognostic, or therapeutic efficacy • Blood-based profiles/signatures including panels of molecules related to immune regulation and inflammatory pathways have been discovered • Issues in plasma/serum proteomics, including pre-analytical variables, requiring standardization for specimen collection/processing, quantitation, and setting strategies for managing biomarkers after their detection, currently exist. These markers do not seem to be ready for clinical applications
Structural neuroimaging	• Reduction in hippocampus volumetry, derived from structural MRI, has been consistently found in AD and MCI across a wide range of mono- and multicenter studies • Hippocampus volumetry has also been used as a secondary endpoint in clinical trials on potential disease modifiers in AD or MCI • The EMA regulatory authorities have endorsed a qualification process for the use of low hippocampus volume for enrichment of study samples • Few automated protocols have already been cleared for marketing as a medical device by the FDA • The attractiveness of MRI as endpoint in clinical trials is related to the assumption that regional brain volume can serve as *in vivo* surrogate of neuronal density • Neuropathological evidence suggests a selective involvement of specific subcortical areas, most notably the cholinergic nuclei of the basal forebrain and noradrenergic nuclei in the *locus coeruleus* in AD • Diffusion Tensor Imaging has become a leading method in investigating white matter microarchitecture and integrity and has been widely employed in AD and MCI
Functional neuroimaging	• Functional MRI, studying the neuronal activity through non-invasive means during specific cognitive states, has been able to detect functional alterations prior to onset of cognitive impairment or AD-related structural neurodegeneration • Functional MRI studies are focused on the "default mode network", *i.e.* the interplay between a set cortical areas and the hippocampal memory system
In vivo molecular neuroimaging	• FDG-PET has demonstrated to be of great value because it allows the detection of different patterns of neurodegeneration; it is also highly useful in differentiating within amyloid-positive subtypes of disease which cannot be distinguished on the basis of their amyloid PET-scan • PiB is the current gold-standard tracer for PET amyloid-imaging. Recently, ^{18}F-labeled compounds have been evaluated to enable allow a more widespread application of this method. [^{18}F]Florbetapir/Amyvid has already been approved by the FDA and the EMA • Concerning early diagnosis, several studies demonstrated a high predictive value of a positive amyloid-scan in the stage of MCI with regard to conversion to AD
Neurodynamics	• Time-resolved EEG and MEG measures have been increasingly explored to identify predementia AD (MCI)
Resting state	• Brainwave band power estimates in the delta, theta, alpha, beta and gamma frequency bands, as well as their ratios, have been used as a major tool to demonstrate RSN changes in AD and predementia AD patients as compared to healthy controls • Studied have shown a connection between clinical (MMSE) measures and frequency band power (alpha)
Functional	• ERP/ERF markers (peak latency, amplitude, brain sources) measure task-related functional changes which are not available in resting state. Deterioration of cognitive/episodic memory measures (P300, P600, *etc.*) has been demonstrated in AD and predementia AD subjects by multiple studies

Abbreviations: AD, Alzheimer's disease; $A\beta_{1-40}$, 40 amino acid-long form of the amyloid beta peptide; $A\beta_{1-42}$, 42 amino acid-long form of the amyloid beta peptide; CSF, cerebrospinal fluid; EEG, electroencephalography; EMA, European Medicine Agency; ERP, event-related potentials; ERF, event-related fields; FDA, Food and Drug Administration; FDG, [^{18}F]Fluorodeoxyglucose; GWAS, genome-wide association studies; LOAD, late-onset AD; MCI, mild cognitive impairment; MEG, magnetoencephalography; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging; p-tau_{181}, hyperphosphorylated tau protein at threonine 181; p-tau_{231} hyperphosphorylated tau protein at threonine 231; PET, positron emission tomography; PiB, [^{11}C]Pittsburgh-Compound-B; RSN, resting state network; t-tau, total-tau.

of prodromal AD in subjects showing symptomatic predementia AD. Therefore, these criteria rely on the implementation of biomarkers to detect a specific subset of MCI individuals who are in the predementia phase [22,24].

From a conceptual perspective, the IWG criteria foster the perception of AD as a dimensional clinico-biological entity and have been positively applied in clinical therapy trials approved by the US Food and Drug Administration (FDA) [22]. They have been

Table 2
Short- to mid-term perspectives and future directives of biological markers in AD.

Area of markers	Key points
Genetics	• Additional AD susceptibility variants are expected to be identified in upcoming GWAS based on larger sample sizes and/or higher resolution genetic maps • Resequencing part (*e.g.* by targeting specific functional regions such as the exome) or all of the human genome using "next-generation sequencing" technologies • "Next- and third generation sequencing" technologies will allow efficiently extending the knowledge of AD genetics to the lower allele frequency spectrum, down to low-frequency variants
Cerebrospinal fluid	• The use of multiple longitudinal CSF specimens is necessary to detect the time point at which CSF biomarkers convert from physiologic to pathologic values • Substantial progresses in the exploratory "omics" disciplines, especially proteomics/metabolomics, will enhance the detection of novel candidate CSF biomarkers • Many candidate biomarkers have the potential to increase the diagnostic accuracy of the "core" biomarkers $A\beta_{1-42}$, t-tau, and p-tau (*e.g.* BACE1)
Blood	• Progress in blood-based biomarker discovery relies on the establishment of Standard Operating Procedures (SOPs) for the appropriate selection of patients and specimens • The Human Plasma Proteome Project (HPPP) is an initiative launched that will face matters related to pre-analytical variability and to make attempts to establish SOPs • The Blood-Based Biomarker Interest Group (BBBIG), an international working group of leading AD scientists from academia and industry, will inspect the present scenario of biomarker discovery in blood in order to identify current needs that will enable the field to progress • It seems to be doubtful that a blood marker alone will be in itself adequate for the diagnosis of AD • In contrast, it seems most likely to have combinations of markers: several proteins coupled with other blood-based or non-blood-based markers, such as imaging
Structural neuroimaging	• The EADC-ADNI hippocampal harmonization project is providing an internationally consented protocol for manual hippocampus segmentation that will serve to validate automated hippocampus volumetry methods • Future studies are needed to address more specifically associations between regional brain atrophy pattern and regional markers of neuronal degeneration • The next years will see increasing use of automated volumetry of hippocampus or regional brain atrophy pattern as secondary endpoints in clinical trials in prodromal AD and AD dementia stages • Structural imaging markers are being used to enrich the risk for AD in clinical samples of MCI subjects for clinical trials. In addition, structural MRI will help to enrich study samples of asymptomatic subjects with positive molecular biomarkers of AD • The presence of hippocampus atrophy together with amyloid positivity will help to select subjects with a high risk of conversion to AD or MCI within a timeframe that is relevant for a clinical trial • The effect of a novel structural imaging marker of predementia AD on care systems worldwide, that have difficulties to provide adequate care even to patients in clinically manifest stages of disease, needs to be assessed in future studies • Novel methods, including high-field MRI at 3 Tesla and ultra-high field MRI at 7 Tesla, will gain increasing importance to understand the morphological/neuroanatomical basis of cognitive decline in AD • Based on mappings of subcortical nuclei from *postmortem* analyses, MRI scans *in cranio* will help to identify early changes in cholinergic and noradrenergic projecting nuclei in predementia and dementia stages of AD • MRI-based detection of amyloid plaques in humans will become a major topic of research in coming years. The use of 7 Tesla MRI in human studies may allow *in vivo* detection of cortical amyloid deposition in the future
Functional neuroimaging	• Functional MRI will be increasingly applied in the area of novel pharmaceutical strategies, in AD and MCI. Although drug-induced modulation of memory-related networks have been detected by functional MRI, few studies have demonstrated abnormal activation following pharmacological treatment in MCI and AD
In vivo molecular neuroimaging	• Novel imaging instrumentation such as hybrid PET/MRI scanners may offer the opportunity to merge the complementary information from different imaging modalities into new integrated *in vivo* biomarkers of neurodegeneration
Neurodynamics	• Design of enhanced EEG/MEG-based AD biomarkers: - Neurodynamic measures (such as brain connectivity, global synchronization, synchronization likelihood, detrended fluctuation analysis, approximate entropy, mutual information, source localization, and other non-linear signal features) will be used within the framework of both the resting-state and functional biomarker paradigms to adapt better to the complex characteristics and dynamics of progressive neurodegeneration and aging - Future functional EEG/MEG biomarkers will rely on multidimensional (spatio-spectro-temporal characteristics) in order to handle efficiently single-trial EEG/MEG data and increase sensitivity/specificity - Efficient biomarker selection with the final goal to evaluate the current state of AD-related functional brain networks for each individual subject • Standardization and validation of selected EEG/MEG-based AD biomarkers: - A selected battery promising neurodynamic biomarkers will pass through a rigorous multi-step and multi-center standardization/validation process before they can be used as diagnostic aids - Modular approach will be required for new biomarker standards. A robust review procedure will be put in place to facilitate fast and efficient biomarker upgrades

Abbreviations: AD, Alzheimer's disease; $A\beta_{1-42}$, 42 amino acid-long form of the amyloid beta peptide; CSF, cerebrospinal fluid; EADC-ADNI, European Alzheimer's Disease Centers-Alzheimer's Disease Neuroimaging Initiative; EEG, electroencephalography; GWAS, genome-wide association studies; MCI, mild cognitive impairment; MEG, magnetoencephalography; MRI, magnetic resonance imaging; p-tau, hyperphosphorylated tau protein; PET, positron emission tomography; t-tau, total-tau.

recognized by the European Medicine Agency (EMA) [25] for the employment in clinical drug trials as well.

3. National Institute on Aging/Alzheimer's Association criteria

Following the emerging development of the IWG/Dubois criteria, the National Institute on Aging (NIA) and the Alzheimer's Association (AA) summoned three working parties aimed at establishing criteria for the staging of AD [26–28]. Differently from the IWG that use an integrated clinico-biological approach covering all of the AD symptomatic phases, the NIA-AA employs three different categories of criteria for cases in which biomarkers have been measured: one for the asymptomatic phase ("preclinical AD"), one for the AD-MCI phase ("MCI due to AD"), and one for the

AD-dementia phase (“dementia due to AD”) [20]. Notably, the NIA-AA criteria distinguish between amyloid and neuronal injury markers. This distinction is based on the hypothesis that Aβ generation drives other pathophysiological changes, an idea strongly supported by genetic evidence from familial autosomal dominant AD, Down’s syndrome, and the recent demonstration of a protective mutation in the amyloid precursor protein (*APP*) gene [29]. The biomarkers of Aβ accumulation are represented by significant amyloid tracer retention using PET imaging and/or low CSF concentrations of $A\beta_{1-42}$. The biomarkers of neuronal degeneration or injury consist of increased levels of CSF tau (t-tau or p-tau), reduced fluorodeoxyglucose uptake on PET in specific areas encompassing temporoparietal cortex, and atrophy on structural MRI primarily including medial temporal lobes and parietal cortices [30,31].

Subjects with preclinical AD can be categorized into three stages using cognitive markers and biomarkers. In particular, individuals showing only anomalous amyloid markers are classified in stage 1; those with both atypical amyloid and injury markers are considered in stage 2; those showing both unusual amyloid and injury markers accompanied by minimal cognitive impairments, such as SCI, are classified in stage 3. Individuals with MCI due to AD or dementia due to AD are categorized in a risk staging model according to amyloid and neuronal injury markers, as follows: (I) high likelihood for AD if both amyloid and neuronal injury markers are aberrant, (II) intermediate likelihood for AD if only one of the two markers has been assessed and is anomalous, (III) uninformative if one marker is atypical and the other normal, or *vice versa* [20].

The IWG group considers the presence of brain amyloid accumulation in the absence of clinical features in the sporadic population to be indicative of an “at risk” group. In contrast, the NIA-AA group considers such individuals to indeed already have preclinical AD, suggesting that in time they would develop cognitive decline and the clinical dementia syndrome. This presents a fundamental hypothetical and conceptual difference of the two approaches with practical consequences for trials which needs to be further elucidated.

4. The genetics of Alzheimer’s disease

AD has been designated as a multifaceted pathology characterized by a high-degree of genetic heterogeneity. This implies both that the same phenotype can be generated or modified by a number of different genetic loci and alleles, and that mutations or polymorphisms at different positions in the same gene lead to the same clinical syndrome [32]. This situation is aggravated by the fact that, in some instances, different mutations in the same gene can lead to clinically distinct syndromes. Hence, AD is considered to belong to the growing fraction of “genetically complex” diseases.

A peculiar feature observed in AD is the dichotomy of (I) familial *versus* (II) “apparently” non-familial forms of disease. The former, referred to as familial AD, accounts for less than 5% of all AD cases and is often conferred by individual disease-causing mutations transmitted in classic Mendelian fashion, mostly typically by autosomal dominant transmission. Since age of onset in these forms of AD is usually early ($<$65 years) or very early ($\leq$50 years), it is often also called early-onset familial AD (EOFAD). The latter, commonly defined as non-Mendelian, “polygenic”, or “sporadic” AD, accounts for about 95% of all AD cases. It is typically characterized by an onset age well beyond 65 years of age, and it is also designated as late-onset AD (LOAD) [32,33]. However, it should be highlighted that this dichotomization scheme is over simplistic, as there are cases of EOAD without evidence for familial clustering or Mendelian transmission while, on the other hand, these clustering and transmission patterns are frequently observed in LOAD [33]. In addition to these genetic causes, non-genetic (*e.g.* environmental or epigenetic) factors are likely significantly affecting an individual’s risk to develop AD. However, the exact mechanisms underlying the possible pathogenic effects of these non-genetic factors are still mostly elusive which is, at least in part, owing to the fact that it is still relatively difficult to detect and evaluate them experimentally [34].

The introduction of high-throughput DNA genotyping and sequencing technologies, allowing to systematically screen the genomes of a large number of individuals simultaneously, has led to the completion of a high number of genome-wide association studies (GWAS) in AD. These studies allow simultaneously investigating literally millions of genetic markers (mostly so-called single-nucleotide polymorphisms, SNPs) in one experiment to assess their effect on disease risk, or quantitative phenotypes. Not unexpectedly, these GWAS have led to more reproducible and more consistent findings than three decades of candidate-gene-driven research before [35].

4.1. EOFAD with Mendelian transmission

EOFAD is caused by rare and highly penetrant mutations in three genes, namely: amyloid precursor protein (*APP*, located at chromosome region 21q21.2), presenilin 1 (*PSEN1*, located at 14q24.3), and presenilin 2 (*PSEN2*, located at 1q42.13) [33]. Presently, more than 220 distinct disease-causing mutations have been discovered across these genes (for an up-to-date summary, see the Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB) at http://www.molgen.vib-ua.be/ADMutations/ [36]). Currently, over 30 AD-causing mutations have been reported in *APP*, encoding for the precursor protein for Aβ. Interestingly, most of the *APP* mutations occur near the putative γ-secretase site between amino acidic residues 714 and 717, suggesting that the γ-cleavage event of APP or its (dys)regulation are crucial for the development of AD [32]. The vast majority of EOFAD mutations are observed in *PSEN1* located on chromosome 14. *PSEN1* encodes for a highly conserved polytopic membrane protein, presenilin 1, which is involved in mediating intramembranous, γ-secretase processing of APP to generate Aβ peptides [37]. At present, the overall number of known AD-causing mutations in *PSEN1* exceeds 180. Lastly, EOFAD is rarely caused by mutations in *PSEN2* which encodes for presenilin 2, which represents another member of the presenilin family of proteins, displaying substantial homology to presenilin 1, both at the genomic and protein level [38,39]. In summary, the currently known AD-causing mutations occur in three different genes located on three different chromosomes. Functionally, the proteins encoded by all three genes share a common biochemical pathway, *i.e.* the altered production of the Aβ peptide. Together, these findings provide strong support for the “amyloid hypothesis” indicating that an abnormal production and/or regulation of Aβ is one of the main factors underlying AD pathogenesis [40]. While the currently known mutations in these three EOFAD genes account for a large fraction of Mendelian AD, they do not account for all cases, suggesting that AD-causing mutations in other genes exist. The successful identification of these hitherto unknown Mendelian AD genes could, thus, provide entirely new insights into AD pathogenesis [33].

Recently, a study has detected mutations in the *SORL1* gene in EOFAD patients [41]. *SORL1* encodes for the protein SorLA that belongs to a set of protein-trafficking molecules in the endocytic and retromer pathways and is implicated in modulating the production of Aβ peptide [41]. These findings suggest that *SORL1* may represent a genetic risk factor for AD, although these data need independent replication.

4.2. Sporadic AD/LOAD

In contrast to EOFAD, LOAD exhibits a significantly more complex and intricate pattern of interplay between genetic and non-genetic factors. This situation, combined with the fact that each factor only exhibits exceedingly small effect sizes, has been proven to make the identification of these factors a complicated issue.

The earliest and by far best established genetic risk factor for LOAD is the presence of one or two copies of the *ε4* allele in the apolipoprotein E gene (*APOE*), located on chromosome 19q13.2 [42]. The risk effect of *APOE ε4* has been replicated in many studies across various ethnic groups. Besides the increase in AD risk conferred by the *ε4* allele, a less pronounced protective effect has been reported, albeit somewhat less consistently, for the least common *ε2* allele [43]. Despite its comparatively large effect size, it is important to note that the presence of the *APOE ε4* allele is neither necessary nor sufficient to actually cause AD. Instead, it works as a *bona fide* genetic risk modifier, likely by diminishing the age of onset in a dose-dependent manner. In spite of the accomplishments of over two dozen published GWAS in AD, *APOE ε4* remains to be the single most important genetic risk factor for AD, both in terms of effect size and statistical significance [32].

Despite its well-known genetic association, the biochemical aspects of *APOE ε4* in AD pathogenesis are still only incompletely understood. The encoded protein, apolipoprotein E (apoE), is synthesized in a large number of tissues, primarily in the liver. Hepatic apoE accounts for roughly three-quarters of circulating plasma levels of the protein [44,45]. The human brain is the second most prominent site of synthesis, chiefly occurring in the astrocytes [46] and microglia [47]. There is experimental evidence from transgenic mice that the expression of the human *ε4* allele and mutant APP promotes Aβ accumulation during the course of the disease, suggesting that amyloid may accumulate progressively with time [48]. Moreover, apoE participates in cholesterol transport and lipid metabolism and, in addition to AD, the *ε4* allele also represents a confirmed risk factor in vascular disease, likely owing to its link to augmented plasma cholesterol levels [49]. Amyloid angiopathy involving capillaries is much more prevalent in *APOE ε4* carriers [50].

After the original report suggesting *APOE ε4* to be a genetic risk factor in AD, literally hundreds of genes have been investigated for evidence of genetic association and disease risk, mostly to no avail (for an up-to-date overview of the accumulated evidence, see the AlzGene database at http://www.alzgene.org/ [51]). As outlined above, this situation changed substantially with the advent of GWAS which have led to the identification of at least ten novel loci linked to mostly LOAD risk: *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *MS4A6A*, *MS4A4E*, *CD33*, *CD2AP*, and *EPHA1* [52–56]. Functionally, these genes appear to be mostly linked with three (interdependent) molecular pathways: (I) the amyloidogenic cascade, (II) cholesterol-lipid metabolism, and (III) immune-inflammatory mechanisms [57]. Extending these leads, Jones et al. (2010) have assessed the functional role of SNPs not quite reaching genome-wide significance in AD and arrived at a very similar conclusion, *i.e.* that especially pathways related to immune system response and lipid metabolism appear to be particularly overrepresented [58]. More recently, rare amino-acid changing variants in *TREM2* (encoding for the triggering receptor located on myeloid cells 2) have been implicated as additional risk factors for LOAD [59,60]. Intriguingly, the protein encoded by *TREM2* is an immune receptor participating in the clearance of neural debris from the central nervous system (CNS), *via* processes including phagocytosis and reactive oxygen species production [61]. In all likelihood, additional AD susceptibility variants will be identified in upcoming GWAS based on larger sample sizes and/or higher resolution genetic maps. Equally, efforts are already under way to resequence part (*e.g.* by targeting specific functional regions such as the exome) or all of the human genome using "next-generation sequencing". Other than GWAS – which are based on microarray technology primarily targeting common genetic variations – these methods will allow efficiently extending our knowledge of AD genetics to the lower allele frequency spectrum, down to low-frequency variants such as the ones already observed in *TREM2*. However, even the increasingly widespread application of these powerful new technologies will not abolish the need for extensive subsequent functional genetic experiments to elucidate the pathogenic mechanisms underlying the observed genetic effects [32].

5. Cerebrospinal fluid biomarkers

Owing to its contiguity to the brain parenchyma and the free exchange with the brain extracellular space, the biochemical composition of CSF is able to provide information on the brain chemistry. The distinctive features of CSF, together with the low incidence of complications after lumbar puncture [62] have supported the introduction of lumbar puncture and analyses of CSF biomarkers into routine clinical practice in some centers [63,64]. CSF biomarkers are also increasingly used in clinical drug trials, both for enrichment of the target population at inclusion and to evaluate the biochemical effects of treatment [65–67].

5.1. AD dementia

In the early' 90s, a first publication has documented elevated CSF amounts of t-tau in patients with AD dementia [68]. After that, augmented CSF concentrations of p-tau [69] and reduced levels of $A\beta_{1-42}$ [70] have been described. A large number of studies have replicated these findings. A decrease in CSF $A\beta_{1-42}$ to about 50% of the level in cognitively normal elderly subjects has been regularly reported, whereas an increase in CSF t-tau to approximately 300% of the level in cognitively normal elderly subjects and a less evident growth in CSF p-tau to about 200% have been repeatedly detected [71]. Such biomarkers show 80–95% of sensitivity and specificity in the dementia phase of the pathology [71,72].

The CSF concentration of these markers is within the normal range in several differential diagnoses, including depression and Parkinson's disease [5,69,72]. Additionally, measurement of p-tau in CSF is of help to distinguish AD from other dementing pathologies, such as frontotemporal dementia and Lewy-body dementia. Only minimal differences among immunoassays specific for various epitopes of p-tau, including p-tau_{181}, p-tau_{231}, and p-tau_{199}, have been found [73]. The diagnostic accuracy of these CSF biomarkers has also been substantiated in analyses in which the diagnosis was then proven by autopsy [74,75] with comparable or superior discriminatory power than in studies utilizing patients with clinical diagnoses only.

5.2. Prodromal AD

CSF biomarkers exhibit a high predictive value in detecting prodromal AD in MCI subjects [72]. A study with a protracted clinical follow-up period has revealed that the combination of all three core CSF biomarkers shows a sensitivity of 95% to recognize prodromal AD in MCI [76]. Moreover, these markers are able to predict the rate of cognitive decline in patients with MCI/very mild AD dementia [77].

A high diagnostic accuracy of CSF biomarkers for prodromal AD has also been corroborated in large multicenter studies, such as the US-ADNI [75], the European Development of Screening guidelines and Criteria for Predementia Alzheimer's disease (DESCRIPA) study

[78], and the Swedish Brain Power (SBP) project [79]. These findings emphasize the role of CSF biomarkers as clinical diagnostic tools to detect enhanced risk in MCI subjects to have prodromal AD.

5.3. Preclinical AD

The notion of preclinical AD designates cognitively normal subjects harboring early AD pathology, not severe enough to induce cognitive signs. The efficacy of CSF biomarkers in the preclinical stage to recognize patients who will progress to AD dementia has been assessed. Skoog et al. (2003) have found a reduction in CSF $A\beta_{1-42}$, but normal t-tau and p-tau levels in cognitively normal 85-year-olds who later developed dementia [80]. These results are corroborated in a population-based cohort of healthy elderly subjects aged 70–78 years with 8 years follow-up [81] and in a clinical study on asymptomatic elderly subjects aged 60–94 years [82]. According to these data, CSF levels of $A\beta_{1-42}$ start declining in the preclinical phase of sporadic AD, prior to any manifest increase in t-tau or p-tau.

With reference to familial AD, Moonis et al. (2005) have uncovered that asymptomatic subjects carrying familial AD mutations exhibit both low CSF $A\beta_{1-42}$ and high t-tau concentrations [83]. This finding is confirmed in an analysis by Ringman et al. (2008) showing that mutation carriers have the full AD pattern of CSF biomarker changes long before symptom onset [84]. Bateman et al. (2012) have also suggested that CSF $A\beta_{1-42}$ may start to decrease already 25 years before the estimated clinical onset in familial AD mutation carriers, whereas increased CSF tau may be observed 15 years before predicted symptom onset [85]. Altogether, these results suggest that CSF biomarkers, especially $A\beta_{1-42}$, convert to positive several years before the first appearance of clinical signs, also in the familial form of the disease. Notably, familial AD mutation carriers – in their early 20s – may commence at higher CSF $A\beta_{1-42}$ concentrations than non-mutation carriers [84,86]. It should be noted that most of the studies published to date are "pseudo-longitudinal" in their design; they relate cross-sectional biomarker data to longitudinal clinical or neuroimaging markers or time before expected disease onset. Longitudinal examinations with repeated CSF samplings are required to define when and how fast the shift to lower CSF $A\beta_{1-42}$ and higher tau levels occurs, indicating onset of amyloid deposition and neurodegeneration.

5.4. Combined analyses of Aβ and tau biomarkers

Combining $A\beta_{1-42}$ with tau offers good discriminative value for AD patients compared to age-matched healthy controls, with a sensitivity of 85% and a specificity of 86%. Nevertheless, when these ratios are employed to discriminate AD from other dementias, a lower degree of specificity is achieved [87]. Other examinations have used the tau x $A\beta_{1-40}/A\beta_{1-42}$ ratio – referred to as the AD index – showing sensitivity 69% and specificity 88% [88] or the combination among $A\beta_{1-42}$, $A\beta_{1-38}$, and tau to make a diagnosis of AD [89]. In the latter analysis, increased p-tau and the ratio $A\beta_{1-42}/A\beta_{1-38}$ account for accuracies higher than 80 and 85%, respectively, to differentiate AD *versus* non-Alzheimer dementias (NAD). The combination of p-tau with $A\beta_{1-42}/A\beta_{1-38}$ leads to a sensitivity of 94% to identify AD and 85% specificity to exclude NAD. The ratio $A\beta_{1-42}/A\beta_{1-38}$/p-tau, robustly distinguishing AD *versus* NAD, is believed to satisfy the accuracy requirements for an appropriate screening and differential diagnostic AD biomarker [89]. When reviewing this type of sensitivity and specificity figures for the AD CSF biomarkers, it should be noted that these figures come from studies based on clinically diagnosed patients, which means that a biomarker can never show a better performance than the clinical diagnosis in such studies.

5.4.1. Progression from cognitively normal subjects to MCI

The increased ratio of tau/$A\beta_{1-42}$ and p-tau/$A\beta_{1-42}$ in normal subjects has been related to an amplified risk of conversion to MCI. A study has demonstrated that about 70% of those with a high ratio *versus* only 10% of those with a normal ratio change to MCI over a 3-year period [90]. Later, it has been observed that all subjects who have converted to MCI display increased tau/$A\beta_{1-42}$ ratios (over a follow-up of 42 months), while no conversions take place in the normal ratio group [91]. In light of this, the subset of normal elderly with high ratios seems to have already developed amyloid deposition and neurodegeneration. This might denote a subgroup with a diagnosis of preclinical AD.

5.4.2. Progression from MCI to AD

Numerous studies assessing the efficacy of CSF markers in predicting the risk of progression from MCI to AD indicate that diminished $A\beta_{1-42}$ and elevated t-tau and p-tau show in MCI a sensitivity equivalent to that observed in more advanced AD [92]. Lower CSF $A\beta_{1-42}/A\beta_{1-40}$ ratios suggest risk of progression to AD in subjects with very mild dementia [92]. A large longitudinal study of MCI subjects (18 months follow-up) has allowed the detection of a grown tau/$A\beta_{1-42}$ ratio in 90% of MCI subjects who have later converted to AD compared to 10% of those who have not converted [93]. Combining tau with the $A\beta_{1-42}$/p-tau_{181} ratio has significantly predicted progression of MCI into more advanced AD in another longitudinal study (average follow-up: 4–6 years) [76].

As emphasized by Blennow et al. (2012) [94], given that the diagnostic accuracy for the combination of CSF $A\beta_{1-42}$, t-tau, and p-tau has been reported to be higher than for any biomarker alone [76,93,95,96], a multiparameter assay, utilizing the Luminex™ xMAP technology (Luminex Corporation, Austin, TX, USA) to enable simultaneous quantification of these CSF biomarkers, has been developed [97]. The employment of this assay in multicenter studies on CSF biomarkers has yielded a good diagnostic performance [75,76,79,98].

5.5. Time course of AD biomarkers

Great consideration has been given to the hypothetical model for the sequence of pathologic events in AD suggested by Jack et al. (2010) according to which biomarkers reflecting Aβ pathology become positive before those reproducing neuronal degeneration and tangle development [99]. Two recent examinations have addressed this issue in detail. Both studies, after scrutinizing MCI cohorts with long clinical follow-up, have identified an evident reduction in CSF $A\beta_{1-42}$ along with grown levels of t-tau and p-tau. In particular, one study has demonstrated that MCI subjects with prodromal AD present with low CSF $A\beta_{1-42}$, regardless of time to dementia, whereas t-tau and p-tau are highest in patients with shorter time to conversion, thus indicating that $A\beta_{1-42}$ is completely altered before t-tau or p-tau [100]. These data support the hypothesis that modified Aβ metabolism precedes tau-related disease and neuronal degeneration. The other study has disclosed that MCI subjects with elevated concentrations of injury markers – namely, t-tau and p-tau – may develop faster, therefore presenting shorter time to conversion [101]. Since both analyses are cross-sectional in regards to the biomarker data, the use of multiple longitudinal CSF specimens is necessary to detect the time point at which CSF biomarkers convert from physiologic to pathologic values.

5.6. CSF biomarkers variability

Substantial interlaboratory discrepancies, with reference to CSF biomarker levels, make assessments and comparisons of data from different laboratories problematic. As a result, globally recognized

reference and cut-off values have not been established. For this reason, standardization efforts have been introduced to harmonize laboratory practices [102], define procedures on CSF collection and handling [103], create reference materials for assay calibration [104], and delineate reference measurement protocols [105]. In particular, the establishment of certified reference materials is presently executed as a concerted effort among the Alzheimer's Association, the International Federation of Clinical Chemistry and Laboratory Medicine, and the Institute for Reference Materials & Measurements [106].

A universal quality-control program to evaluate total analytical variability of the best-established CSF biomarkers – $A\beta_{1-42}$, t-tau, and p-tau – has been recently initiated by the Alzheimer's Association. The aim is the standardization of CSF biomarker measurements between research and clinical laboratories to increase the analytical precision and improve the longitudinal stability of biomarker measurements [79].

To date, the major cause of the experimental variability for CSF biomarkers is due to between-laboratory factors [107]. Since global biomarker cut-off levels cannot be defined owing to the high extent of variability, each laboratory should employ internally validated cut-off values and guarantee longitudinal stability in its measurements. Progresses in standardization of laboratory protocols in conjunction with the enhancement of kit performance and the use of fully automated tools are expected to improve the effectiveness of CSF AD biomarkers for both researchers and clinicians [107].

5.7. Upcoming candidate biomarkers

The composition of CSF is subject to fluctuations that mirror the complexity of AD pathophysiology, involving SPs deposition, NFTs formation, gliosis/neuroinflammation, and synaptic and neuronal loss. Accordingly, lots of molecules have been proposed as potential AD biomarkers in the CSF [108]. As the power and the complexity of the "omics" disciplines such as proteomics and metabolomics – that promise to revolutionize biomedicine – has greatly advanced over the last decade, proteins encompass the majority of viable candidates. In this context, both hypothesis-driven strategies – allowing the study of definite molecules participating to Aβ metabolism, neurodegeneration, neuroinflammation, and oxidative stress – and unbiased as well as targeted multianalyte profiling approaches – for instance, proteomic screening and molecular arrays – have been employed (see Fagan and Perrin (2012) for an exhaustive review on novel candidate CSF biomarkers [108]). Individually, various candidates are useful to evaluate statistical differences between cohorts of AD and control samples, and many of these also have the potential to increase the diagnostic accuracy of the "core" biomarkers $A\beta_{1-42}$, t-tau, and p-tau. Certain promising molecules seem to be of help for diagnosis/differential diagnosis, prognosis, and therapeutic monitoring ("theragnosis") [108].

Notably, in the framework of the "omics" revolution, mounting evidence is emphasizing the role of metabolomics to determine diagnostic biomarkers for AD. The metabolome designates a set of small-molecule metabolites discovered within a biological sample in a specific physiologic or developmental condition. Thus, different disease states disturbing biochemical networks will lead to dissimilar metabolic signatures [109]. This groundbreaking approach recognizes metabolic disturbances by assessing the activity of various metabolites at the same time. The discovery of uncommon disruptions in the metabolic network could serve to better elucidate the pathological mechanisms [110]. Notably, two analyses have reported alterations in CSF metabolome of AD. One study has showed the increase of the concentrations of eight amino acids in AD *versus* MCI [111]. Another larger examination, after measuring 343 analytes has also led to detect eight molecules with statistical significance; interestingly, one of these markers, cortisol, correlated with the advancement of the disease [112]. A disadvantage with metabolomics as compared with proteomics and peptidomics may be that, in contrast to several proteins, there is no data showing an established role for small (non-protein) molecules in AD pathogenesis.

6. Blood prospective candidate biomarkers

The attention on blood-based biomarkers for the diagnosis of AD has rapidly developed during the past decade. Although conventional AD biomarkers from CSF are highly accurate, barriers to their clinical application are still present. Since blood is a biofluid much more easily reached and manageable than CSF, searching for consistent blood-borne biomarkers is needed. In this connection, the Blood-Based Biomarker Interest Group (BBBIG), an international working group of leading AD scientists from academia and industry, has been established to scrutinize the present scenario and to support the progress in the field (see Henriksen et al. (2013) for a critical perspective on the status of blood-based biomarkers for AD [13]).

Although the association of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations with incipient AD has been repeatedly investigated, definite data are still lacking. Increased $A\beta_{1-40}$ or $A\beta_{1-42}$ levels have been shown to predict the development of AD [113,114]; however, other analyses have revealed no associations [115,116] or opposite [117] results. A low $A\beta_{1-42}/A\beta_{1-40}$ ratio is assumed to predict future AD [113,118,119]. On the other side, an increased ratio [114,120] or no major difference [115] in subjects with incipient AD, compared with those that have not developed AD, have been described. A recent meta-analysis has suggested that a low $A\beta_{1-42}/A\beta_{1-40}$ ratio could predict the progression of AD, but no such association has been observed for the single peptides [121].

With reference to tau, some studies have highlighted differences in the modulation of CSF tau levels as compared to blood. In case of hypoxic brain damage subsequent to cardiac arrest, tau is promptly released into the bloodstream but efficiently cleared, within 24 h, in patients showing positive neurological outcome [122]. In contrast, CSF tau levels remain elevated for several weeks after an acute neurological insult [123]. In addition, tau concentrations are significantly increased in CSF of AD patients, but less in the equivalent plasma samples. Actually, measurements of tau in CSF and plasma compartments are not associated [124]. More recently, the association of plasma tau concentrations with AD has been appraised in a cross-sectional study including AD patients, MCI subjects, and cognitively healthy controls, using a newly developed ultra-sensitive immunoassay for the quantification of tau protein [125]. Plasma concentrations of tau appear increased in AD in relation to MCI and healthy controls. MCI-AD subjects (*i.e.* MCI converters to AD) display tau levels comparable to those detected in MCI-stable (*i.e.* MCI non-converters to AD) and healthy subjects. This overlap among ranges is believed to diminish the efficacy of plasma tau as diagnostic test [125].

During the last decade, the development of mass spectrometry-based technologies has elected proteomics as the chief platform to inspect the plasma/serum proteome for the discovery of next generation biomarkers showing diagnostic, prognostic, or therapeutic efficacy [126]. Mass spectrometry-based methods, together with innovative tools in progress, are welcomed because they will significantly improve the ability to detect blood markers [127,128]. By simultaneously quantifying the levels of many plasma analytes, biomarker patterns successfully distinguishing AD patients from controls [129] or associated with MCI or AD have been disclosed [130]. Since the activities of most molecules are connected to immune regulation and inflammatory pathways, the existence of

an inflammatory process in AD has been firmly proposed [131,132]. Nevertheless, such protein panels have been hard to reproduce in independent studies [133].

However, two analyses, utilizing large and well-characterized clinical cohorts, have discovered that a set of inflammatory molecules display modified expression as a function of AD [130,134]. Moreover, both Doecke et al. (2012) [134] and O'Bryant et al. (2011) [135] have found diagnostic accuracy across cohorts employing biomarker algorithms/profiles. These encouraging results provide additional support for the blood-based profiles/signatures.

The discovery of plasma/serum biomarkers for a CNS disease as AD meets both conceptual and practical challenges [136]. No findings of transcripts/proteins/metabolites in blood have been successfully replicated to be definitively approved as AD biomarkers. Moreover, based on the data from the literature, it seems to be doubtful that a blood biomarker alone will be in itself adequate for the diagnosis of AD. In contrast, it seems most likely to have a combination of markers: several proteins coupled with other blood-based or non-blood-based markers, as imaging [127]. It is also uncertain the existence of only one set of biomarkers for all conceivable uses in AD. It is probable that there will be a group of biomarkers to support AD diagnosis, a different set of molecular markers to predict outcome in AD patients or conversion in MCI, and, probably, another cluster to allow monitoring the evolution of the disease [128].

It should be also emphasized the presence of many issues in plasma/serum proteomics, including the existence of pre-analytical and analytical variables. Consequently, there is an urgent need for standardization of specimen collection/processing, quantitation, and setting strategies for managing biomarkers after their detection [137]. Progress also relies on the establishment of Standard Operating Procedures (SOPs) for the appropriate selection of patients and specimens, thus decreasing the complexity of samples to be analyzed [138]. In this regard, the Human Plasma Proteome Project (HPPP) (see http://www.hupo.org/initiatives/plasma-proteome-project/) is an initiative conceived and launched by the Human Proteome Organization (HUPO) (available at http://www.hupo.org/) to solve matters related to pre-analytical variability and to make attempts to establish SOPs [139]. In addition, the development of informatic tools for data management and collaborations with other disease-related initiatives of the HUPO to extend the area of plasma/serum biomarker discovery should be encouraged [140].

7. Neuroimaging markers

7.1. Structural MRI markers

Reduction of hippocampus volume, derived from structural MRI, is one of the key biomarkers of AD in the IWG [23] and NIA-AA criteria [30]. This reflects the consistent findings of reduced hippocampus volumes in AD and MCI subjects across a wide range of mono- and multicenter studies (for meta-analysis, see Clerx et al. (2012) [141]). Hippocampus volume has also been used as a secondary endpoint in several clinical trials on potential disease modifiers in AD or MCI, including vaccination [142], muscarinic receptor agonists [143], and glutamate modulators [144]. Although widely used since more than 20 years, standardization of manual hippocampus volumetry has only begun in 2011 with the European Alzheimer's Disease Centers-Alzheimer's Disease Neuroimaging Initiative (EADC-ADNI) hippocampal harmonization project [145] that now provides an internationally consented protocol for manual hippocampus segmentation (available at http://www.hippocampal-protocol.net/SOPs/index.php). This protocol will serve to validate automated hippocampus volumetry methods [146]. The EMA regulatory authorities have endorsed a qualification process for the use of low hippocampus volume to help enrichment of study samples (available at http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_-procedural_guideline/2011/10/WC500116264.pdf). Few automated protocols have already been cleared for marketing as a medical device by the US FDA. However, hippocampal atrophy is not specific to AD and is found in other conditions, including fronto-temporal dementia [147] vascular dementia [148], Lewy-body dementia [149], and depression [150].

Another structural marker beyond hippocampus volumetry is whole brain volume as longitudinal marker of disease progression and treatment effects. Automated algorithms such as voxel-based compression mapping [151] allow stable estimates of whole brain volume over time and across centers [152]. Whole brain volume has been used as secondary endpoint in several clinical trials [142,153], but has become less attractive with the advent of regionally more specific protocols. These are based on local measures of gray matter concentration or cortical thickness at each point of the space and on high dimensional warping of brain scans into a common standard space [154] to estimate regional pattern of atrophy in data driven automated analyses. Longitudinal evaluation of these pattern of atrophy has begun to be used in phase IIa type clinical trials [155]. In addition, multivariate approaches such as machine learning with support vector machines have successfully been employed to derive patterns of brain atrophy that discriminate AD patients from healthy controls and MCI converters from MCI-stable subjects [156–158]. By highlighting specific topographical patterns of atrophy, these approaches have the potential to be useful to discriminate between different types of dementia [158]. Presently, scanner manufacturers are developing radiological expert systems based on these algorithms to help the radiologist to rate the presence or absence of AD from the pattern of atrophy derived from a single brain scan. It is important to note that these technical devices need to be employed in a multidisciplinary clinical setting where the diagnostic relevance of an imaging finding is put in the context of all other relevant clinical information by a clinical dementia specialist.

The attractiveness of MRI as endpoint in clinical trials is related to the assumption that regional brain volume can serve as *in vivo* surrogate of neuronal number. Clinico-pathological comparison studies have shown that hippocampus volume obtained *antemortem* accounted for at least 50% of variability in neuron numbers determined during autopsy [159]. The amount of variation explained by MRI-based hippocampus volumetry was above 90% when MRI scans was obtained *postmortem* [160]. Thus, hippocampus volumetry can be considered as an *in vivo* surrogate measure of hippocampal neuronal number. However, one should be careful to simply interpolate these findings to *in vivo* measures of cortical atrophy. In 27 *antemortem* cognitively intact subjects, cortical thinning determined *postmortem* across age cohorts was not associated with regional neuron numbers and density, but was suggested to reflect changes in neuronal and dendritic architecture [161]. Therefore, future studies need to address more specifically associations between regional brain atrophy and regional markers of neuronal degeneration. A platform implementing *postmortem* MRI *in cranio* can help such an approach through access to *postmortem* MRI data whose signal distribution is close to *in vivo* MRI scans [162]. Moreover, hippocampal sclerosis may occur in the absence of AD pathology and hippocampal atrophy is common in fronto-temporal lobar degeneration related to mutation of the progranulin (*GRN*) or chromosome 9 open reading frame 72 (*C9ORF72*) genes [163].

An explicit framework has been proposed for a specific temporal sequence of biomarker changes during progression from

asymptomatic AD to AD dementia [99]. A study of the Dominantly Inherited AD Network has provided evidence supporting such a sequence in asymptomatic mutation carriers of familial AD [85]. In these subjects, hippocampus atrophy was estimated to follow amyloid accumulation by 10 years and to precede onset of dementia by up to 15 years. Findings from familial forms cannot simply be transferred to sporadic AD. A recent study in sporadic AD interpolated 6-year follow-up data onto a timeline from asymptomatic to clinical manifest disease covering several decades [164]. This study estimated an onset of hippocampus atrophy 4 years before onset of clinical dementia, much later than in the familial cases. It is necessary to keep in mind that these data represent interpolations from cross-sectional [85] or maximum 6 years follow-up [164] data that are projected onto a timeline of disease progression. This projection, however, relies on assumptions of disease stages that shall actually be tested in the specific study. Therefore, there is some circularity in testing these models that need to be validated in further studies.

7.1.1. Future directions: application of existing methods in a new context

The next years will see increasing use of automated volumetry of hippocampus or regional brain atrophy pattern as secondary endpoints in clinical trials in prodromal AD and AD dementia stages. Regulatory authorities are seeking for biological surrogate markers for disease modification in a situation where neuropsychological endpoints require large cohorts and complex study designs to differentiate symptomatic from disease modifying effects [6]. Structural imaging markers will play a key role in this respect, because they provide stable measures over time and across scanners and are closely associated with underlying changes of neuronal integrity [159]; moreover, the functional relevance of regional atrophy for some specific cognitive impairments has been established [165]. Therefore, the use of structural imaging endpoints will help to reduce sample size in future clinical trials. Due to the wide availability of structural imaging markers, they are also being used to enrich the risk for AD in clinical samples of MCI subjects for clinical trials. In addition, structural MRI will help to enrich study samples of asymptomatic subjects with positive molecular biomarkers of AD. The presence of amyloid alone does not predict progression to cognitive decline with sufficient accuracy, as only 25% of amyloid positive cognitively healthy subjects progress to MCI or AD within 3 years [166]. Therefore, the presence of hippocampus atrophy together with amyloid positivity will help to select subjects with a high risk of conversion to AD or MCI within a timeframe that is relevant for a clinical trial.

Use of such markers in the well-controlled setting of a clinical trial will be in the interest of probands participating in such trials. However, these protocols will also be increasingly used for diagnostic purposes outside of clinical trials. If embedded into a multidisciplinary diagnostic setting and applied to symptomatic patients, the use of such protocols will probably help make full use of the anatomical information in a structural MRI scan to the benefit of the patient. The situation is different, when such measures are employed as screening instruments. Even today, private companies offer an analysis based on regional brain and hippocampus volumetry to people who pay to get a confirmation that their brain is still structurally intact. The problem with this business model is that we are far from knowing what an atrophic hippocampus or regional brain atrophy means in terms of risk for AD and dementia in an asymptomatic person without further clinical information. Moreover, there is no point in identifying a hypothetical risk of AD without offering an intervention and support scheme to an individual. There are still many issues to be resolved on how to adequately communicate the negative aspects of a screening to a client, such as the risk of false-positive findings, the lack of a treatment option, and the probable lack of clinical relevance of a true positive finding. The "litmus test" for the usefulness of an imaging marker is its application in the "intent to diagnose" population, *i.e.* in those patients that will be confronted with this diagnostic test in primary care. There is almost no evidence available on the usefulness of imaging markers, including MRI, to support an early diagnosis of AD outside of clinically highly selected samples. Future studies are needed to determine the efficacy of MRI to detect AD type pattern of atrophy in the presence of comorbidities that had usually been excluded in studies so far. In addition, the effect of a novel structural imaging marker of predementia AD on care systems worldwide that have difficulties to provide adequate care even to patients in clinically manifest stages of disease needs to be assessed in future studies [4].

7.1.2. Future directions: novel methods

Novel methods will gain increasing importance to understand the neurobiological basis of cognitive decline in AD. The wide availability of high-field MRI at 3 Tesla and the increasing availability of ultra-high field MRI at 7 Tesla render subfield measurements of the hippocampus a feasible diagnostic approach in selected samples. Pathological evidence suggests a selective vulnerability of hippocampal subfields in AD [167]. Manual methods to determine hippocampal subfields are based on the direct identification of anatomical boundaries and serve as gold-standard to assess the performance of automated methods. Using hippocampus subfields can significantly decrease the rate of false positive findings in the prediction of future conversion from MCI to AD using manual [168] or automated [169] measurement. Sequences at 7 Tesla provide higher spatial resolution, new contrasts and access to even finer substructures of the hippocampus [170,171], but the clinical relevance of these measures needs to be explored in future studies [172].

Neuropathological evidence suggests a selective involvement of specific subcortical areas, most notably the cholinergic nuclei of the basal forebrain [173,174] and noradrenergic nuclei, in the *locus coeruleus* in AD. Based on mappings of subcortical nuclei from *postmortem* analyses, MRI scans *in cranio* will help to identify early changes in cholinergic and noradrenergic projecting nuclei in predementia and dementia stages of AD [175,176].

MRI-based detection of amyloid plaques has been successfully implemented in transgenic animals [177–180]. Further, a recent study indicates that detection is also possible in non-transgenic mouse lemur primates, in which plaques are formed naturally and are more similar to those found in humans [181]. MRI detection of plaques in humans will thus become a major topic of research in coming years. Using 7 Tesla MRI in human studies may allow *in vivo* detection of cortical amyloid deposition in the future, based on susceptibility related imaging [182] or direct visualization of amyloid plaques using intrinsic or extrinsic contrast agents. The validity of first findings and their relevance for early diagnosis will be explored in the coming years.

7.2. Diffusion tensor imaging

Diffusion Tensor Imaging (DTI) is a magnetic resonance (MR) technique that measures the random thermal motion of water molecules, *i.e.* Brownian motion, within tissue [183]. This modality does not require the injection of contrast material or radiation exposure and provide, non-invasively, unique information of the axonal organization of the brain, which is not feasible with standard MRI techniques. During the last decade, this technique has become a leading method in investigating white matter (WM) microarchitecture and integrity and has been widely employed in AD and MCI [184–187].

In a clinical context, modern MR high-field scanners (between 1.5 and 3.0 Tesla) allow rapid whole-brain assessment (4–10 min) of the apparent water diffusion tensor (DT) field using echo-planar imaging sequences. Images generated from DTI data may be qualitatively interpreted by using directionally encoded color (DEC) maps in which each color represents the axonal orientation of WM tracts. By contrast, using quantitative scalar metrics, most commonly the mean diffusivity (MD) and fractional anisotropy (FA), tissue integrity may be inferred [188]. DT-derived rotational invariants such as single eigen-values may be exploited in quantifying WM tracts integrity through region of interest (ROI), voxel- or tract-based spatial statistics approaches [185]. Furthermore, information regarding WM architecture may be quantified through deterministic and/or probabilistic tractography algorithm [189].

Normal human brain exhibits higher hindrance to water motion (diffusion) perpendicular to the long axis of WM bundles than parallel. This restriction is mostly attributed to macromolecules and cellular barriers (cell membrane) [190]. Neuronal damage, because of loss of the barriers, causes an increase in MD and a decrease FA.

Increased MD and decreased FA values have been reported in AD and MCI in parietal and temporal areas, including the hippocampal region, suggesting unspecific bundle degeneration [191,192]. Abnormal DT derived indices have also been demonstrated in frontal region, and specifically in the *cingulum posterior*, *corpus callosum*, *fasciculus longitudinalis superior*, and *fasciculus uncinatus* [193–195].

A recent study including both AD and MCI subjects [196] demonstrated a circumscribed increase in FA. These findings were aided by examining variations of a third tensor invariant, tensor mode [197] allowing to differentiate the type of anisotropy (planar, *e.g.* in regions of crossing fibers *versus* linear, in regions with one predominant orientation). Using this method, authors postulated a selective degeneration of only one of two crossing fibers suggesting a relative sparing of motor-related projection fibers crossing the association tracts of the *fasciculus longitudinalis superior*. In addition, DTI has been able to track the age-related WM degeneration in AD and, in agreement with the retrogenesis model (regions that mature late are more vulnerable to age- and disease-related degeneration), WM changes have been shown to appear earlier in specific areas such as temporo-parietal regions, the *fasciculus longitudinalis inferior*, and prefrontal regions [186,198,199].

Importantly, the reproducibility and robustness play a major role in DT data acquisition; this is a delicate point as tensor techniques employ extremely noisy echo-planar sequences, requiring strict quality control and quality assurance routines [200]. A recent meta-analysis highlights the high variability in both the anatomy of regions studied and DTI metrics [201]. Also, a recent European multicenter study, the European DTI Study in Dementia (EDSD) [202], revealed significant center-related effect in DT-derived measures.

One shortcoming of conventional DTI methods is related to the use of the simplistic model of a Gaussian propagator, which is not sufficiently accurate in regions where mixed tissue types can give rise to significant partial volume effects and/or where two or more WM fiber cross [203]. To this aim, more advanced methods such as Kurtosis Imaging [204–207], Diffusion Spectrum Imaging [208], higher-order tensor models [209], compartment models [210,211], and anomalous diffusion [212,213] have been introduced in order to augment their suitability in a clinical setting [214]. These upcoming techniques have been succesfully used in some pathologies, including AD, to enhance information of earlier microstructural tissue alterations linked to disease progression [215,216]. Among these, Kurtosis Imaging seems to be the most promising developing modality in relation of its easy setup/optimization and relatively short time acquisition in clinical MR scanners.

7.3. Functional MRI markers

Functional MRI (fMRI) represents an extraordinary technique that can study the neuronal activity through non-invasive means during specific cognitive states. This technique exploits the blood-oxygen-level-dependent (BOLD) contrasts in the vascular capillary network around the cerebral cortex. The regional metabolic demand, due to cortical activity (specific tasks/paradigms), determines an increase in local capillary hemodynamic and in the oxygenated/deoxygenated blood ratio. The increase of local deoxyhemoglobin concentration, because of its paramagnetic properties, generates an increase in local signal intensity. This technique has a relatively high spatial and temporal resolution and can be acquired along with structural MR images during the same scan session.

Several fMRI studies have been able to detect functional alterations prior to onset of cognitive impairment or AD-related structural neurodegeneration [217–219]. Task-based fMRI has been employed to study memory-related activation in the hippocampus and medial temporal lobe, typically reporting a decrease in hippocampal or parahippocampal activity during information encoding [220–224]. Also, several other studies have reported a decreased activation in the medial temporal lobe in MCI subjects [225–227].

A growing body of fMRI studies have focused on the "default mode network" (DMN), *i.e.* the interplay between a set cortical areas and the hippocampal memory system [228], the activity of which is thought to be reduced during memory intensive tasks to favor encoding and to be increased during retrieval [229]. Several studies have found dysfunctional modulation of encoding-related network activity in AD [227,230–233] or abnormal default mode pattern activity in AD and MCI patients using resting-state fMRI [234–238]. Interestingly, these results in MCI subjects have been correlated with a higher risk of progressing to AD-related dementia [239].

A bright future of fMRI or resting-state fMRI in AD and MCI might come in the area of novel pharmaceutical strategies, to date underexploited. Although drug-induced modulation of memory-related networks have been detected by fMRI [240], only few studies have demonstrated abnormal activation following, for example, long-term treatment with cholinesterase inhibitors in MCI and AD [241–244]. Therfore, additional studies are needed to test the potential role of fMRI as biomarker in clinical trials [245].

The speed of the innovation and the optimization of all these emerging modalites will be strictly related to stronger and faster MR gradients. Also, the integration of complementary information through a multimodal approach will be very useful to overcome the shortcomings of each single protocol, requiring advanced analysis tools which are able to integrate information from different protocols into the same processing pipeline. Similar approaches are likely to aid in better discrimination and staging of AD [8,246–248]. In this context, information from different modalities may be simultaneously combined using the support of machine learning algorithms enabling the classification of a single subject into a predefined group while dealing with any type of input features (*e.g.* genetic, clinical, and neuropsychological imaging data). Importantly, the classification performance is not significantly degraded if same-modality data are collected in different centers [249]. Recent results based on multimodal approaches have achieved encouraging results in discriminating AD and MCI subjects [250,251]. In the coming years, machine learning algorithms will be incorporated into scanner software to

enhance the semi-automated detection of prodromal AD stages based on high-dimensional pattern recognition.

7.4. Amyloid PET and fluorodeoxyglucose-PET markers

7.4.1. Fluorodeoxyglucose-PET

PET imaging biomarkers represent highly valuable tools for non-invasive assessment of molecular and functional pathologies which are considered to be early phenomena in the development of AD. [^{18}F]Fluorodeoxyglucose (FDG) is a well-established tracer, which allows the imaging of cerebral glucose metabolism, known to be tightly associated with neuronal function. Synaptic activity leads to an increased energy demand, which is covered by glial cells surrounding the synapse by increased glucose uptake from the blood [252]. Inversely, synaptic/neuronal dysfunction results in a decreased energy demand which is mirrored in regional metabolic decline.

Typical patterns of hypometabolism have been described in AD, including posterior parietal regions, precuneus, and also frontal cortical regions, sparing sensorimotor and visual cortex. These characteristic findings have been demonstrated to be superior to neuropsychological testing, regarding early and differential diagnosis of AD, even when *postmortem* histopathological analysis of brain tissue served as a gold-standard [253–255].

Numerous studies were able to demonstrate that early abnormalities particularly in posterior cingulate/precuneus cortical regions have a high positive and negative predictive value with regard to prediction of conversion to AD in the stage of MCI [256,257]. Even in some subjects with subjective memory impairment changes in metabolism have been observed, potentially reflecting early AD-typical pathological changes in the brain [258]. Interestingly, Reiman et al. (1996) were able to demonstrate abnormalities even in *APOE ε4*-positive subjects in younger age without any cognitive symptoms, underlining the high sensitivity of this method [259].

Regarding differential diagnosis, FDG-PET has demonstrated to be of great value because it allows the detection of different patterns of neurodegeneration, which are specific for various non-AD (amyloid-negative) forms of neurodegeneration. This includes the subtypes of frontotemporal lobar degeneration (frontotemporal dementia, progressive aphasia, semantic dementia) as well as subtypes of Parkinson-plus syndromes such as multiple system atrophy, corticobasal degeneration, and progressive supranuclear palsy [260]. Most importantly, FDG-PET is also highly useful in differentiating within amyloid-positive subtypes of disease which cannot be distinguished on the basis of their amyloid PET-scan. This includes Lewy-body dementia, posterior cortical atrophy, and the logopenic variant of progressive aphasia [255,261].

A tight correlation between the level of metabolic decline with the degree of cognitive impairment has been demonstrated consistently [262], which qualifies this method for follow-up and therapy control studies [263]. This correlation can, however, be somewhat influenced by cognitive reserve effects, expressed in variable magnitude [264]. It has also been demonstrated that FDG-PET is capable to capture therapy effects of cognitive as well as pharmacological intervention trials [265,266].

Regarding the plethora of data underlining the clinical value of FDG-PET for early and differential diagnosis of neurodegenerative disorders, as well as its complementary features as compared to amyloid-imaging, it can be expected that this method will remain an important biomarker in the coming years. Suitable MR-procedures such as resting-state fMRI or arterial spin labeling may generally bear the potential to provide information on neuronal dysfunction relatively similar to FDG-PET findings. However, the individual clinical value of these methods remains to be established in the future.

7.4.2. Amyloid-PET imaging

Today, several tracers for PET amyloid-imaging have been evaluated successfully, including clinical phase I–III studies in humans. The greatest overall number of studies has been performed with the tracer [^{11}C]Pittsburgh-Compound-B (PiB), which can be considered as the current gold-standard [267]. More recently, several ^{18}F-labeled compounds have been evaluated which would allow more widespread application of this method. One of these compounds ([^{18}F]Florbetapir/Amyvid^{TM}) has already been approved by the FDA and the EMA for commercial distribution and several others will follow in the near future. For a comprehensive review, see Rowe and Villemagne (2011) [268].

Consistently, in the great majority of all studies, a distinct uptake of the amyloid tracers has been observed in AD-patients throughout the cerebral cortex, including frontal, temporoparietal regions, and the precuneus. Whereas the basal ganglia are also regularly affected, sensorimotor and visual cortical regions show less uptake and the cerebellum is free of any relevant gray matter tracer accumulation. In young healthy control subjects, no gray matter binding of the amyloid tracers is observed but only non-specific tracer uptake in the white matter has been demonstrated. In general, this white matter uptake has been described to be less pronounced for [^{11}C]PiB as compared to the ^{18}F labeled compounds, which may somewhat decrease the sensitivity of the ^{18}F-labeled versions of amyloid tracers. The tracer ^{18}F-AZD4694 may form an exception, because it has been demonstrated to show comparably lower white matter retention [269]. The apparent differences in tracer distribution between different types of amyloid tracers have raised concerns about the comparability/standardization of amyloid-PET results. In this context, different initiatives are underway, trying to define a common standard for quantification of different amyloid-imaging results [270]. This may be particularly important with regard to clinical studies.

In vivo versus postmortem histopathological cross-evaluation studies have been performed, in general confirming that increased cortical tracer-uptake corresponds to amyloid aggregation in the brain [271,272]. The tracers are also considered to be specific for amyloid deposition with the exception of [^{18}F]FFDNP, which has been demonstrated to bind also to tau aggregates [273]. Although the tracers are specific for the protein aggregation (*i.e.* amyloid-plaques), the protein aggregation is not specific for AD. For example, it is known from histopathological studies that in Lewy-body dementia, amyloid plaques aggregation will be found in the brain in addition to the pathognonomic synuclein deposits, in most cases [274]. Thus, amyloid-imaging may not be able to differentiate between Lewy-body dementia and AD. Furthermore, amyloid-imaging alone may not be helpful with regard to distinguishing between amyloid-positive subtypes of AD (typical AD, logopenic variant of progressive aphasia, and posterior cortical atrophy) [261].

With regard to early diagnosis, a number of studies demonstrated a high predictive value of a positive amyloid-scan in the stage of MCI with regard to conversion to AD [275,276]. Even in subjects with subjective memory impairment, increased levels of amyloid deposition have been described [277] and Reiman et al. (2009) were able to demonstrate elevated amyloid-levels in asymptomatic carriers of the *APOE ε4* allele [278]. Furthermore, in a relevant proportion of elderly subjects without any cognitive complaints elevated cortical tracer-uptake was observed consistently. The meaning of these findings is not definitely clear so far, but a number of findings indicate that these subjects may indeed suffer from early AD-pathology, potentially leading to dementia later in life. This includes relatively worse performance in cognitive tests [279,166] as well as abnormal findings in other imaging tests such as resting-state connectivity [280]. In addition, recent trials in

autosomal dominantly inherited forms of AD were able to demonstrate cerebral amyloid deposition decades ahead of the expected onset of disease, using amyloid-PET. However, currently the expected time to a potential conversion to AD cannot be estimated on the basis of a positive amyloid-scan alone. Furthermore, it has to be taken into account that amyloid-imaging is not suitable to detect soluble amyloid oligomers, which have been discussed to potentially represent the most toxic species [281].

Only a limited correlation has been observed between *in vivo* measured amyloid burden and cognitive decline. This may particularly depend on the stage of disease: (I) in cognitively healthy elderly subjects amyloid pathology may not yet have induced neurotoxic effects downstream from amyloid aggregation sufficient enough to have an impact on cognition; (II) in patients with manifest Alzheimer's dementia, a plateau of amyloid deposition has been observed, indicating that amyloid deposition reaches saturation, whereas subsequent neurodegeneration (and cognitive decline) continues [282]. As for FDG-PET, in the presence of cerebral compensation mechanisms, expressed to different degree in different subjects, it may also lead to a discrepancy between cortical amyloid load and symptomatic appearance. These factors do not necessarily limit the value of amyloid-imaging for therapy trials. First, the value of amyloid-imaging with regard to patient selection is undoubted. Second, amyloid-imaging may allow the measurement of the increase in amyloid deposition over time particularly in early stages, *i.e.* ahead of a plateau phase. Finally, it has been demonstrated that the response to anti-amyloid therapy may be quantified at least in a group based evaluation [283].

Regarding the commercial availability of amyloid-imaging tools, appropriateness of use criteria have recently been published in the *Journal of Nuclear Medicine and Molecular Imaging* [284]. These criteria suggest a useful application of amyloid-imaging in patients with MCI, in AD with atypical presentation (*e.g.* early-onset) and when the diagnosis is uncertain after evaluation by a dementia expert. Without doubt, amyloid-imaging may represent one of the most important biomarkers for scientific and clinical assessment of AD in the future. The establishment of this sophisticated method for *in vivo* assessment and quantification of a molecular neuropathology will certainly also depend on reimbursement issues and on the question if anti-amyloid therapy trials will be followed further and yield in first promising pharmacotherapeutic approaches.

7.4.3. Complementary value of FDG-PET and amyloid-PET and order of abnormalities

As mentioned above, recently introduced guidelines recommend the integration of biomarkers into the classification/estimation of likelihood of preclinical, prodromal, and manifest stages of AD. According to these guidelines, both FDG-PET and amyloid-PET are suited to play an important role as diagnostic biomarkers in all stages of disease. In short, the proof of amyloid pathology (as possible with amyloid-PET) accompanied by proof of neuronal injury (as possible with FDG-PET) and finally proof of cognitive impairment sum up to an increasing probability for AD [25–27]. All these guidelines are based on the assumption that amyloid pathology is the first biomarker to become positive, followed by neuronal injury/tau-pathology and finally cognitive decline. Some recent data from a study in subjects with inherited AD in presymptomatic stages seems to confirm this notion in principle [85]. On the basis of the currently available information, it seems that amyloid-PET and $A\beta_{1-42}$-changes in the CSF behave relatively similar with regard to early detection of ongoing AD-pathology. Bateman et al. (2012) were able to demonstrate a very early decline in CSF $A\beta_{1-42}$-levels in mutation carriers but coming from a higher preexisting overall $A\beta_{1-42}$-level [85]. Thus, an early detection of ongoing disease on the basis of single time-point absolute $A\beta_{1-42}$ CSF levels would not be possible. A significant difference in $A\beta_{1-42}$-levels between carriers and non-carriers was detected relatively later as compared to the onset of significant abnormalities detected with amyloid-PET.

The mentioned guidelines and recent models of biomarker time courses treat CSF tau, FDG-PET, and structural MRI as equivalent markers of neuronal injury, appearing subsequently to amyloid pathology [285]. However, this assumption may represent an oversimplification for several reasons. First, it is known that FDG mirrors neuronal dysfunction and, from a pathophysiological point of view, it appears obvious that functional changes should be detectable ahead of neuronal loss/brain atrophy. In fact, studies were able to demonstrate higher sensitivity of FDG-PET as compared to structural MRI with regard to prediction of AD in the stage of MCI. FDG-PET may also be able to monitor changes in neuronal function in response to therapy, which may not be detectable with MRI or CSF tau measurements.

Furthermore, several recent findings are challenging the classic amyloid-hypothesis. This includes imaging studies demonstrating the presence of neuronal injury in absence of any proof of amyloid pathology [286,287]. Thus, further studies are required to gain deeper insights into the actual order of appearance of the pathologies and the threshold of their detectability. In this context, novel PET-tracers for tau-imaging may be of extraordinary importance. Fortunately, first successful experiments to establish such novel imaging biomarkers are currently on their way [288].

As mentioned above, the advantage of imaging biomarkers as compared to CSF biomarkers can be found in the provision of information not only on the presence of a certain pathology but also about the topography and the actual extent in the brain. This may be an important advantage with regard to disease staging, follow-up/therapy control, and differential diagnosis. Novel imaging instrumentation such as hybrid PET/MR scanners may offer an additional opportunity to merge the complementary information from different imaging modalities into new integrated *in vivo* biomarkers of neurodegeneration.

8. Neuroelectrical and neuromagnetic markers

The full potential of neurodynamic time-sensitive biomarkers using electroencephalography (EEG) [289] and magnetoencephalography (MEG) [290] for quantification of degenerative brain changes during various stages of AD has yet to be realized. Subtle but consistent deviations in the electromagnetic neuronal dynamics have been shown to precede explicit cognitive manifestations in AD [291] which could enable a future role of EEG/MEG biomarkers not only as a clinical diagnosis and treatment option, but also as a new mode for AD stage discovery. Dramatic progresses in dense-array active-EEG and MEG sensor technology, as well as in advanced signal processing techniques [292] have generated a recent surge of interest to use these promising capabilities in the context of improved clinical AD diagnosis. The added value of the EEG/MEG markers as an inexpensive, fast, and time-resolved tool is set to be explored rigorously both as a standalone approach and as a complementary measure together with other biomarker modalities.

8.1. Resting-state neuroelectrical/neuromagnetic markers

The spontaneous activity of the brain's resting-state networks (RSN), while the subject is idle with eyes closed or open, can be characterized by quantitative EEG/MEG measures (qEEG/qMEG), often using frequency band power or time-frequency estimates. Brainwave components of the resting EEG could be altered in the early stages of AD. There is evidence that EEG power in the alpha band declines with AD-related cognitive impairment [293]. Other

studies have shown enhanced low-frequency brain oscillation in the theta [294] and delta bands in temporal and occipital areas as well as reduction of beta power in temporal and occipital areas in MCI [295]. However, frequency band-power methods need to address some current limitations, notably regarding the necessity to adjust band limits depending on task and individual, as well as to study more completely each band's significance in relation to neuronal phenomena.

A next generation of more sophisticated resting-state signal analysis approaches [292] is set to improve upon and to replace band-power markers in the next decade by capturing better the complex characteristics and dynamics of progressive neurodegeneration and aging. Promising methods involve brain connectivity [296], global synchronization, synchronization likelihood [291], detrended fluctuation analysis, approximate entropy, mutual information, source localization, and a host of further non-linear signal features. This will open new possibilities and raise new questions such as a recent study showing in AD not only that an EEG synchronization marker was suppressed in the 10–30 Hz range (upper-alpha and beta bands) but also that the temporal fluctuations of this synchronization measure carry additional diagnostic value in the lower alpha and beta bands [297].

8.2. Functional neuroelectrical/neuromagnetic markers

Functional neuroelectrical/neuromagnetic biomarkers represent an emerging candidate for a diagnostic tool in AD clinical practice, created to evaluate specific functional activities of the brain, as opposed to resting-state. Their main purpose is the dynamic detection of cognitive-task-related deviations in brain function following the onset of AD due to impairment of neuronal connections or neuronal components participating in the functional response. Such deviations are not always manifested during the resting-state due to the targeted activation of task-related pathways and areas of the brain. Although existing topographical and pathophysiological biomarkers have shown substantial capabilities for identification or follow-up of AD [298], functional neurodynamic measures provide differential information that is advantageous and complementary in relation to cognitive impairment and progression of the disease [299]. AD biomarkers of pathophysiological type using amyloid-imaging can expose early changes in cognitively normal individuals leading to dementia, yet the subsequent structural brain changes during the various stages of the disease are more optimally followed using topographical biomarkers such as MRI and FDG-PET [300]. There is a clear need to bridge the drawbacks of these biomarker approaches in view of the challenging tasks of detection and follow-up of subjects on the way to convert to clinical AD [301].

Currently, most functional EEG biomarkers [302] are based on spatio-temporal features such as the peak amplitude or latency of event-related potentials (ERPs) [303] (*e.g.* the N400/P600 ERPs which are cognitive indicators of episodic memory encoding [304]). Yet, the event-related potential/event-related field (ERP/ERF) approach is in need to address further some well-known usability issues [305]. Similarly to resting-state measures, in the future a wider application of new biomarkers based on evoked spatio-spectro-temporal measures and task-related dynamic synchrony methods will be needed to bring in additional capability for handling single-trial EEG/MEG data more reliably, and to reflect the state of the functional brain networks for each individual subject.

8.3. Future steps toward establishing the neuroelectrical/neuromagnetic markers

The main challenge for establishing the EEG/MEG biomarkers as an AD diagnostic instrument is the diversity of approaches in existing studies. While this richness of possibilities is quite promising, a first practical step would be to select a first battery of neurodynamic biomarkers based on existing results and to initiate proposals for full standardization and implementation in practice. A modular approach would ensure that future advances can be efficiently integrated. Possible standardization modules could include data recording procedures, specific guidelines on suppression of signal noise interferences, as well as recommendations on feature extraction and diagnostic decision-making. Special attention is necessary to ensure an adaptive approach as a prerequisite for success, including the integration of individual biomarker baselines for the subjects. The goal to recognize reliably each AD stage using EEG/MEG biomarkers is particularly challenging since it is necessary to overcome known brain plasticity effects due to compensatory mechanisms in the preclinical and prodromal stages of AD as neurodegenerative and cerebrovascular lesions impose progressive impairment. The final steps would involve an extensive multi-step, multi-center validation of the biomarker standards, as well as a modality integration with other measures (a compatibility study).

The existing record of neuroelectrical/neuromagnetic biomarker performance in the scientific literature suggests a promising potential in enhancing the reliability and specificity of AD prognosis while circumventing technical, experimental, financial and logistic limitations of other biomarker measurements [306].

9. Regulatory perspectives

Despite remarkable progress in understanding the molecular underpinnings of AD during the last three decades, there are no effective interventions for altering the progression of the disease. Even those few medicinal products approved for symptomatic treatment of mild to moderate stages the disease are inadequate for long term amelioration of symptoms in more severe cases. The positive results of pre-clinical studies aimed at rescuing synaptic dysfunction or preventing behavioral impairment in animal models [307] have yet to be translated into disease-modifying compounds in humans. The latest clinical trial failure of bapineuzumab and the very modest results from two major phase III studies for solanezumab raises several questions regarding: (I) prevailing ideas-theories about the pathogenesis of the disease, (II) the appropriates of the therapeutic targets, (III) selection or inclusion criteria of subjects into clinical trials, *e.g.* pre-clinical subjects *versus* mild-moderate, and (IV) study design. US and EU regulatory agencies are facing these questions as well recent recommendations of various task forces for clinical trials in AD.

Recently, the FDA has proposed a draft guideline for Industries (available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM338287.pdf) allowing alternative targeting of intervention at the early stages of AD. According to this new guidance FDA suggests potential approaches to clinical trial design and execution that allow for regulatory flexibility and innovation [308]. There they cover the selection of patients for trials at early stages of AD and for this there is a consensus within the AD research community that clinical diagnosis of early cognitive impairment might be coupled with specific appropriate biomarkers of disease. Diagnostic criteria have been established and are under validation by various working groups [6]. Such biomarkers include brain Aβ load, as measured by PET and CSF levels of Aβ and tau proteins [309] as outlined earlier in this article.

However, adequate validation of these biomarkers is still lacking despite over 19,000 published papers. Approximately 150 longitudinal studies related to the biomarkers of interest were identified which included subjects who had objective cognitive impairment but no dementia at baseline. The authors report that

the body of evidence for these imaging and CSF biomarkers is still limited and variable across the different types of biomarkers [310]. As far as the CSF biomarkers are concerned, it was recently reported that the overall variability of data coming from a total of 84 laboratories remains too high to allow the validation of universal biomarker cut-off values for the specific intended use [107], which underscores the urgent need for better harmonization and standardization of these methods.

The use of biomarkers as endpoints in earlier stages of drug development is well established for regulators, and there are examples to approve medicinal products on the basis of their effects on validated surrogate markers, *e.g.* antihypertensives, or cholesterol-lowering products. However, these examples have been considered as validated surrogate markers as they allow substitution for a clinically relevant end point. In their validation a link between a treatment-induced change in the biomarker and long-term outcome of the relevant clinical measure was undoubtedly established. Therefore, the regulatory requirements on biomarkers used as endpoints in clinical trials are high as outlined earlier [309]. In consequence EU regulators help applicants in their research and development by issuing opinions on the acceptability of using such biomarkers or a distinct methodology in clinical trials. Since 2011, EMA's Committee for Medicinal Products for Human Use (CHMP) has adopted and published four of these qualification opinions for use in the development of medicines for AD. Three of these qualification opinions are for biomarkers to help identify and select patients at the pre-dementia stage of the disease. The fourth one is for a biomarker to be used to select patients for clinical trials in mild and moderate AD. In August 2013, a public consultation ended on a qualification opinion for a novel model of disease progression and trial evaluation in mild and moderate AD. The simulation tool is intended to provide a quantitative rationale for the selection of study design and inclusion criteria for the recruitment of patients.

In the diagnostic area, the approval of the first radiopharmaceutical for PET imaging of Aβ neuritic plaques in the brain by the European Commission, in January 2013, on the recommendation of the CHMP has been another step forward. This diagnostic agent can be used in patients who are being evaluated for AD and other causes of cognitive decline. Two other diagnostic radiopharmaceuticals for AD ([^{18}F]Florbetaben and [^{18}F]Flutemetamol) are currently under evaluation by the CHMP. However, interpretation of amyloid scans is not without hurdles: amyloid positivity does not reliably distinguish between clinical diagnoses, so that neuropsychiatric normal people as well as those with MCI, AD, and other neurodegenerative diseases can all be "amyloid positive". Therefore, a positive amyloid scan must be considered in the full clinical picture of a patient, on the other hand a negative amyloid scan indicates that the likelihood of cognitive impairment due to AD is low [311,312].

Another issue in future clinical trials is the appropriate choice of clinical endpoints. In established AD the CHMP guidance requires co-primary endpoints in cognition (mandatory) together with functional or global outcome measures; moving now to earlier asymptomatic or prodromal stages of AD might change this requirement. Thus, the FDA suggests for clinical trials focusing on patients in whom overt dementia seems imminent the use of a single scale that combines assessment of both cognition and function such as the score on the Clinical Dementia Rating Sum of Boxes (CDR-SB) [308]. For patients whose disease is at an even earlier clinical stage, it might be possible to approve a drug through an accelerated procedure pathway on the basis of assessment of only cognitive symptoms in the US. The accelerated approval mechanisms will allow drugs that address an unmet medical need to be approved on the basis of a surrogate endpoint or an intermediate clinical endpoint (*i.e.* a sensitive cognitive measure). In the EU, a similar approach is possible *via* a "conditional" approval, which implies that the applicant accepts after such an preliminary approval the obligation to carry out further long-term clinical studies to confirm clinical efficacy and safety. Only after the approval and long-term treatment, it would be possible to properly follow the amelioration of cognitive and behavioral disorders as well as the slowing of the progression of neurodegenerative lesions as shown by neuroimaging techniques [309]. Pharmaceutical industry is encouraged to seek scientific advice on their development program as soon as possible with the regulators, if they intend to use new methods to define the patient population or specific study designs and assessment tools. For instance, Richard et al. (2013) have proposed a new memory test for improving the diagnostic accuracy in patients with mild cognitive impairment recently. In particular, the Net Reclassification Improvement (NRI), followed by MRI and CSF analysis, might be an attractive and easy way to interpret certain measures for clinicians [313]. The development and validation of such new assessment tools is encouraged by regulators.

By the end of 2013, CHMP will decide whether or not there is a need to revise the guideline on the clinical investigation of medicines for the treatment of AD on the basis of new knowledge obtained from the use of biomarkers in clinical evaluation and new trends in research and development. It has already been acknowledged that AD is more a "continuum" of different stages and that the focus of new drug development has shifted to earlier stages. It is desirable that regulators and all involved stakeholders work together to decide the best design at the various stages of disease of the new clinical trials for AD prevention and treatment.

10. Conclusions

According to the new diagnostic criteria of AD recommended by the IWG [21,23] and the revised NIA-AA [26–28] initiatives, biomarkers are expected to play a prominent role in future development-validation of technologies-algorithms for: (I) accurate detection of people in the early stage of the disease, (II) more reliable diagnosis, and (III) accurate prognosis or prediction of asymptomatic people at elevated risk. This will be also possible in equivocal cases with unusually presenting clinical symptoms and problematic classification/differential diagnosis [314]. As argued by Visser et al. (2012), the IWG and the NIA-AA criteria display both commonalities and important differences [20]. Notably, they concur in recognizing the onset of AD prior to dementia [24] and highlight the employment of biomarkers as critical and supportive data for the early diagnosis of prodromal AD. In clinical trials, biomarkers can be utilized to enrich early or asymptomatic AD, thus decreasing both the extent of heterogeneity within diagnostic groups and the number of individuals necessary to detect statistically significant group differences. As a result, the statistical power will be increased [315].

Besides their diagnostic significance, biomarkers may contribute to the progress in the development of novel drugs for the treatment of AD related molecular mechanisms. They may be employed for the *in vitro* monitoring of drug discovery plans intended to identify new molecules inhibiting amyloidogenic mechanisms and to provide surrogate measures assessing treatment efficacy of novel Aβ-targeting drugs, which would decrease the time and cut the costs of clinical trials [94]. In addition, biomarkers may help demonstrate the usefulness of a certain therapy in a specific patient, thus assisting the physician to find the proper medication. Intriguingly, Lu et al. (2013), employing solid-state nuclear magnetic resonance (NMR) approach, have reported the existence of an original structural model of Aβ fibrils

from AD brain, characterized by significant differences from *in vitro* fibrils [316]. These novel structural data can be utilized to construct novel structure-specific PET radioligands for *in vivo* amyloid-imaging and conceptualize more selective small molecule inhibitors, and therapeutic antibodies [317]. These unique structure-specific PET radioligands, once validated by future follow-up studies, might be used in cooperation with CSF and blood biomarkers to help refining patient stratification [317].

Controlled and observational longitudinal studies utilizing combinations of biofluid markers in conjunction with other types of diagnostic and therapeutic approaches are required. In the absence of such studies, it is challenging to recommend exhaustive diagnostic algorithms that integrate fluid biomarkers. Moreover, the paucity of standardized procedures to quantify the existing biomarkers impedes the use of validated biomarker cut-off values to guide and monitor clinical decision-making. Attaining the validation of these cut-off points is one of the key objectives of present research performed into biomarker discovery both for AD and for other neurological disorders [318].

Finally, the standardization of the methodologies and the development of external control assays/tools/methodologies are compulsory requirements to enable the successful use of biomarkers in the diagnosis and management of AD [6,319].

At present, trials aiming at exploring early AD have been developed. In this regard, an umbrella group–the Collaboration for Alzheimer's Prevention (CAP), sponsored by Fidelity Biosciences Research, Inc., and the Alzheimer's Association – has been established which incorporates three separate, but interconnected, long-term prevention initiatives [18]: the Dominantly Inherited Alzheimer's Network (DIAN) [85], the Alzheimer's Prevention Initiative (API) [320], and the Anti-amyloid Treatment of Asymptomatic Alzheimer's (A4) trial [321]. CAP has been promoted to harmonize the studies and encourage data sharing: it exists as a setting for DIAN, API, and A4 to keep a systematic discussion among them as they plan and execute their preclinical treatment trials [18]. All of the three trials will focus on the concept that AD pathological mechanisms initiate long before the onset and progress of dementia and that amyloid is critically involved in the disease pathogenesis [322].

The paradigm shift toward early AD detection/characterization/diagnosis is essential to redefine and launch successful interventional trials. Such a paradigm embraces both secondary prevention (*i.e.* preventing the progression of pathological mechanisms and subsequent symptoms) and primary prevention (*i.e.* preventing the beginning of molecular and cellular mechanisms/signaling pathways). This objective may be attained by integrating the clinical trial approach to disease into a public health model, using long-term longitudinal databases that include large populations [323]. In this connection, significant initiatives showing a worldwide perspective are: the Organization for Economic Co-operation and Development (OECD) Task Force on AD (available at http://www.oecd.org/), the EU/US Task Force on Clinical Trial Development in AD [18,324], and the non-profit corporation Prevent Alzheimer's Disease 2020 (PAD2020) (available at http://www.pad2020.org) [17], all stressing that a world-wide database should be established by integrating/expanding existing cohorts and registries [323].

Given the vibrant and as of yet relatively unexploited future potential of the multimodal biomarker development, the current status of the integration of biomarkers in clinical trials seems only the beginning of the evolving paradigmatic "systems biology and neural network" era of AD [7,12]. This seems to be the most promising road ahead to breakthrough advances in this highly complex scientific arena. It is recognized that we can learn much from existing research in early asymptomatic populations as well as in familial autosomal dominant AD. However, it will be necessary to chart the full spectrum biomarker map in complex, non-linear sporadic AD [7,12] to progress and improve effective treatment perspectives.

Systems biology is an emerging interdisciplinary approach to AD research [12] that allows the integrated examination and assessment of interrelated biological pathways where structurally/functionally different biomolecules are simultaneously measured over time in cells, networks of cells, organs, or whole organisms [325]. Systems biology, embracing a large set of divergent methodological approaches, has become realistic owing to multiple high-throughput "omics" technologies, namely genomics/epigenomics, transcriptomics, proteomics, and metabolomics/lipidomics. These platforms, in association with accurate bioinformatic analyses using powerful computational and statistical modeling tools, will permit the investigation of various types of molecular interactions [325], such as transcriptional modules [326], gene-interaction networks [326], protein–protein interaction networks [327], and signaling networks [327]. Studying these network models will help unveil previously unknown molecular network properties of AD as well as identify genes, proteins, and cellular pathways critically involved in AD mechanisms. This, in turn, will be of support for the detection of the most appropriate gene and protein targets for AD treatment.

Conflict of interest

H.H. declares no competing financial interests related to the present article. During the last two years he has received lecture honoraria and/or research grants and/or travel funding and/or participated in scientific advisory boards and/or as a consultant to diagnostic, biotechnology, and pharmaceutical companies involved in the manufacture and marketing of biomarkers and/or diagnostics and/or drugs or medicinal products for cognitive impairment and Alzheimer's disease including Boehringer-Ingelheim, Bristol-Myers Squibb, Elan Corporation, Wyeth, Novartis, Eisai Inc., Pfizer, Schwabe, Sanofi-Aventis, Roche Pharmaceuticals and Diagnostics, GE Healthcare, Astra-Zeneca, Avid, Eli Lilly and Company, Janssen-Cilag, Merz Pharmaceuticals, GlaxoSmithKline-Biologicals, Jung-Diagnostics, Thermo Fisher Scientific Clinical Diagnostics, Cytox. He is co-inventor in pending patent submissions relating to biological markers and/or diagnostics and has not received any royalties.

K.B. has served at Advisory Boards for Innogenetics, Kyowa Hakko Kirin Pharma, Pfizer, and Roche.

H.B. declares no conflicts of interest. He does not have any financial support from commercial companies regarding this study.

A.D. declares the following potential conflicts of interest: Piramal, AVID Pharmaceuticals/Lilly, GE Healthcare, and Siemens Healthcare.

O.C. declares no financial activities related to the present article. He reports having received lecture fees from Lundbeck, consulting fees from Guerbet and no other biomedical financial interests or any potential conflicts of interest.

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P.S.A. declare no competing financial interests related to the present article. He serves on a scientific advisory board for NeuroPhage; he has served as a consultant to Elan Corporation, Wyeth, Eisai Inc., Schering-Plough Corp., Bristol-Myers Squibb, Eli Lilly and Company, NeuroPhage, Merck & Co., Roche, Amgen, Genentech, Inc., Abbott, Pfizer Inc, Novartis, Bayer, Astellas, Dainippon, Biomarin, Solvay, Otsuka, Daiichi, AstraZeneca, Janssen,

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The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease

Guy M. McKhann[a,b,*], David S. Knopman[c], Howard Chertkow[d,e], Bradley T. Hyman[f], Clifford R. Jack, Jr.[g], Claudia H. Kawas[h,i,j], William E. Klunk[k], Walter J. Koroshetz[l], Jennifer J. Manly[m,n,o], Richard Mayeux[m,n,o], Richard C. Mohs[p], John C. Morris[q], Martin N. Rossor[r], Philip Scheltens[s], Maria C. Carrillo[t], Bill Thies[t], Sandra Weintraub[u,v], Creighton H. Phelps[w]

[a]*Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[b]*Zanvyl Krieger Mind/Brain Institute, Johns Hopkins University, Baltimore, MD, USA*
[c]*Department of Neurology, Mayo Clinic, Rochester, MN, USA*
[d]*Department of Neurology, McGill University School of Medicine, Montreal, QC, Canada*
[e]*Bloomfield Centre for Research in Aging, Lady Davis Institute, Montreal, QC, Canada*
[f]*Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[g]*Department of Radiology, Mayo Clinic, Rochester, MN, USA*
[h]*Department of Neurology, University of California, Irvine, CA, USA*
[i]*Department of Neurobiology and Behavior, University of California, Irvine, CA, USA*
[j]*Alzheimer Disease Research Center, University of California, Irvine, CA, USA*
[k]*Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
[l]*National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA*
[m]*Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, New York, NY, USA*
[n]*Gertrude H. Sergievsky Center, Columbia University Medical Center, New York, NY, USA*
[o]*Department of Neurology, Columbia University Medical Center, New York, NY, USA*
[p]*Eli Lilly and Company, Indianapolis, IN, USA*
[q]*Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA*
[r]*Dementia Research Centre, Department of Neurodegeneration, UCL Institute of Neurology, University College London, Queen Square, London, United Kingdom*
[s]*Department of Neurology and Alzheimer Center, VU University Medical Center, Amsterdam, The Netherlands*
[t]*Alzheimer's Association, Chicago, IL, USA*
[u]*Department of Psychiatry, Northwestern Feinberg School of Medicine, Chicago, IL, USA*
[v]*Department of Neurology, Northwestern Feinberg School of Medicine, Chicago, IL, USA*
[w]*Alzheimer's Disease Centers Program, National Institute on Aging, Bethesda, MD, USA*

Abstract The National Institute on Aging and the Alzheimer's Association charged a workgroup with the task of revising the 1984 criteria for Alzheimer's disease (AD) dementia. The workgroup sought to ensure that the revised criteria would be flexible enough to be used by both general healthcare providers without access to neuropsychological testing, advanced imaging, and cerebrospinal fluid measures, and specialized investigators involved in research or in clinical trial studies who would have these tools available. We present criteria for all-cause dementia and for AD dementia. We retained the general framework of probable AD dementia from the 1984 criteria. On the basis of the past 27 years of experience, we made several changes in the clinical criteria for the diagnosis. We also retained the term possible AD dementia, but redefined it in a manner more focused than before. Biomarker evidence was also integrated into the diagnostic formulations for probable and possible AD

*Corresponding author: Tel.: 410-516-8640; Fax: 410-516-8648.
E-mail address: guy.mckhann@jhu.edu

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dementia for use in research settings. The core clinical criteria for AD dementia will continue to be the cornerstone of the diagnosis in clinical practice, but biomarker evidence is expected to enhance the pathophysiological specificity of the diagnosis of AD dementia. Much work lies ahead for validating the biomarker diagnosis of AD dementia.

Keywords: Alzheimer's disease; Dementia; Diagnosis; Magnetic resonance brain imaging; Positron emission tomography; Cerebrospinal fluid

1. Introduction

In the fall of 1983, a group was convened by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) to establish criteria and to describe the clinical diagnosis of Alzheimer's disease (AD). The group addressed issues of medical history, clinical examination, neuropsychological testing, and laboratory assessments and then produced a report, which was published in July 1984 [1]. The criteria in this report, commonly referred to as the NINCDS-ADRDA criteria, have been quite successful, surviving for over 27 years. These criteria have been reliable for the diagnosis of probable AD, and across more than a dozen clinical pathological studies have had a sensitivity of 81% and specificity of 70% [2]. They have been widely used in clinical trials and clinical research.

However, now 27 years later, these criteria require revision. Therefore, the National Institute on Aging and the Alzheimer's Association charged a workgroup with the task of revising the 1984 criteria for AD dementia. Details of the charge to the workgroup are described in the Introduction that accompanies this article [3]. The characterization of the preclinical [4] and mild cognitive impairment (MCI) [5] phases of the AD pathophysiological processes is described in the companion articles.

Our knowledge of the clinical manifestations and biology of AD has increased vastly. The features of the original criteria that required revision include the following:

1. The fact that the histological pathology of AD (or surrogates for this pathology) may be found across a broad clinical spectrum (including individuals who are cognitively normal, those with MCI, and those with dementia) [6,7]. Therefore, throughout this article, we use the term AD patho-physiological process to encompass the antemortem biological changes that precede the postmortem neu-ropathological diagnosis of AD as well as the neuropathological substrate. AD dementia refers to the clinical syndrome that arises as a consequence of the AD pathophysiological process.
2. Lack of acknowledgment of distinguishing features of other dementing conditions that occur in a similarly aged population, which were not completely recognized decades ago. For example, Dementia with Lewy bodies [8], vascular dementia [9], behavior variant frontotemporal dementia [10–12], and primary progressive aphasia [13] have been characterized extensively.
3. No inclusion of results of magnetic resonance imaging, positron emission tomography (PET) imaging, and cerebrospinal fluid (CSF) assays (that we will refer to subsequently as biomarkers) in decision-making. Initial efforts to incorporate biomarkers into the diagnosis of AD dementia and MCI [14] need to be coupled with a more comprehensive approach to the diagnostic process.
4. The implication that memory impairment is always the primary cognitive deficit in all patients with AD dementia. Experience has shown that there are several nonamnestic presentations of the pathophysiological process of AD, the most common ones being the syndrome of posterior cortical atrophy [15] and the syndrome of logopenic-primary progressive aphasia [16].
5. Lack of information about genetics of AD. Mutations in three genes—amyloid precursor protein, presenilin 1, and presenilin 2—cause an early onset, autosomal dominantly inherited AD [17].
6. Proposed age cutoffs for the diagnosis of AD dementia. Work over the past decades has established that AD dementia in those aged <40 years, although rare, does not differ in its pathophysiology from older persons [18]. AD dementia in persons aged >90 years is also part of that same spectrum as that of younger persons, even though clinical–pathological correlations are attenuated [19].
7. Extreme heterogeneity of the "Possible" AD dementia category, including a group of patients who would now be diagnosed as "Mild cognitive impairment (MCI)."

The objective of our committee was to focus on the criteria for AD dementia, that is, dementia secondary to the pathophysiology of AD. It was our intention to first review the NINDS–ADRDA criteria and then to update them, incorporating more modern innovations in clinical, imaging, and laboratory assessment. We will first propose (1) Criteria for all-cause dementia and then, (2) Criteria for dementia caused by AD. We set ourselves the goal of ensuring that the revised criteria would be flexible enough to be used by both general healthcare providers without access to neuropsychological testing, advanced imaging, and CSF measures, as well as specialized investigators involved in research or in clinical trial studies who would have these measures available.

2. Criteria for all-cause dementia: Core clinical criteria

In this section, we outline core clinical criteria to be used in all clinical settings. Because there are many causes of dementia, we will first outline the criteria for all-cause dementia.

The diagnosis of dementia is intended to encompass the spectrum of severity, ranging from the mildest to the most severe stages of dementia. The methodology for staging of dementia severity was beyond the charge of the workgroup. Dementia is diagnosed when there are cognitive or behavioral (neuropsychiatric) symptoms that:

1. Interfere with the ability to function at work or at usual activities; and
2. Represent a decline from previous levels of functioning and performing; and
3. Are not explained by delirium or major psychiatric disorder;
4. Cognitive impairment is detected and diagnosed through a combination of (1) history-taking from the patient and a knowledgeable informant and (2) an objective cognitive assessment, either a "bedside" mental status examination or neuropsychological testing. Neuropsychological testing should be performed when the routine history and bedside mental status examination cannot provide a confident diagnosis.
5. The cognitive or behavioral impairment involves a minimum of two of the following domains:
   a. Impaired ability to acquire and remember new information—symptoms include: repetitive questions or conversations, misplacing personal belongings, forgetting events or appointments, getting lost on a familiar route.
   b. Impaired reasoning and handling of complex tasks, poor judgment—symptoms include: poor understanding of safety risks, inability to manage finances, poor decision-making ability, inability to plan complex or sequential activities.
   c. Impaired visuospatial abilities—symptoms include: inability to recognize faces or common objects or to find objects in direct view despite good acuity, inability to operate simple implements, or orient clothing to the body.
   d. Impaired language functions (speaking, reading, writing)—symptoms include: difficulty thinking of common words while speaking, hesitations; speech, spelling, and writing errors.
   e. Changes in personality, behavior, or comportment—symptoms include: uncharacteristic mood fluctuations such as agitation, impaired motivation, initiative, apathy, loss of drive, social withdrawal, decreased interest in previous activities, loss of empathy, compulsive or obsessive behaviors, socially unacceptable behaviors.

The differentiation of dementia from MCI (see companion article [5] on the diagnosis of MCI) rests on the determination of whether or not there is significant interference in the ability to function at work or in usual daily activities. This is inherently a clinical judgment made by a skilled clinician on the basis of the individual circumstances of the patient and the description of daily affairs of the patient obtained from the patient *and* from a knowledgeable informant.

3. Proposed classification criteria for AD dementia

We propose the following terminology for classifying individuals with dementia caused by AD: (1) Probable AD dementia, (2) Possible AD dementia, and (3) Probable or possible AD dementia with evidence of the AD pathophysiological process. The first two are intended for use in all clinical settings. The third is currently intended for research purposes.

4. Probable AD dementia: Core clinical criteria

4.1. Probable AD dementia is diagnosed when the patient

1. Meets criteria for dementia described earlier in the text, and in addition, has the following characteristics:

A. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days;

B. Clear-cut history of worsening of cognition by report or observation; and

C. The initial and most prominent cognitive deficits are evident on history and examination in one of the following categories.
   a. Amnestic presentation: It is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least one other cognitive domain, as defined earlier in the text.
   b. Nonamnestic presentations:
      - Language presentation: The most prominent deficits are in word-finding, but deficits in other cognitive domains should be present.
      - Visuospatial presentation: The most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia. Deficits in other cognitive domains should be present.
      - Executive dysfunction: The most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present.

D. The diagnosis of probable AD dementia ***should not*** be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by

a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) core features of Dementia with Lewy bodies other than dementia itself; or (c) prominent features of behavioral variant frontotemporal dementia; or (d) prominent features of semantic variant primary progressive aphasia or non-fluent/agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

Note: All patients who met criteria for "probable AD" by the 1984 NINCDS–ADRDA criteria [1] would meet the current criteria for probable AD dementia mentioned in the present article.

4.2. Probable AD dementia with increased level of certainty

4.2.1. Probable AD dementia with documented decline

In persons who meet the core clinical criteria for probable AD dementia, documented cognitive decline increases the certainty that the condition represents an active, evolving pathologic process, but it does not specifically increase the certainty that the process is that of AD pathophysiology.

Probable AD dementia with documented decline is defined as follows: evidence of progressive cognitive decline on subsequent evaluations based on information from informants and cognitive testing in the context of either formal neuropsychological evaluation or standardized mental status examinations.

4.2.2. Probable AD dementia in a carrier of a causative AD genetic mutation

In persons who meet the core clinical criteria for probable AD dementia, evidence of a causative genetic mutation (in *APP, PSEN1, or PSEN2*), increases the certainty that the condition is caused by AD pathology. The workgroup noted that carriage of the $\varepsilon 4$ allele of the apolipoprotein E gene was not sufficiently specific [20] to be considered in this category.

5. Possible AD dementia: Core clinical criteria

A diagnosis of possible AD dementia should be made in either of the circumstances mentioned in the following paragraphs.

5.1. Atypical course

Atypical course meets the core clinical criteria in terms of the nature of the cognitive deficits for AD dementia, but either has a sudden onset of cognitive impairment or demonstrates insufficient historical detail or objective cognitive documentation of progressive decline,

Or

5.2. Etiologically mixed presentation

Etiologically mixed presentation meets all core clinical criteria for AD dementia but has evidence of (a) concomitant cerebrovascular disease, defined by a history of stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) features of Dementia with Lewy bodies other than the dementia itself; or (c) evidence for another neurological disease or a non-neurological medical comorbidity or medication use that could have a substantial effect on cognition

Note: A diagnosis of "possible AD" by the 1984 NINCDS-ADRDA criteria [1] would not necessarily meet the current criteria for possible AD dementia. Such a patient would need to be re-evaluated.

6. Probable AD dementia with evidence of the AD pathophysiological process

The rationale for including biomarkers for the pathophysiological process of AD in the diagnostic criteria is summarized in the Introduction to this series of articles [3]. The major AD biomarkers that have been widely investigated at this time (see [21] for review) may be broken into two classes based on the biology which they measure. Biomarkers of brain amyloid-beta (Aβ) protein deposition are low CSF $A\beta_{42}$ and positive PET amyloid imaging [22,23]. The second category is that of biomarkers of downstream neuronal degeneration or injury. The three major bio-markers in this category are elevated CSF tau, both total tau and phosphorylated tau (p-tau); decreased ^{18}fluorodeoxyglucose (FDG) uptake on PET in temporo–parietal cortex; and disproportionate atrophy on structural magnetic resonance imaging in me-dial, basal, and lateral temporal lobe, and medial parietal cortex. Total tau and p-tau are treated equivalently in this study, although p-tau may have more specificity for AD than other dementing diseases.

In persons who meet the core clinical Criteria for probable AD dementia biomarker evidence may **increase the certainty that the basis of the clinical dementia syndrome is the AD pathophysiological process**. However, we do not advocate the use of AD biomarker tests for routine diagnostic purposes at the present time. There are several reasons for this limitation: (1) the core clinical criteria provide very good diagnostic accuracy and utility in most patients; (2) more research needs to be done to ensure that criteria that include the use of biomarkers have been appropriately designed, (3) there is limited standardization of biomarkers from one locale to another, and (4) access to biomarkers is limited to varying degrees in community settings. Presently, the use of biomarkers to enhance

certainty of AD pathophysiological process may be useful in three circumstances: investigational studies, clinical trials, and as optional clinical tools for use where available and when deemed appropriate by the clinician.

Biomarker test results can fall into three categories–clearly positive, clearly negative, and indeterminate. We envision that application of biomarkers for the AD pathophysiological process would operate as outlined in the Table 1.

7. Possible AD dementia with evidence of the AD pathophysiological process

This category is for persons who meet clinical criteria for a non-AD dementia but who have either biomarker evidence of AD pathophysiological process, or meet the neuropathological criteria for AD. Examples would include persons who meet clinical criteria for dementia with Lewy bodies or for a subtype of frontotemporal lobar degeneration, but who have a positive AD biomarker study or at autopsy are found to meet pathological criteria for AD. In the biomarker table, we indicate that both categories of biomarkers must be positive for an individual who presents clinically with a non-AD phenotype to meet criteria for possible AD. This is a conservative approach that may change as more information is gained concerning the long-term outcomes of different combinations of biomarker findings. A diagnosis of possible AD dementia with evidence of AD pathophysiological process does not preclude the possibility that a second pathophysiological condition is also present.

8. Considerations related to the incorporation of biomarkers into AD dementia criteria

As described in the two companion articles on the preclinical [4] and MCI [5] phases of the AD pathophysiological process, AD dementia is part of a continuum of clinical and biological phenomena. AD dementia is fundamentally a clinical diagnosis. To make a diagnosis of AD dementia with biomarker support, the core clinical diagnosis of AD dementia must first be satisfied.

According to their nature, CSF biomarkers rely on a quantitative interpretation in comparison with normative standards. Imaging biomarkers can be interpreted in both a qualitative or quantitative manner. In many cases, biomarker results will be clearly normal or abnormal. In these cases, a qualitative interpretation of a biomarker test will unequivocally identify "positive" findings that imply the presence of the underlying AD pathophysiological process, or negative findings that unequivocally imply absence of an AD pathophysiological process. However, in some cases, ambiguous or indeterminate results will be obtained. This is inevitable given that all biomarkers are continuous measures, and the diagnostic labels of "positive" or "negative" require that cutoff values be applied to continuous biological phenomena. Although sophisticated quantitative and objective image analysis methods do exist, at present, accepted standards for quantitative analysis of AD imaging tests are lacking. Standard clinical practice in diagnostic imaging is qualitative in nature. Therefore, quantification of imaging biomarkers must rely on local laboratory specific standards. The same holds true for CSF biomarkers, although standardization efforts are more advanced for CSF biomarkers than for the imaging tests. Quantitative analytic techniques are, and will continue to be in evolution for some time. Therefore, practical use of biomarkers must follow best-practice guidelines within laboratory-specific contexts, until standardization has been fully accomplished.

A sequence of events has been described with Aβ pathophysiological processes becoming abnormal first and downstream neuronal injury biomarkers becoming abnormal later [6,7]. This might imply a hierarchical ranking of Aβ biomarkers over downstream neuronal injury biomarkers for diagnostic purposes. However, at this time, the reliability of such a hierarchical scheme has not been sufficiently well established for use in AD dementia. Given the number of

Table 1
AD dementia criteria incorporating biomarkers

Diagnostic category	Biomarker probability of AD etiology	Aβ (PET or CSF)	Neuronal injury (CSF tau, FDG-PET, structural MRI)
Probable AD dementia			
Based on clinical criteria	Uninformative	Unavailable, conflicting, or indeterminate	Unavailable, conflicting, or indeterminate
With three levels of evidence of AD pathophysiological process	Intermediate	Unavailable or indeterminate	Positive
	Intermediate	Positive	Unavailable or indeterminate
	High	Positive	Positive
Possible AD dementia (atypical clinical presentation)			
Based on clinical criteria	Uninformative	Unavailable, conflicting, or indeterminate	Unavailable, conflicting, or indeterminate
With evidence of AD pathophysiological process	High but does not rule out second etiology	Positive	Positive
Dementia-unlikely due to AD	Lowest	Negative	Negative

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-beta; PET, positron emission tomography; CSF, cerebrospinal fluid; FDG, ^{18}fluorodeoxyglucose; MRI, magnetic resonance imaging.

different AD biomarkers, it is inevitable that different combinations of test results can occur. For example, individual cases might be encountered with a positive Aβ and negative neuronal injury biomarker, or a positive FDG PET and negative tau measure, and so on. At present, the data are insufficient to recommend a scheme that arbitrates among all different biomarker combinations. Further studies are needed to prioritize biomarkers and to determine their value and validity in practice and research settings.

9. Pathophysiologically proved AD dementia

The diagnosis of pathophysiologically proved AD dementia would apply if the patient meets the clinical and cognitive criteria for AD dementia outlined earlier in the text, and the neuropathological examination, using widely accepted criteria [24], demonstrates the presence of the AD pathology.

10. Dementia unlikely to be due to AD

1. Does not meet clinical criteria for AD dementia.
2. a. Regardless of meeting clinical criteria for probable or possible AD dementia, there is sufficient evidence for an alternative diagnosis such as HIV dementia, dementia of Huntington's disease, or others that rarely, if ever, overlap with AD.
   b. Regardless of meeting clinical criteria for possible AD dementia, both Aβ and neuronal injury biomarkers are negative (see section 6, earlier in the text).

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Positron emission tomography molecular imaging for drug development

Paul M. Matthews,[1,2] Eugenii A. Rabiner,[1,2] Jan Passchier[1] & Roger N. Gunn[1,2,3]

[1]GSK Clinical Imaging Centre, GlaxoSmithKline Research and Development Ltd, Hammersmith Hospital, London, [2]Centre for Neurosciences, Department of Medicine, Imperial College, London and [3]Department of Engineering Sciences, University of Oxford, Oxford, UK

Correspondence
Professor P.M. Matthews, GSK Clinical Imaging Centre, Hammersmith Hospital, DuCane Road, London W12 0NN, UK
Tel.: +44 20 8008 6036
Fax: +44 20 8008 6491
E-mail: paul.m.matthews@gsk.com or p.matthews@imperial.ac.uk

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Human *in vivo* molecular imaging with positron emission tomography (PET) enables a new kind of 'precision pharmacology', able to address questions central to drug development. Biodistribution studies with drug molecules carrying positron-emitting radioisotopes can test whether a new chemical entity reaches a target tissue compartment (such as the brain) in sufficient amounts to be pharmacologically active. Competition studies, using a radioligand that binds to the target of therapeutic interest with adequate specificity, enable direct assessment of the relationship between drug plasma concentration and target occupancy. Tailored radiotracers can be used to measure relative rates of biological processes, while radioligands specific for tissue markers expected to change with treatment can provide specific pharmacodynamic information. Integrated application of PET and magnetic resonance imaging (MRI) methods allows molecular interactions to be related directly to anatomical or physiological changes in a tissue. Applications of imaging in early drug development can suggest approaches to patient stratification for a personalized medicine able to deliver higher value from a drug after approval. Although imaging experimental medicine adds complexity to early drug development and costs per patient are high, appropriate use can increase returns on R and D investment by improving early decision making to reduce new drug attrition in later stages. We urge that the potential value of a translational molecular imaging strategy be considered routinely and at the earliest stages of new drug development.

Introduction

Development of the first human positron emission tomography (PET) scanner was reported in 1975 by Michael Ter-Pogossian and Mike Phelps of Washington University in St Louis, USA. Over the next decade, applications to new therapeutics development were limited, but from the late 1980s applications began to grow at a rapid rate. Almost 3500 papers or online radiopharmaceutical reports now are accessible within PubMed (searched at http://www.ncbi.nlm.nih.gov/sites/entrez), using terms PET AND drug AND (biodistribution OR target occupancy OR pharmacodynamics).[1] with almost 400 reports from 2010 alone!

Molecular imaging using PET enables a new kind of 'precision pharmacology' able to address questions central to drug development in humans *in vivo*: Does a new drug molecule reach the tissue of interest in potentially pharmacologically active concentrations? Is it interacting with the target of interest? What is the quantitative relationship between the extent of this interaction and the administered dose? What are the consequent pharmacological effects and how long do they last? Extension of answers to the latter two questions can suggest ways of stratifying clinical populations, either to speed clinical trials or to deliver higher value in the use of a new drug following registration (Table 1).

Principles of PET

PET imaging is based on the principle that an emitted positron collides with a local electron, resulting in a mutual annihilation and the production of a pair of photons that travel at 180° to each other. The photons can be detected

Table 1
Selected applications of imaging in drug development

Early phase development
• Molecule biodistribution studies confirming molecule reaches the target tissue and does not accumulate in non-target sites of potential toxicity
• Target PK (dose–target occupancy) measurements guiding dose selection
• Pharmacodynamic biomarkers for proof of pharmacology, stronger 'reasons to believe' or contributing key rationale for proof of concept
• Translational preclinical imaging to identify or validate new imaging biomarkers and or provide early differentiation between candidates based on target PK or PD responses
• *In vivo* measures for monitoring safety or toxicity
Late phase development
• Surrogate markers of response more sensitive than clinical measures
• Stratification of patients based on potential for treatment efficacy
• Pharmacological differentiation of asset from marketed drugs or new competitor compounds
Marketed drugs
• Differentiation between available treatments
• Earlier detection of disease or associated pathology:
• Improved disease classification/diagnosis
• Diagnosis of pre- symptomatic or minimally symptomatic disease
• Improved identification of chronic disease exacerbation/recurrence
• Patient stratification based on disease sub-phenotype or early treatment response

as coincident events by γ-detectors surrounding the subject. Knowledge of which detector pairs sense the coincident events and their precise timing enables localization of the annihilation events and reconstruction of the spatial distribution of the emitting radio-labelled molecule. Quantitative measurements of absolute concentrations of the labelled molecule over time can be made with dynamic acquisition of data, corrections to normalize sensitivity to emissions across the region of interest and application of appropriate tracer kinetic models to these data for estimation of the rates of delivery of the radiotracer and the amount retained in tissues of interest.

PET relies on the design and manufacture of radiolabelled tracers or ligands which interact selectively with a target of interest. Ligands will have the characteristics to enable the quantification of a specific binding signal, such as a suitably high ratio of specific to non-specific binding and favourable tissue kinetics. Most commonly used positron emitting radioisotopes decay with a relatively short half-life (e.g. about 20 min for ^{11}C and 110 min for ^{18}F), allowing administration of doses high enough to provide a strong imaging signal without substantially increasing long-term health risks associated with the ionizing radiation. However, a short half-life imposes the limitation that radiotracer production needs to be performed close enough to the PET scanner to allow injection within a few half-lives. Only microdoses of radioligands or other radio-labelled molecules need to be used. PET is exquisitely sensitive (even only picomoles of labelled material can be detected), and thus can be conducted under conditions in which the ligand occupies <5% of the target and has no pharmacologically relevant activity ('tracer conditions').

PET data can be co-registered with structural data from computed tomography (CT) or magnetic resonance imaging (MRI) to aid in anatomically localizing any signal. However, the spatial resolution of PET, even in modern tomographs, is lower (typically about ~4 mm) than that achieved by CT or MRI.

Biodistribution studies applying PET molecular imaging

Pharmacological activity of a new chemical entity depends directly on the free concentration that can be achieved in the relevant tissue. Establishing the tissue free concentration with confidence can be a critical starting position for early phase development and difficult to establish confidently using conventional approaches, especially for a 'privileged' tissue compartment such as the central nervous system (CNS). Bengt Langstrom and colleagues including Mats Bergstrom introduced the powerful concept of determining molecule distribution and concentration *in vivo* in humans using PET after labelling the molecule with a positron-emitting isotope that does not change the chemical structure or properties [1].

The principles of a PET biodistribution study are straightforward. A dynamic PET scan measures the concentration–time course of the radiolabelled compound in the tissue of interest. In conjunction with associated measurements of the concentration in blood, it is possible to use bio-mathematical kinetic models to derive estimates of the clearance from plasma to tissue (a function of the blood flow and the tissue extraction of the molecule from the blood) and the ratio of the concentration of labelled drug (and drug metabolites) in tissue to blood that would be achieved at equilibrium (the tissue : blood partition coefficient). Associated HPLC analysis of blood samples *ex vivo* allows additional consideration of any metabolism of the radiolabelled compound.

To varying extents, different compounds will distribute in the tissue, where they will be either 'free' or 'bound' to tissue components. This binding can be either displaceable (high affinity, low capacity binding, e.g. to a receptor) or non-displaceable (low affinity, high capacity binding, as with lipophilic interactions). However, the calculation of the tissue 'free' concentration from the total tissue concentration is typically not feasible from PET data alone. Accurate estimation of the fraction of the non-displaceable compartment which is attributable to 'free' drug is performed by combining the PET estimates of the total blood : tissue partition coefficient with *in vitro* equilibrium dialysis assays that account for any non-specific binding in the tissue [2]. Combination of PET and equilibrium dialysis

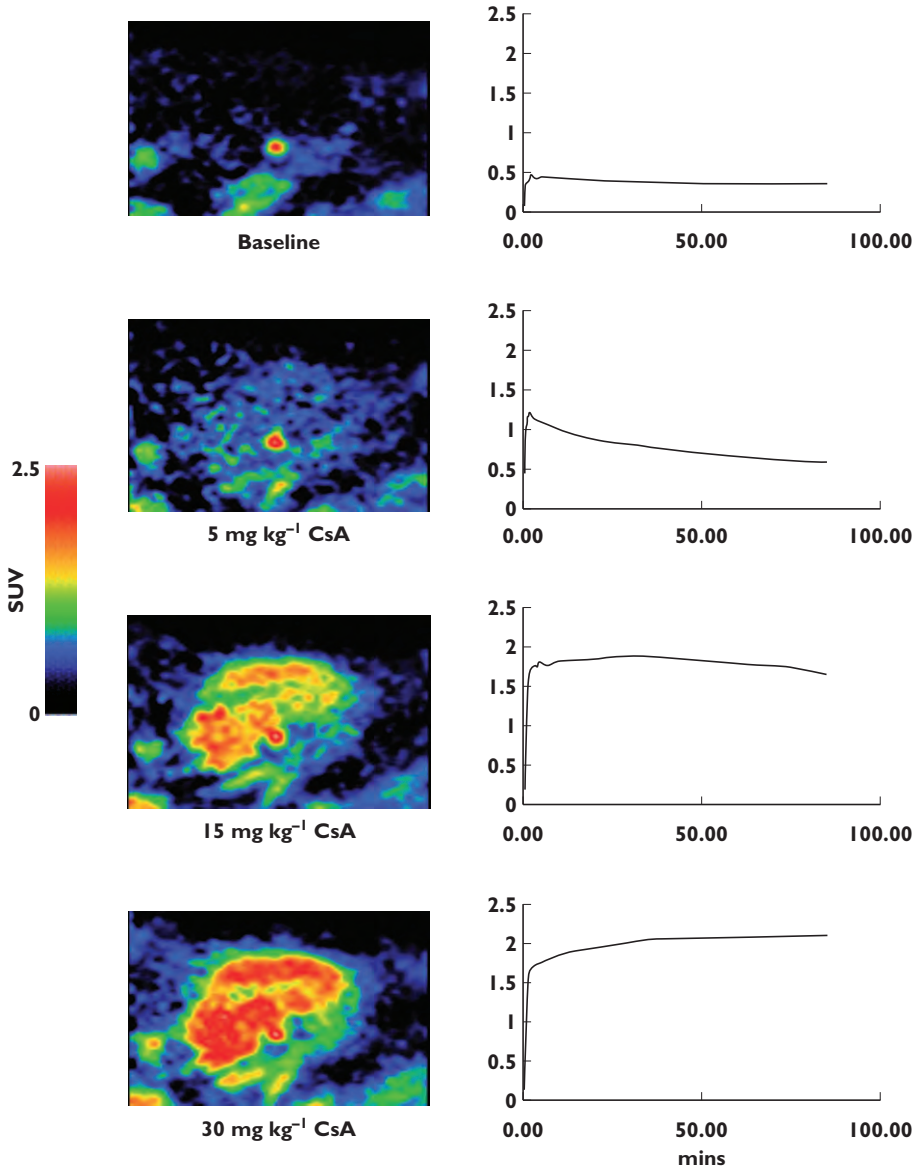

Figure 1

Effect of increasing doses of the competitive P-gp substrate cyclosporine A (CsA) on [^{11}C]-loperamide uptake in porcine brain. Increasing doses of CsA lead to increased net uptake of the [^{11}C]-loperamide with greater competition for the transporter. Note that the prominent 'hot spot' in the upper two images localizes to the pituitary gland, which sits outside the blood–brain barrier. Images were acquired from the same animal scanned sequentially on the same day

data can also allow one to infer whether the tissue uptake is by passive diffusion or by active (or facilitated) transport [3].

If it can be assumed that tissue uptake occurs by passive diffusion, the 'free' tissue concentration can be calculated from measurements of the 'free' plasma concentration of the labelled molecule at equilibrium [4]. Target occupancy (O) then can be estimated by assuming that the *in vitro* and *in vivo* K_D are equivalent.

$$O = C_{\text{free plasma}} / (C_{\text{free plasma}} + K_D)$$

The passive diffusion assumption can be explored further by measuring changes in the tissue concentration of the drug after administration of relevant transporter inhibitors (e.g. P-glycoprotein [P-gp] antagonists [3, 5] (Figure 1) or by pre-treatment with large doses of the unlabelled compound [6].

While the most common application of PET biodistribution studies thus far has been to the development of drugs targeting the central nervous system (CNS), they also can play an important role in other areas, e.g. in optimizing anti-cancer drugs [7], as up-regulation of pumps that exclude drugs from tumours is well described [8]. PET biodistribution studies can be integrated with conventional stable isotope DMPK studies [9] or with pharmacodynamic measures [10].

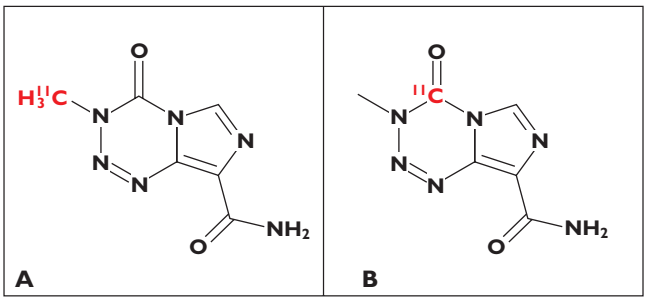

Figure 2

Chemical structures of 3-N-[^{11}C-methyl]-temozolomide (A) and [4-^{11}C-carbonyl]-temozolomide (B)

It is important to recognize, however, that it is the distribution of the radionuclide, not the molecule, that is directly measured in the PET experiment. A creative extension of the traditional biodistribution experiment that provided information on drug metabolism directly from the PET study illustrates this well [11]. Temozolomide, an alkylating agent used in cancer chemotherapy, undergoes decarboxylation and ring opening in the 3–4 position to produce the highly reactive methyldiazonium ion (which then can alkylate DNA for pharmacological action of the molecule). To evaluate this directly *in vivo* in humans, a dual radiolabelling strategy was employed in which [^{11}C]-temozolomide was radiolabelled separately both in the 3-N-methyl and 4-carbonyl positions (Figure 2A,B, respectively). ^{11}C in the C-4 position of [4-^{11}C-carbonyl]-temozolomide was converted to [^{11}C]-CO_2 and an inactive metabolite. Paired studies were performed with the two labelled forms of [^{11}C]-temozolomide in a small number of patients with gliomas. A third PET scan was performed with ^{11}C-radiolabelled bicarbonate to provide data allowing quantitative modelling of the labelled CO_2 release. Data were obtained on activities of [^{11}C]-temozolomide and [^{11}C]-metabolites in plasma collected during scanning and [^{11}C]-CO_2 was measured in the expired air. Greater amounts of [^{11}C]-CO_2 in the plasma and exhaled air and lower tumour [^{11}C]-temozolomide signal with the [4-^{11}C-carbonyl]-temozolomide relative to that labelled in the 3-N-methyl position confirmed ring-opening as a mechanism for metabolic activation of temozolomide.

A new extension of the above techniques is being pioneered with first efforts to characterize the biodistribution behaviours of monoclonal antibodies and other biopharmaceuticals. A range of methods are available for labelling such large molecules [12]. Because of the much slower approach to an equilibrium biodistribution (typically expected to be days to weeks) for these large molecules, long-lived positron emitters such as ^{89}Zr, ^{64}Cu or ^{124}I have been used. Considerable information is potentially available from such experiments, but confounds arising from the slow approach to steady-state, the need to account for physical barriers to free diffusion and different kinds of non-specific interactions (e.g. with molecule uptake into the reticuloendothelial system) make these studies technically more challenging than those with small molecules. Although promising, this area is still in an early stage of development.

Assessing target interactions with PET

Demonstration of interaction of a drug molecule with its target in a tissue also provides direct evidence of biodistribution into that tissue. If possible, it should be considered the approach of choice for defining drug–target pharmacokinetics.

Target interaction studies are most informative if there is a strong hypothesis regarding the extent of target interaction needed for a pharmacological effect. In such cases, data relating plasma concentration to target occupancy can guide dose selection directly. For example, for inhibitors of G-protein coupled receptors, preclinical (and clinical) studies typically suggest that free concentrations sufficient to provide above 70% receptor occupancy are needed (see e.g. [13, 14]). If information concerning the relationship between plasma concentration and target interactions is available before dose ranging studies, the range of doses that need to be explored in early phase studies can be deduced.

Target interaction studies require a radioligand that binds selectivity to the target of interest with a high enough affinity to provide useful signal-to-noise in a PET study. The usual outcome measure of interest from a radioligand study is the 'binding potential' (BP), which is proportional to the specific binding divided by the free concentration of the radioligand [15]. If the PET study is performed after administration of an unlabelled (drug) molecule that binds to the same target, the measured radioligand BP will vary with the local free drug concentration. Conducting such studies over a range of doses, allows binding affinity of the unlabelled molecule to be estimated the variation in radioligand BP (Figure 3). Characterization of the relationship between plasma concentration and target interaction for alternative candidate molecules can be important particularly if there are dose limiting toxicities (Figure 4).

PET studies are expensive and there is an ethical imperative to minimize exposure of volunteers to even the low additional radiation exposure of PET studies. Adaptive designs that use information gained from each single observation to improve the selection of the subsequent dose can optimize the efficiency of a study [16]. The less prior knowledge concerning dosing that is available, the greater the potential efficiency gains with adaptive designs [16].

Human *in vivo* target interaction studies can reduce sources of substantial uncertainty in drug development. For example, in some cases the affinity of a molecule in humans *in vivo* is very different from that measured in isolated tissue in *ex vitro* or in preclinical models. A histamine H_3-receptor

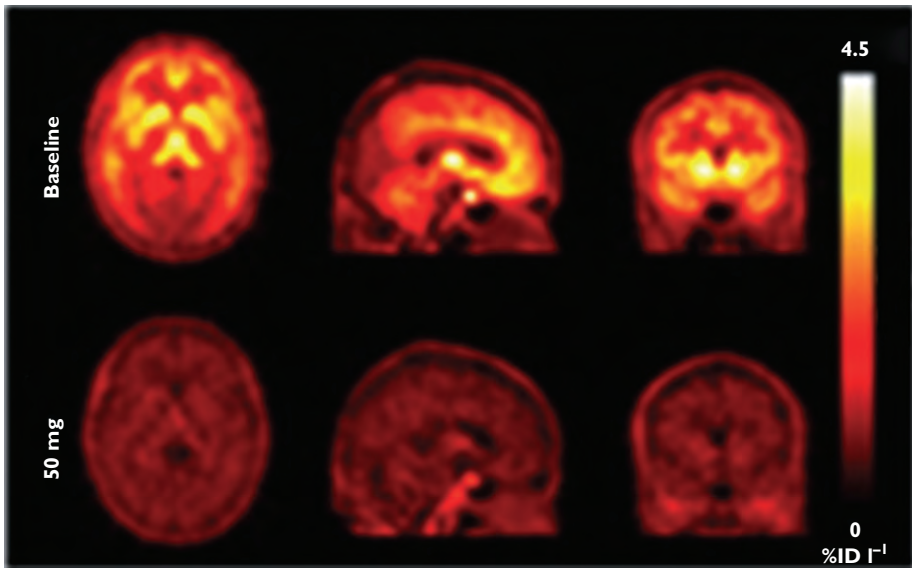

Figure 3

One image set from an illustrative drug occupancy study illustrating the radioligand signal before (upper) and after (lower) administration of cold drug competing for the same binding site. Brighter regions define increased radioisotope concentration. With repetition of a similar image pair over a range of doses of cold drug (or by varying timing of radioligand injection after cold drug administration), varying plasma concentrations at the time of scanning allow estimation of an *in vivo* IC_{50} for the cold drug based on measures of relative displacement of the radiotracer

antagonist we have studied, for example, was shown to have an *in vivo* human affinity a full order of magnitude higher from that measured in preclinical studies [17]. This observation had a substantial impact on the drug development programme, as it gave rationale and confidence for a major reduction in dosing into a range that was well-tolerated by patients. The study also defined unexpectedly slow receptor 'off' rates for the molecule, leading to a re-estimation of the optimal dosing frequency. This example thus also highlights that, in general, the time course for target interaction does not reflect plasma pharmacokinetics except for the limiting case of molecules with fast equilibrium binding properties that diffuse passively between compartments. An interesting variant of this application of target occupancy studies is to the 'reverse engineering' of empirically established treatments to define better those interactions that may be driving therapeutic efficacy [18].

Target interaction studies have typically been conducted as single dose studies for experimental convenience. Repeat dose occupancy studies may induce changes in target expression, in which case application of single dose occupancy measures will be inaccurate. However, if the pharmacokinetic model appropriate to the drug can be estimated, repeat dose brain target occupancy can be estimated based on the basis of the combined occupancy data obtained after administration of a single dose and plasma pharmacokinetic data [19]. Theoretical arguments show that the models used in these analyses can predict repeat dose occupancy even when the relationship following single dose is not described by a simple direct model dependent on the instantaneous plasma concentration.

Applications of PET to studies of pharmacodynamics

Some radiotracers (e.g. [^{18}F]-fluorodeoxyglucose (FDG), [^{18}F]-6-fluoro-L-3,4-dihydroxyphenylalanine (FDOPA), [^{18}F]-3′-fluorothymidine (FLT)) can be used to assess specific metabolic or synthetic rates, allowing inferences concerning the functional state or integrity of a tissue. Radioligands can be used to measure the concentration of specific receptor or transporter sites, allowing for the assessment of the integrity or distribution of a specific target that may correspond to its expression. Quantitative compartmental analysis methods can be used to take account of the potential confounds from differences in blood flow (and, thus, availability of the radioligand) between tissues.

Two applications illustrate the complementary ways in which radiotracers and radioligands can be used for pharmacodynamic studies. FDG has been used as a PET *radiotracer* for defining brain metabolic activity in Alzheimer's disease (AD) and its pharmacological modulation. For example, effects of treatments expected to enhance metabolism or slow rates of its impairment with the progression of neurodegeneration can be assessed with serial FDG PET scanning [20]. By contrast, [^{11}C]-PIB has been used as a *radioligand* to provide specific information concerning

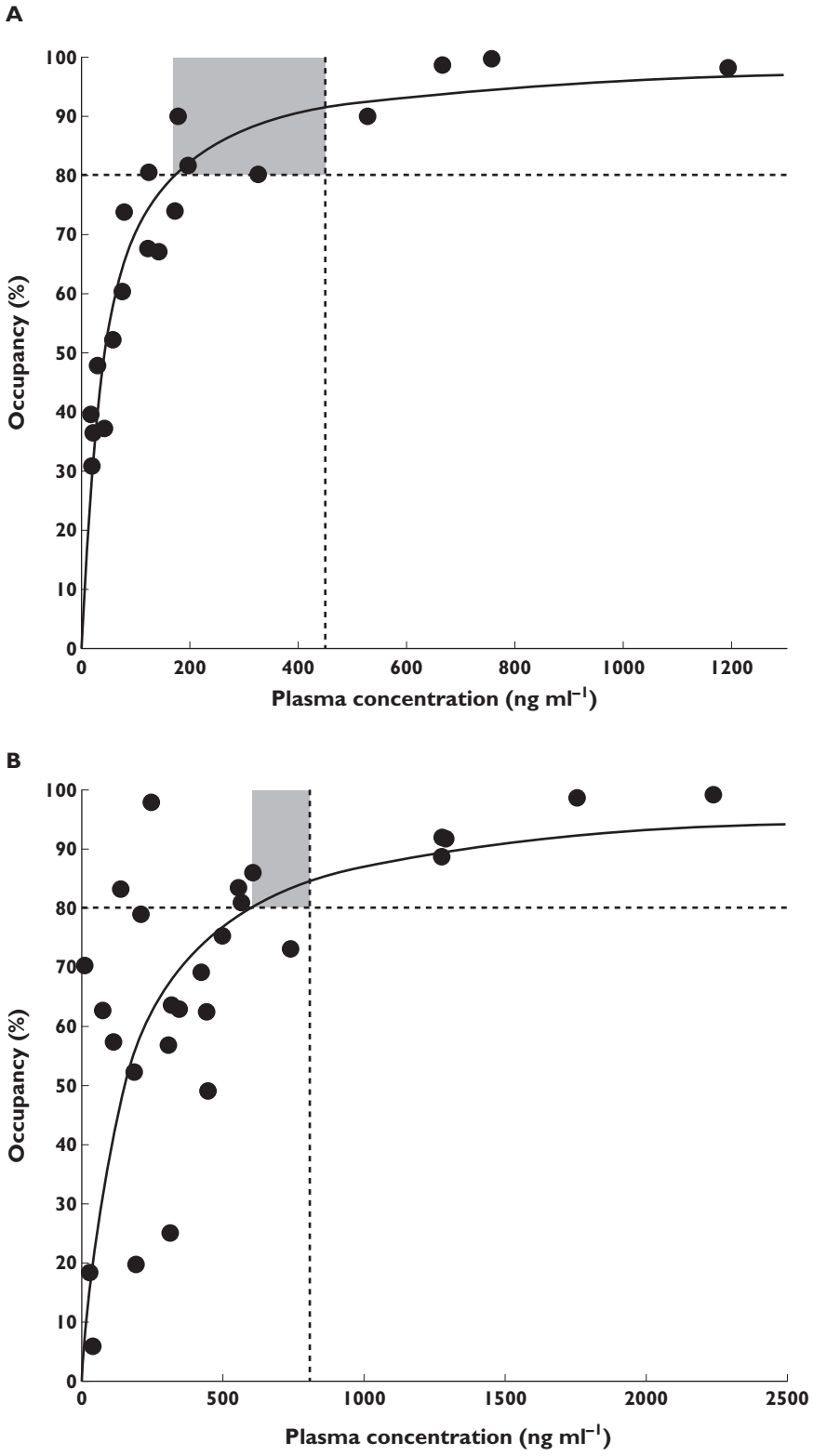

Figure 4

An example of data from target occupancy studies with two molecules in candidate selection. The molecules were antagonists and previous work suggested that occupancy by approximately 80% or more would be needed for the desired pharmacological effects (horizontal broken line). However, both molecules had recognized potential toxicities at plasma concentration shown by the vertical solid line. The *in vivo* human target occupancy-plasma concentrations defined in separate sets of PET experiments (see panels A and B) established a range of plasma concentrations (and thus doses) over which pharmacological effects were likely to be seen. In doing so, they also estimated the therapeutic index for the molecules (grey areas). The molecule used in the study for panel A, which has the higher therapeutic index, was selected for further development

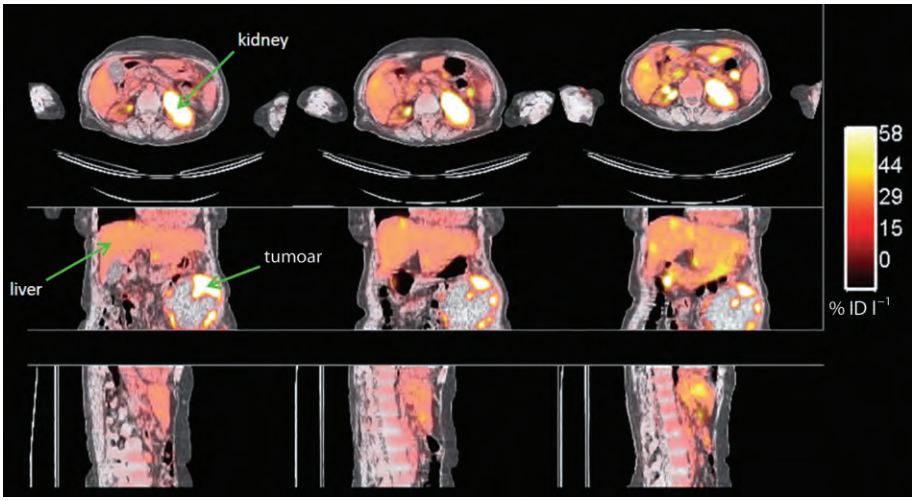

Figure 5

[^{18}F]-FDG PET study of a patient with an abdominal ovarian tumour (arrow). A significant decrease in [^{18}F]-FDG uptake (SUV_{max}) together with volumetric tumour reduction was observed at the second visit. Images courtesy of Dr A. Saleem, GSK Clinical Imaging Centre

the deposition of amyloid, which is thought to be related directly to mechanisms of neurodegeneration and is a current target for AD therapy. A number of pharmaceutical companies are developing anti-amyloid antibodies intended to provide a 'peripheral sink' which binds blood amyloid and thus reduces brain amyloid concentrations. The radioligand [^{11}C]-PIB, which has a high affinity for the beta sheet structure of the deposits, can be used to localize and estimate changes in relative concentrations of amyloid, as demonstrated in a recent phase IIa study of bapineuzumab [21].

Similar considerations hold for applications of pharmacodynamic PET to development of therapeutics in oncology. For example, use of FDG PET as a *radiotracer* provides an index of the enhanced glucose transport and phosphorylation in many tumours (the 'Warburg' effect). Qualitative assessment of the FDG PET signal is used routinely in the clinic as a diagnostic marker for tumours. Quantitative measurements before and after treatment can define pharmacodynamic effects expressed as changes in glucose transport, glycolytic enzyme activity or cell viability [22] (Figure 5). An atypical but illustrative example of this for drug development came with the demonstration of dramatic FDG PET responses to imatinib within 24 h of dosing for gastrointestinal stromal tumours in some patients [23]. Complementary information comes with use of specific *radioligands*. An ^{18}F-tagged peptide dimer of arginine-glycine-aspartate (E[c(RGDyK)]$_2$) that binds to the $\alpha_v\beta_2$ integrin that is up-regulated with tumour angiogenesis defines integrin-positive tumours more specifically, for example [24]. A growing 'toolkit' of radiotracers able to assess the activity of biological processes commonly altered by many therapies and specifically-targeted radioligands is available (Table 2).

Table 2

Selected positron emission tomography radiotracers well characterized for pharmacodynamic studies (adapted from [43], but see also http://www.ncbi.nlm.nih.gov/books/NBK5330/)

PET radiotracer	Clinical application
[^{13}N]-ammonia	Myocardial perfusion
[^{18}F]-fluorodeoxyglucose FDG)	Glucose uptake and phosphorylation
[^{11}C]-methionine	Protein synthesis
[^{18}F]-fluoromizonidazole	Tumour hypoxia
[^{11}C]-acetate	Oxidative metabolism
[^{18}F]-DOPA	Presynaptic dopaminergic function
[^{18}F]-fluoride	Bone scintigraphy
[^{82}Rb]-rubidium	Myocardial perfusion
[^{18}F]-fluorotyrosine	Amino acid uptake, protein synthesis
[^{11}C]-thymidine	DNA synthesis
[^{18}F]-fluorothymidine (FLT)	Tumour cell proliferation
[^{64}Cu]-ATSM or [^{18}F]MISO	Hypoxia

While the major applications of PET in drug development thus far have been to CNS or oncology therapeutics, there is potential for much wider applications of PET in drug development. One of the most promising new general areas of application is to inflammatory diseases [25]. The best characterized radioligand target has been the 18 kDa translocator protein (TSPO, previously known as the peripheral benzodiazepine receptor), expression of which is increased with macrophage or microglial activation, to provide a molecular marker of innate immune responses [26]. The most widely used radioligand thus far has been [^{11}C]-PK11195 [18], but interpretation of studies is limited by its relatively poor signal-to-noise ratio [23].

While several alternative radioligand candidates have been evaluated in humans, differences in their binding affinity between subjects raised concerns about whether studies with them can be interpreted quantitatively [27]. However, identification of a genetic polymorphism in the *TSPO* gene that is responsible for this behaviour now promises to make quantitative studies possible after simple genetic testing [28].

Integration of data from PET target occupancy studies and functional MRI (fMRI) methods provides a novel strategy for directly relating information on drug–target interactions directly with a measure of functional effects in the brain. Recent studies illustrating this approach have related the extent of binding of an antagonist to its μ-opioid target with modulation of fMRI reward responses to the administration of a palatable food stimulus [29] or a dopamine receptor occupancy to reward responses in a gambling task (Figure 6). The study simultaneously provided direct evidence validating the target as a modulator of satiety responses in humans and suggested a pharmacological dose range based both on the measures of target interaction and pharmacodynamic effect. New potential for extension of this kind of work has come with advances in detector technology that have made possible a first generation of fully integrated human PET/MRI systems [30, 31]. Integrated acquisition of data will increase the precision of registration of the MRI and PET data particularly for applications outside of the head.

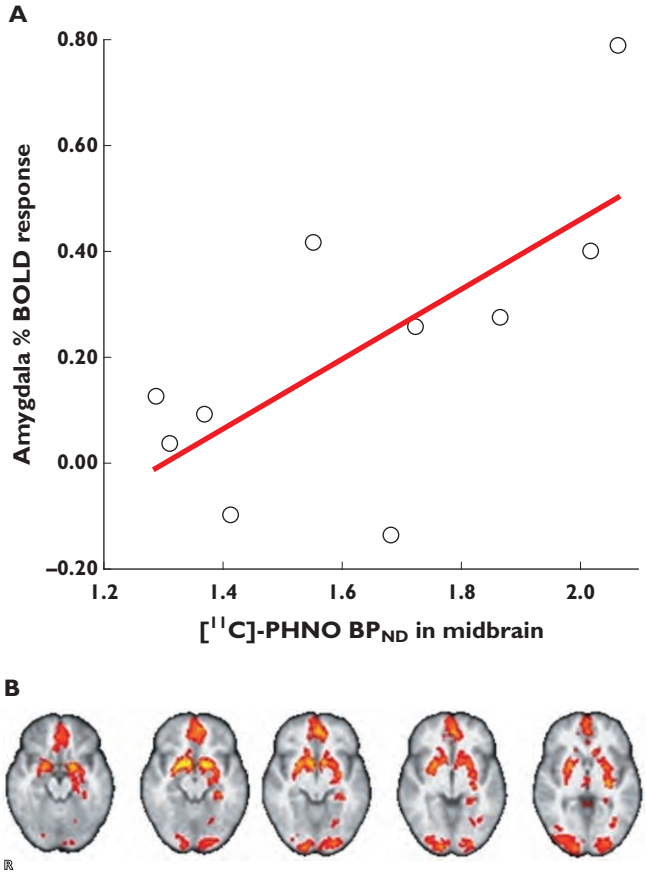

Figure 6

(A) Group statistical maps (overlaid on structural MRI image) showing baseline fMRI BOLD response to receipt of monetary rewards. (B) Relationship between study population variability in midbrain dopamine receptor subtype 3 (D3R) availability and amygdala response to reward. Correlation between midbrain [^{11}C]-(+)-PHNO BP_{ND} and amygdala activation to monetary rewards for individual subjects studied, demonstrating that subjects with higher midbrain D3R availability have greater amygdala BOLD response to rewards

Development of target-specific radioligands for PET

Availability of appropriate radioligands is a major challenge for PET molecular imaging in applications to drug development. Target interaction studies demand availability of a radioligand that has good affinity for the target, and binds with high specificity and selectivity [32]. There are many examples of such molecules, particularly for targets in the CNS [33], but novel drug targets will demand novel radioligands and the discovery of radioligands for new targets is a complex and resource-intensive undertaking requiring highly specialized and skilled staff.

Typically, the first steps in the discovery of a novel radioligand involve screening relevant compounds (e.g. from a library of molecules with some selectivity for binding to the target of interest) for feasibility of introduction of a positron-emitting radioisotope label (e.g. ^{11}C or ^{18}F) and for chemical parameters such as lipophilicity (e.g. log P or log [solubility in octanol]/[solubility in aqueous solution at pH 7.4]) and affinity and selectivity for the target (e.g. through measures of K_i, IC_{50}, EC_{50}). Additional pharmacological criteria include plasma clearance, metabolic fate, plasma protein binding and the potential to access the target tissue. For applications in the brain, assessment of the potential for crossing the blood–brain barrier is critical. Compounds need to be sufficiently lipophilic to allow passive diffusion across cell membranes while not being so lipophilic that a substantial fraction interacts non-specifically with membranes. As a 'rule of thumb', initial consideration is given to compounds with a measured log P of 1–3.

The affinity of a radioligand for its target and the amount of target available are fundamental parameters that will determine the observed signal. The desired affinity range for radioligand candidates therefore depends on the expected target density. The signal-to-noise ratio can be approximated by the BP ($BP = (B_{max} - B) \times (k_{on}/k_{off})$, where B_{max} is the concentration of the target of interest and B is the concentration of the target occupied by the ligand). The ratio of first order kinetic rate constants for radioligand binding and release from the target, k_{on}/k_{off}, can be expressed as $1/K_D$. Under true tracer conditions ($B << B_{max}$), the equations can be simplified as $BP = B_{max}/K_D$. A practical

range for the binding potential, when allowing for non-specific binding in the tissues of interest, is between 0.5 –15. Values less than 0.5 or greater than 15 suggest that a candidate radioligand may suffer from either undesirably high variability or low precision, respectively.

Target selectivity is governed by the relative affinity, density and tissue distribution of potentially competing interactions. Under usual circumstances, adequate target specificity is expressed with a similar density in the same tissue demands at least an order of magnitude difference in affinity. However, for applications in which receptors have known distributions and are not anatomically co-localized, similar affinities for receptors can be allowed.

The potential for a compound to access the tissue of interest also should be carefully considered. Our personal experience in discovery efforts for radioligands targeting the brain has been that the brain : blood ratio observed in preclinical rodent or porcine biodistribution studies should be >1 for practical utility. Invasive, equilibrium dialysis measurements of the fraction of ligand free in plasma (f_P) and tissue proteins (f_{ND}) can provide even *in vitro* data that predicts brain penetration; the ratio $f_P : f_{ND}$ correlates well with the non-specific volume of distribution (V_{ND}) [3, 15].

Care should be taken to ensure the ligand is not subject to fast active transport from the target tissue back into the blood. Examples of such active transport systems are P-gp, the organic anion transporters (OATP), lung cancer resistant protein (LRP), brain cancer resistant protein (BCRP) and multidrug resistant proteins (MRP) [8]. In brain the most prominent of these is P-gp. *In silico* or *in vitro* methods can be used to screen out compounds that may be substrates for this transporter [2, 34]. However, weak to moderate substrates can still be useful radioligands, e.g. [^{11}C]-carfentanil and [^{18}F]-4-(2′-methoxyphenyl)-1-[2′-(N-2-pyridinyl)-p-fluorobenzamido]-ethyl-piperazine ([^{18}F]-*p*-MPPF) [35].

In order to optimize signal-to-noise, encourage rapid tracer kinetics and facilitate equilibrium between plasma and tissue concentrations within the period of the scan (typically 90–120 min), clearance of the ligand from plasma should be relatively fast. This is usually challenging with chemical structures derived directly from molecules developed as drugs, because most therapeutics are designed for dosing no more frequently than once or twice daily. The compounds with high plasma clearance that are preferred for radioligands are typically not seen as viable drug candidates, so the drug development process actively screens against them.

Radioisotope labelling is perhaps the most flexible part of initial candidate screening, as there is a well-developed arsenal of chemistry methodology for both ^{11}C and ^{18}F chemistries. Work with ^{18}F has focused primarily on nucleophilic substitution reactions (both aliphatic and aromatic). Nonetheless, feasibility of the chemistry can be challenging for ^{11}C syntheses. As a rule of thumb, a PET radiopharmaceutical should be available for clinical use within three half-lives of receiving the radioactivity from the cyclotron. For ^{11}C, this means that the entire process, including quality control release testing to specification, should ideally take less than 1 h. As a result, most chemistry with ^{11}C involves introduction of the label as a single, final step to limit loss of product as a result of radioactive decay [36].

Finally, the position of labelling should be carefully considered with regard to the known or probable metabolic fate of the PET radioligand. Where possible, the ligand should be labelled in a position which, upon oxidation or hydrolysis, leads to a labelled hydrophilic fragment, as these are less likely to enter tissues. An example illustrating this is provided by the 5-HT$_{1A}$ receptor radioligand, [^{11}C]-WAY 100635. Initially, this compound was labelled in the *O*-methyl position (Figure 7A) to achieve similar criteria based on the reported primary route of metabolism in rat. Rodent preclinical studies supported the potential suitability of this radioligand with demonstration of a high signal in the hippocampus and a very low signal in the cerebellum, tissues known to have high and very low 5-HT$_{1A}$ receptor expression, respectively [37]. However, translation to humans gave a surprising result: the observed medial temporal cortex (MTC) : cerebellum signal ratio was more than five-fold lower than expected [38]. Contrary to the experience with rats, it was found that in man the primary route of metabolism in humans is through hydrolysis of the amide bond (Figure 7B). The hydrolysis product ([^{11}C]-WAY 100634) readily enters the brain and has a high affinity for α_1-adrenergic receptors, reducing the specific signal-to-noise for the 5-HT$_{1A}$ receptor. Subsequent labelling in the carbonyl position generated a radioligand ([^{11}C-carbonyl]-WAY 100635) that gave a much more specific signal (as supported by the much improved MTC/cerebellum signal ratio) [39].

Despite an apparent thorough knowledge of critical physicochemical parameters that define the boundaries the overall rate of discovery of new PET tool compounds compared with the effort invested by the field is low. Recent advances in design-based biomathematical modelling [40], better understanding of factors predicting non-specific binding [41] and new approaches to medicinal and PET chemistry promise opportunities for more efficient development in the future.

Conclusions

PET allows a new 'precision pharmacology' that can have an important role in drug development. While imaging experimental medicine can add complexity to planning clinical development and increase the cost per patient studied, well-designed studies can answer key questions earlier and with smaller numbers of subjects for more confident decision-making. In the future, applications of molecular imaging to the development of drugs can add further value with their translation to clinical use as a

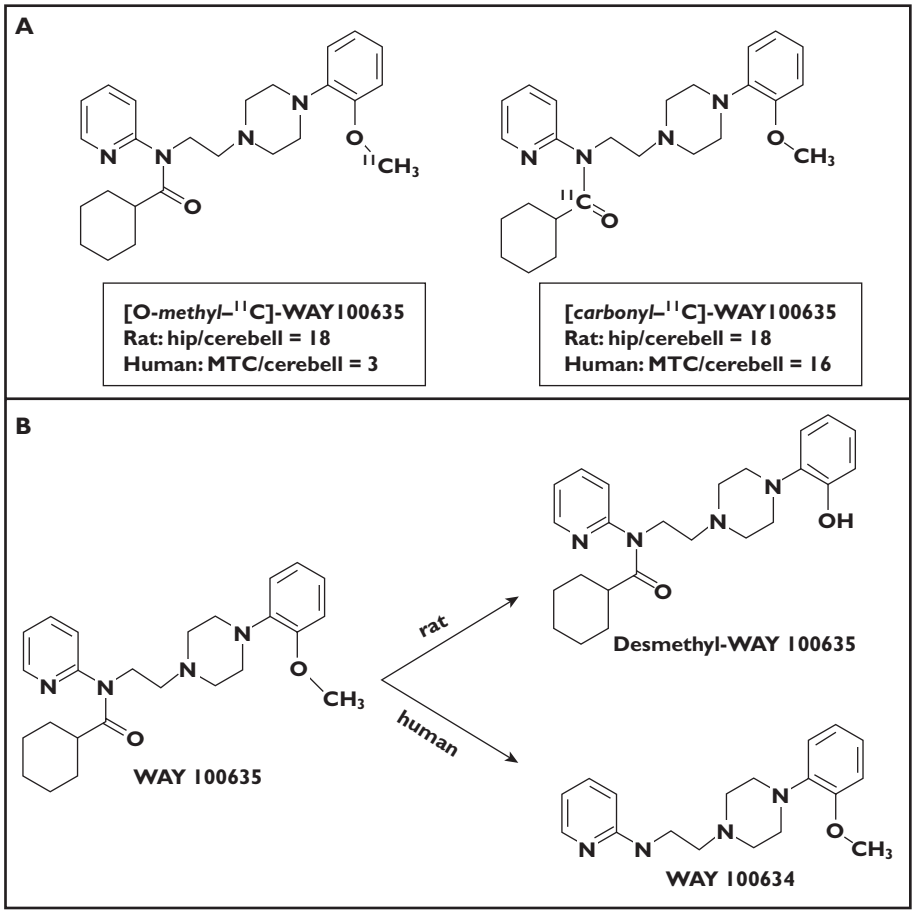

Figure 7

(A) Structures for alternately radiolabelled forms of the radioligand WAY100635 which show different relative radioisotope accumulation in hippocampus (hip) and medial temporal cortex (MTC) relative to the cerebellum (cerebell) in rat and human because of different routes of metabolism in the two species (B)

companion diagnostic for *patient stratification* enabling higher efficacy and value. More responsive patient populations can be identified not just to enable smaller, more informative clinical trials, but also to direct medicines to patients who will experience the greatest benefit [42]. We urge that the potential value of a translational molecular imaging strategy be considered routinely and at the earliest stages of planning for the development of new drugs.

Competing Interests

The authors all are full-time employees of GlaxoSmithKline and hold stocks and options in the company.

PMM is supported by the Medical Research Council for work that has contributed to this review. The authors also want to acknowledge the support of their colleagues at the GSK Clinical Imaging Centre for work described in this review and particularly to Professor Marc Laruelle, whose leadership and insight helped all of them to appreciate better how best to deliver value from molecular imaging for drug development. The authors additionally thank Jasper van der Aart for help with the figures and Rachel Green for general editorial support.

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STATE OF CONNECTICUT

DEPARTMENT OF PUBLIC HEALTH

Office of Health Care Access

January 20, 2015 VIA FACISIMILE ONLY

Kimberly Fabrizio
Sr. Director of Regulatory Affairs and Quality Assurance
Molecular Neuroimaging, LLC
60 Temple Street
New Haven, CT 06510

RE: Certificate of Need Application, Docket Number 14-31965-CON
Molecular Neuroimaging, LLC
Acquisition of a Positron Emission Tomography/Computed Tomography Scanner for Research Studies

Dear Ms. Fabrizio:

This letter is to inform you that, pursuant to Section 19a-639a (d) of the Connecticut General Statutes, the Office of Health Care Access has deemed the above-referenced application complete as of January 20, 2015.

If you have any questions regarding this matter, please feel free to contact me at (860) 418-7007.

Sincerely,

A. Veyberman

Alla Veyberman
Health Care Analyst

* * * COMMUNICATION RESULT REPORT (JAN. 20. 2015 1:11PM) * * *

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STATE OF CONNECTICUT
OFFICE OF HEALTH CARE ACCESS

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TO: KIMBERLY FABRIZIO

FAX: 203.508.1503

AGENCY: MNI

FROM: OHCA

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410 Capitol Ave., MS#13HCA
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Hartford, CT 06134

Greer, Leslie

From: Lazarus, Steven
Sent: Tuesday, February 24, 2015 2:44 PM
To: Veyberman, Alla
Cc: Riggott, Kaila; Greer, Leslie
Subject: FW: 14-31965 PET-CT

Alla,

Please see the email below from DSS regarding your CON application.

Thank you,

Steve

Ps. Leslie, please add to the original file.

From: Wysocki, Richard
Sent: Tuesday, February 24, 2015 2:38 PM
To: Lazarus, Steven
Cc: Martone, Kim; Lavigne, Christopher A.; Riggott, Kaila; Hansted, Kevin
Subject: RE: 14-31965 PET-CT

Steve:

DSS has reviewed the Applicant's information as provided by OHCA, and based on this information, the applicant states "all individuals undergoing PET imaging at MNI facility do so as a participant in a research study. Under no circumstances does MNI or its physicians provide fee for service imaging or participate in any health care reimbursement programs or clinical care to the population being served".

Based on this information, it appears that there should not be any impact to the Medicaid program. Thanks.

Wysocki, Richard
rich.wysocki@ct.gov

DSS
25 Sigourney St. 11th Flr. Rate Setting & CON unit
Hartford, CT 06106
860-424-5103 Direct

www.ct.gov/dss

From: Lazarus, Steven
Sent: Tuesday, February 24, 2015 9:20 AM
To: Wysocki, Richard
Cc: Martone, Kim; Lavigne, Christopher A.; Riggott, Kaila; Hansted, Kevin
Subject: FW: 14-31965 PET-CT

Good morning again Rich,

Please see the information below for another (new) CON application. Please let me know of the impact of this proposal on the Medicaid program.

Thank you,

Steve

Steven W. Lazarus
Associate Health Care Analyst
Division of Office of Health Care Access
Connecticut Department of Public Health
410 Capitol Avenue
Hartford, CT 06134
Phone: 860-418-7012
Fax: 860-418-7053

From: Veyberman, Alla
Sent: Tuesday, February 24, 2015 9:16 AM
To: Lazarus, Steven
Subject: 14-31965 PET-CT

Steve,
Please see the information below.

14-31965-CON Purchase of a Positron Emission Tomography – Computed Tomography Scanner

Molecular NeuroImaging, LLC (MNI) is a neuroimaging services company specializing in the efficient application of scintigraphic biomarkers in drug development and research for neurodegenerative and neuropsychiatric disorders. Molecular Neuroimaging, LLC (MNI) is proposing to acquire a PET/CT camera to meet the increasing demands for PET imaging in its research-dedicated facility. he proposed scanner will be used in research focusing on developing new therapies for neurodegenerative conditions such as Alzheimer disease ("AD"), Parkinson and Huntington diseases, and other neurologic and psychiatric disorders including Schizophrenia, Depression, Multiple Sclerosis, and Fragile X syndrome.
The proposed scanner, Reconditioned Siemens Biograph HI-REZ 6 PET/CT, is a 64 slice PET and 6 slice CT scanner

The current and target population to complete PET imaging at MNI are study participants involved in research trials of new therapies for neurodegenerative disorders. As a research-dedicated imaging center, all individuals undergoing PET imaging at MNI facility do so as a participant in a research study. Under no circumstances does MNI or its physicians provide fee for service imaging or participate in any health care reimbursement programs or clinical care to the population being served.

Patients are not served using the imaging cameras at MNI; the proposed camera is for research only.

Current and Projected Patient Population Mix

TABLE 7
APPLICANT'S CURRENT & PROJECTED PAYER MIX

Payer	Most Recently Completed FY2013		Projected					
			FY2014		FY2015		FY2016	
	Volume	%	Volume	%	Volume	%	Volume	%
Medicare*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Medicaid*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CHAMPUS & TriCare	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Government	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Commercial Insurers	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Uninsured	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Workers Compensation	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Non-Government	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Payer Mix	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Question:
For the Medicaid population only, provide the assumptions and actual calculation used to determine the projected patient volume.

Applicant's response: N/A

Question:
If the proposal fails to provide or reduces access to services by Medicaid recipients or indigent persons, provide explanation for good cause for doing so. *Note: good cause shall not be demonstrated solely on the basis of differences in reimbursement rates between Medicaid and other health care payers.*

Applicant's response: N/A

Question:
Please elaborate on each of your responses of "N/ A" for application questions 6(a)-(d)~ regarding your current and projected patient population mix. In particular, clearly explain why the Applicant states that Medicaid issues are '"not applicable" to the proposed scanner.

Applicant's response:
MNI has responded that the Medicaid issues are not applicable to this proposed camera, as we are not a Medicaid reimbursable or POS entity. MNI conducts research only and neither MNI nor its researchers are registered to receive payment from Medicaid or any other insurers for any clinical services, including PET Imaging that would be conducted on the proposed camera.

Thanks,

Alla Veyberman
CT Department of Public Health
Office of Health Care Access (OHCA)
Phone: 860.418.7007
Fax: 860.418.7053
Email: Alla.Veyberman@ct.gov

STATE OF CONNECTICUT

DEPARTMENT OF PUBLIC HEALTH

Office of Health Care Access

April 9, 2015

IN THE MATTER OF:

An Application for a Certificate of Need filed Pursuant to Section 19a-638, C.G.S. by:	Notice of Final Decision Office of Health Care Access Docket Number: 14-31965-CON
Molecular Neuroimaging, LLC	**Acquisition of a PET/CT Scanner**

To:

Kimberly Fabrizio
Sr. Director of Regulatory Affairs
Molecular Neuroimaging, LLC
60 Temple Street
New Haven, CT 06510

Dear Ms. Fabrizio:

This letter will serve as notice of the approved Certificate of Need Application in the above-referenced matter. On April 9, 2015, the Final Decision, attached hereto, was adopted and issued as an Order by the Department of Public Health, Office of Health Care Access.

Kimberly Martone (ICR)

Kimberly R. Martone
Director of Operations

Enclosure
KRM: amv

Department of Public Health
Office of Health Care Access
Certificate of Need Application

Final Decision

Applicant: **Molecular Neuroimaging, LLC.**
60 Temple Street, New Haven, CT 06510

Docket Number: **14-31965-CON**

Project Title: **Acquisition of a Positron Emission Tomography/Computed Tomography Scanner**

Project Description: Molecular Neuroimaging, LLC ("Applicant") seeks authorization to purchase a Positron Emission Tomography/Computed Tomography Scan camera ("PET/CT") and upgrade its facility to accommodate the proposed PET/CT with a total capital expenditure of $589,149.

Procedural History: The Applicant published notice of its intent to file a Certificate of Need ("CON") application in *The New Haven Register* on August 28, 29 and 30, 2014. On November 18, 2014, the Office of Health Care Access ("OHCA") received the initial CON application from the Applicant for the above-referenced project and deemed the application complete on January 20, 2015. OHCA received no responses from the public concerning the Applicant's proposal and no hearing requests were received from the public per Connecticut General Statutes ("Conn. Gen. Stat.") § 19a-639a(e). Deputy Commissioner Brancifort considered the entire record in this matter.

Findings of Fact and Conclusions of Law

To the extent the findings of fact actually represent conclusions of law, they should be so considered, and vice versa. *SAS Inst., Inc., v. S & H Computer Systems, Inc.*, 605 F.Supp. 816 (Md. Tenn. 1985).

1. Molecular Neuroimaging, LLC ("MNI" or "Applicant") is a for-profit research company located at 60 Temple Street in New Haven, Connecticut specializing in the application of biomarkers in drug development for neurodegenerative and neuropsychiatric disorders. Exhibit A, p. 2

2. MNI currently develops and conducts PET and SPECT imaging studies to support early drug development and investigational trials, emphasizing imaging outcome measures for evaluating disease progression. Exhibit A, p. 2

3. MNI provides services across the pre-clinical and clinical spectrum including radioligand[1] development and manufacturing; design and implementation of clinical trials and customized clinical imaging site coordination and management. These research activities are conducted both independently and in collaboration with 30 global pharmaceutical and biotech companies as well as research organizations such as the Michael J. Fox Foundation, the National Institutes of Health ("NIH") and the Department of Defense. Exhibit A, pp. 2-4, 11

4. The Applicant currently operates a Siemens HR Positron Emission Tomography ("PET") scanner and a Pickler International 3000XP Single Photon Emission Computed Tomography ("SPECT") camera. Exhibit A, p. 3

5. PET imaging is an essential component of the Applicant's research, which is focused on developing new therapies for unmet medical needs in neurodegenerative conditions such as Alzheimer's ("AD"), Parkinson's and Huntington's disease. Exhibit A, p. 2

6. AD and Parkinson's disease are the two most common and rapidly expanding neurodegenerative disorders, with 4 million people in the United States being affected by AD and 1 million people affected by Parkinson's disease. It is estimated that over 50 million people worldwide will have some form of dementing illness by 2020. Exhibit A, p. 6

7. The Applicant's existing PET scanner is 13 years old, has limited efficiencies and requires a significant amount of continued maintenance. While afforded the opportunity to perform multiple PET studies, due to the logistics of the use of radiopharmaceuticals and the limitations of its existing equipment, MNI must often delay the initiation of new studies until existing studies are completed. Exhibit A, pp. 3, 7

[1] A radioligand is a radioactive biochemical substance that is used for diagnosis or for research-oriented study of the receptor systems of the body. In a neuroimaging application the radioligand is injected into the pertinent tissue or infused into the bloodstream.

8. The Applicant is seeking to acquire a reconditioned Siemens Biograph 64 slice PET/6 slice CT Positron Emission Tomography/Computed Tomography scanner ("PET/CT").[2] Exhibit A, pp. 4, 195

9. The proposed scanner will be used in research focusing on developing new therapies for neurodegenerative conditions such as AD, Parkinson's and Huntington's diseases, as well as other neurologic and psychiatric disorders including Schizophrenia, Depression, Multiple Sclerosis, and Fragile X Syndrome. Exhibit A, p. 2, 11

10. NIH and pharmaceutical research sponsors require state-of-the art brain pet imaging for their planned clinical studies and researchers must demonstrate the availability of PET imaging to be considered as clinical sites for these studies. Exhibit A, p. 5

11. The development of therapeutics for such diseases relies on large multi-center studies aimed at evaluating the efficacy of drugs. The availability of PET imaging biomarkers is critical in these large clinical studies to ensure an accurate diagnosis and disease progression monitoring. Exhibit A, p. 5

12. The addition of a second, technologically more sophisticated and efficient camera will expand the number of PET slots available to conduct research and increase the number of research scans that can be conducted. Exhibit A, pp. 2-3

13. The proposed scanner will make the scan acquisition process much more efficient. The scan will be obtained in seconds instead of minutes, resulting in minimal scan time and greater comfort for the research subject. Imaging time for whole body studies, where 8-10 bed positions are required, can be reduced by 45-60 minutes. Exhibit A, p. 3

14. The proposed camera's CT component will provide attenuation correction, resulting in additional clarity and offers an improved image of the human anatomy, which is crucial in analyzing PET images. Exhibit A, p. 3

15. The CT component will also allow for several advanced imaging processing methods to be applied to the PET images, most notably, the ability to use the CT image for a highly accurate registration to the research subject's available MRI scan. Exhibit A, p. 3

16. The PET/CT will support the Applicant's translational research,[3] identifying radioligands for use in clinical studies. Exhibit A, p. 4

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[2] PET/CT scans provide images that pinpoint the anatomic location of abnormal metabolic activity within the body. http://www.radiologyinfo.org/en/pdf/pet.pdf

[3] Translational research is engineering research that aims to make findings from basic science useful for practical applications that enhance human health and well-being. This has been attempted particularly in medicine with translational medicine, research that aims to move "from bench to bedside" or from laboratory experiments through clinical trials to point-of-care patient applications. http://en.wikipedia.org/wiki/Translational_research

17. The Applicant submitted several scholarly articles supporting the increasing role biomarker research/imaging will have in clinical trials. For example, an article "Perspective on the future role of biological markers in clinical therapy trials of Alzheimer's disease: a long range point of view beyond 2020," published in in *Biochemical Pharmacology*, notes that biomarkers appear to be the most promising avenue to scientific advances and may contribute to the progress in development of novel drugs for the treatment of AD and may help demonstrate targeted therapies. Exhibit B, p. 206 and completeness responses dated December 22, 2014.

18. The following table lists existing providers in the area:

TABLE 1
RESEARCH PET/CTS IN AREA

Facility Name	Facility Address	Services	Days/Hours of Operation
Yale University	New Haven, CT	PET/PET CT	*
GE Discovery PET/CT Scanner	New Haven, CT	PET/PET CT	*

Source: CMS.gov; equipment listed as a research site on a PET imaging clinical study on clinicaltrials.gov
*unknown
Exhibit A, p. 8

19. The Applicant's historical and projected utilization is as follows:

TABLE 2
APPLICANT'S HISTORICAL AND PROJECTED UTILIZATION
FISCAL YEARS 2011-2018

			Projected Utilization				
Equipment	FY2012	FY2013	FY 2014*	FY 2015**	FY 2016	FY 2017	FY2018
SPECT	166	187	165	170	170	170	170
PET	148	210	270	280	285	290	290
PET/CT***			0	80	192	225	250
Total	**316**	**397**	**435**	**530**	**647**	**685**	**710**

*Annualized for 2014 based on actual scans January through August 2014
** First year is a partial year for May-December.
***Proposed camera
Note: Base year 2015 estimate of scans developed from current prospect list of new studies (10 scans per month for a total of 80 in 2015). Full year utilization projections based on growth experienced with existing PET scanner, recent experience and market knowledge.
Exhibit A, p. 9; Exhibit B, pp. 209-10

20. The proposal's total capital expenditure is itemized below:

TABLE 3
PROPOSAL CAPITAL EXPENDITURE

Purchase/Lease	Cost
Equipment (Medical, Non-medical Imaging)	$465,000
Construction/Renovation**	$124,149
Total Project Cost	**$589,149**

Exhibit A. p 13

21. Funding will be provided by a line of credit in the amount of $750,000. Exhibit A, p. 14

22. The Applicant will be able to reduce the cost per scan based on the efficiencies yielded from having a second camera with only a modest increase in non-camera costs, such as staffing. A reduced cost per scan will allow MNI to pass savings on to research sponsors. Exhibit A, pp. 14, 16

23. The population to be served by the applicant is study participants involved in research trials for new therapies for neurodegenerative disorders. Exhibit A, p. 6

24. Study participants will primarily include volunteers with neurodegenerative disorders such as AD, Parkinson and Huntington disease and healthy control subjects. All research participants will provide written informed consent in accordance with the Department of Health and Human Services' ("HHS") guidelines and will be compensated for their time as volunteers of the research. Exhibit A, p. 6, 7

25. While the proposal will not have any immediate impact on the quality of health care delivery in the region, the research conducted at MNI will continue to contribute to the development of new diagnostic tools and therapies for patients with neurologic and psychiatric illnesses. Exhibit A, p. 12

26. MNI is exclusively a research company and does not participate in any health care reimbursement programs. Exhibit A. p. 2

27. MNI does not offer any medical services to patients. Exhibit A, p.4

28. The Applicant does not provide direct care to patients and, as such, there will be no change in the patient payer mix. Exhibit A. p. 15

29. The proposed technologically advanced PET imaging camera will further meet the increasing demand for investigational PET imaging studies and will be a critical part of the evaluation

of new therapies for neurodegenerative disorders. It will allow the MNI to meet the demand for PET imaging services for local research investigators and advance PET analysis techniques for worldwide studies. Exhibit A, p. 5

30. OHCA is currently in the process of establishing its policies and standards as regulations. Therefore, OHCA has not made any findings as to this proposal's relationship to any regulations not yet adopted by OHCA. (Conn. Gen. Stat. § 19a-639(a)(1))

31. This CON application is consistent with the overall goals of the Statewide Health Care Facilities and Services Plan. (Conn. Gen. Stat. § 19a-639(a)(2))

32. The Applicant has established that there is a clear public need for its proposal. (Conn. Gen. Stat. § 19a-639(a)(3)).

33. The Applicant has demonstrated that its proposal is financially feasible. (Conn. Gen. Stat. § 19a-639(a)(4)).

34. The Applicant has satisfactorily demonstrated that its proposal is strictly for research purposes. Therefore, it has no impact on the accessibility and cost effectiveness of health care delivery in the region. However, the proposal has the potential to improve the quality of health care delivery in the region. (Conn. Gen. Stat. § 19a-639(a)(5))

35. The Applicant has shown that there will be no change in access to the provision of health care services to the relevant populations and payer mix since the proposed equipment is strictly for research purposes. (Conn. Gen. Stat. § 19a-639(a)(6))

36. The Applicant has satisfactorily identified the population to be served and has satisfactorily demonstrated that this population has a need. (Conn. Gen. Stat. § 19a-639(a)(7))

37. The utilization of existing health care facilities and health care services in the Applicant's service area is not applicable given that this application pertains to research. (Conn. Gen. Stat. § 19a-639(a)(8))

38. The Applicant has satisfactorily demonstrated that this proposal would not result in an unnecessary duplication of existing services in the area. (Conn. Gen. Stat. § 19a-639(a)(9)).

39. The Applicant has satisfactorily demonstrated that the proposal will not result in a reduction or change in access to services for Medicaid recipients or indigent persons. (Conn. Gen. Stat. § 19a-639(a)(10))

40. The Applicant has satisfactorily demonstrated that the proposal will have no impact on the diversity of health care providers and patient choices in the geographical region. (Conn. Gen. Stat. § 19a-639(a)(11))

41. The Applicant has satisfactorily demonstrated that the proposal will not result in any consolidation or adversely affect health care cost or accessibility to care. (Conn. Gen. Stat. § 19a-639(a)(12))

Discussion

CON applications are decided on a case by case basis and do not lend themselves to general applicability due to the uniqueness of the facts in each case. In rendering its decision, OHCA considers the factors set forth in Conn. Gen. Stat. § 19a-639(a). The Applicant bears the burden of proof in this matter by a preponderance of the evidence. *Jones v. Connecticut Medical Examining Board, 309 Conn. 727 (2013).*

Molecular Neuroimaging, LLC ("MNI") is a for-profit research company located at 60 Temple Street in New Haven, Connecticut. MNI specializes in the application of biomarkers in drug development for neurodegenerative and neuropsychiatric disorders. *FF1* More specifically, MNI provides services across the pre-clinical and clinical spectrum, including radioligand development and manufacturing; clinical trial design and implementation as well as clinical imaging site coordination and management. MNI conducts research both independently and in collaboration with 30 global pharmaceutical and biotech companies as well as research organizations such as the Michael J. Fox Foundation, National Institute of Health and the Department of Defense. *FF3*

MNI's imaging studies support early drug development and investigational trials. *FF2* MNI currently operates one PET scanner and one SPECT camera to support its drug development and investigational trials. *FF4* The existing PET scanner is 13 years old and requires a significant amount of continued maintenance. Although MNI has been afforded the opportunity to perform multiple PET studies, due to the logistics involved with the use of radiopharmaceuticals and the limitations of its existing equipment, MNI cannot start the new studies until existing studies are completed. *FF7* Additionally, research sponsors require state-of-the art brain imaging for their clinical studies and researchers must demonstrate the availability of such imaging to be considered as clinical trial sites. *FF10* Moreover, development of pharmaceutical therapies rely on large multi-center studies aimed at evaluating the efficacy of drugs and the availability of imaging biomarkers is critical to ensure an accurate diagnosis and disease progression monitoring. *FF11*

The Applicant is seeking to acquire a 64 slice PET/6 slice CT Positron Emission Tomography-Computed Tomography scanner ("PET/CT"). *FF8* The proposed scanner's CT component will provide attenuation correction, resulting in additional clarity and an improved image of the human anatomy. *FF14* The scanner's CT component will also allow for several advanced imaging processing methods to be applied to the PET images, most notably, the ability to use the CT image for a highly accurate registration to the research subject's available MRI scan. *FF15* In addition, the PET/CT will allow for a more rapid image acquisition process that will result in shorter scan time and greater comfort for research subjects. *FF13* With the addition of the PET/CT scanner, the Applicant will have the ability to increase the number and quality of the scans acquired while performing more studies simultaneously. *FF12*

Because the PET/CT will be used for research purposes only and not for the delivery of health care services, the proposal has no impact on existing service providers in the area. *FF18, 27*

Likewise, it will not have an impact on the services provided to the Medicaid population. *FF 28* It will, however, indirectly benefit the state's population by helping to meet the demand for investigational PET imaging studies and the evaluation of new diagnostic tools and therapies for patients with neurodegenerative disorders. *FF7,16,17,23,25*

Acquisition of the PET/CT scanner will allow MNI to increase the number of its research scans, thus reducing the research cost of a scan. MNI will pass those savings along to its sponsors. *FF22* This has the potential to encourage an increased number of sponsored research studies thereby leading to new scientific knowledge and the hastening of improved treatments for diseases.

Both Alzheimer's and Parkinson's disease are two of the most common and rapidly increasing neurodegenerative disorders in the United States. *FF6* Research imaging is a critical tool in the development of new therapies for these and other neurologic and psychiatric disorders, including Huntington's disease, Schizophrenia, Depression, Multiple Sclerosis and Fragile X Syndrome. *FF5,9* MNI's research efforts will enhance knowledge about such disorders and have the potential to improve future treatment and quality of life outcomes for individuals suffering from these disorders. Therefore, OHCA concludes the Applicant has demonstrated a clear public need for the proposal.

Order

Based upon the foregoing Findings of Fact and Discussion, the Certificate of Need application of Molecular Neuroimaging, LLC for the acquisition of a PET/CT scanner is hereby **approved**.

All of the foregoing constitutes the final order of the Office of Health Care Access in this matter.

By Order of the
Department of Public Health
Office of Health Care Access

April 9, 2015
Date

Janet M. Brancifort
Janet M. Brancifort, MPH
Deputy Commissioner

* * * Communication Result Report (Apr. 9. 2015 5:36PM) * * *

1) OHCA-98604187054
2)

Date/Time: Apr. 9. 2015 5:34PM

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STATE OF CONNECTICUT
OFFICE OF HEALTH CARE ACCESS

FAX SHEET

TO: KIMBERLY FABRIZIO

FAX: 203.508.1503 203.401.4304

AGENCY: MNI

FROM: OHCA

DATE: 4/09/15 **Time:**

NUMBER OF PAGES: 2
(including transmittal sheet

Comments:
Docket Number: 14-31965

PLEASE PHONE IF THERE ARE ANY TRANSMISSION PROBLEMS

Phone: (860) 418-7001 *Fax: (860) 418-7053*

410 Capitol Ave., MS#13HCA
P.O.Box 340308
Hartford, CT 06134

Huber, Jack

From: Huber, Jack
Sent: Thursday, April 30, 2015 2:06 PM
To: 'kfabrizio@mnimaging.com'
Cc: Roberts, Karen
Subject: Notice of CON Expiration Date for the Decision Rendered under Docket Number: 14-31965-CON

Dear Ms. Fabrizio:

On April 9, 2015, in a final decision under Docket Number: 14-31965-CON, the Office of Health Care Access authorized a Certificate of Need ("CON") to Molecular Neuroimaging, LLC, to acquire and operate a PET/CT scanner. Pursuant to Section 19a-639b of the Connecticut General Statutes ("C.G.S."), *"a certificate of need shall be valid for two years from the date of issuance by this office."*

With this letter, please be advised that pursuant to Section 19a-639b, C.G.S., the current CON authorization issued under Docket Number: 14-31965-CON will expire on April 9, 2017. Please contact me at (860) 418-7069 or Karen Roberts, Principal Health Care Analyst at (860) 418-7041, if you have any questions regarding this notification.

Sincerely,

Jack A. Huber

Jack A. Huber
Health Care Analyst
Department of Public Health | Office of Health Care Access |410 Capitol Avenue
P.O. Box 340308 MS #13HCA | Hartford, CT 06134 |Ph: 860-418-7069| Fax: 860-418-7053| email: Jack.Huber@ct.gov