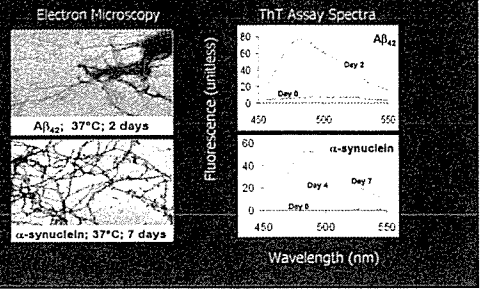
In vitro Characterisation of PIB binding to α-synuclein.

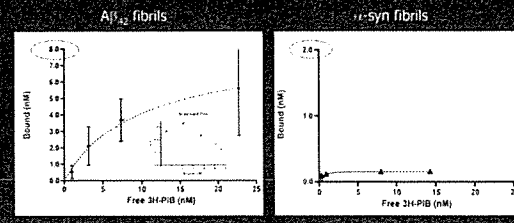
Amyloid Fibrilogenesis



In vitro Characterisation of PIB binding to α-synuclein.

Saturation binding studies

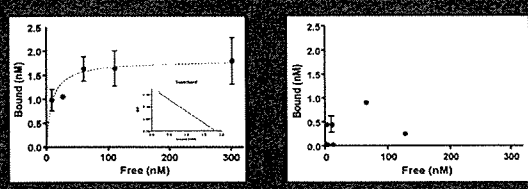
FIBRILS



In vitro Characterisation of PIB binding to α-synuclein.

Saturation binding studies

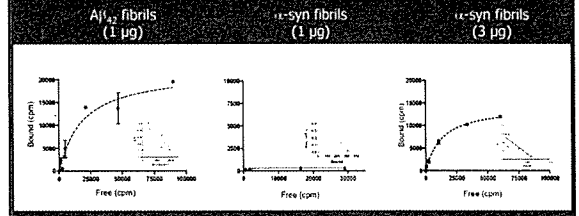
BRAIN HOMOGENATES



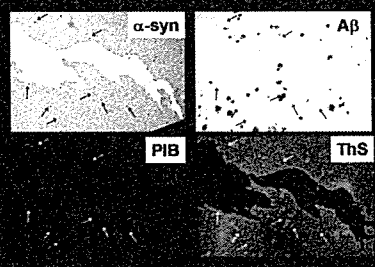
In vitro Characterisation of PIB binding to α-synuclein.

Saturation binding studies

FIBRILS



In vitro Characterisation of PIB binding to α-synuclein.



In DLB subjects, PIB binds to Aβ-containing plaques and not α-synuclein-containing Lewy bodies.

In vitro Characterisation of PIB binding to α-synuclein.

Image Quantification

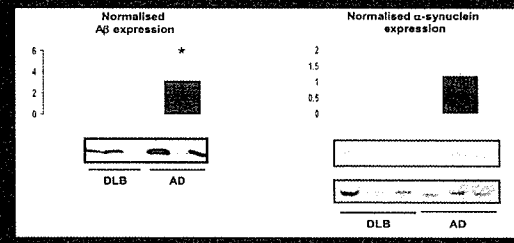
$$\frac{\text{Area Total}}{\text{Area IHC}}$$

In vitro Characterisation of PIB binding to α-synuclein.

Image Quantification

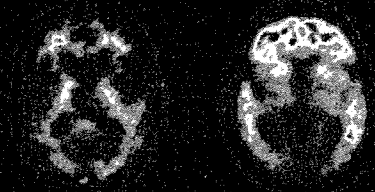
SUBJECT GROUP	REGION	PLAQUE AREA	LEWY BODY AREA
AD (n=12)	FRONTAL	0.0445 ± 0.03	
	TEMPORAL	0.0325 ± 0.01	
DLB (n = 5)	FRONTAL	0.0214 ± 0.02	0.0006 ± 0.0003
	TEMPORAL	0.0177 ± 0.01	0.0006 ± 0.0005

In vitro Characterisation of PIB binding to α-synuclein.



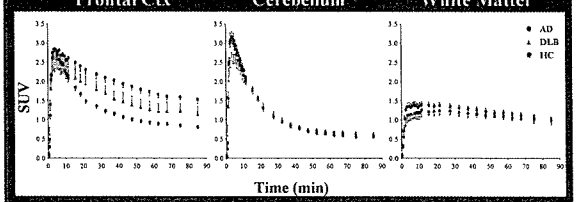
In vitro Characterisation of PIB binding to white matter

^{11}C-PIB

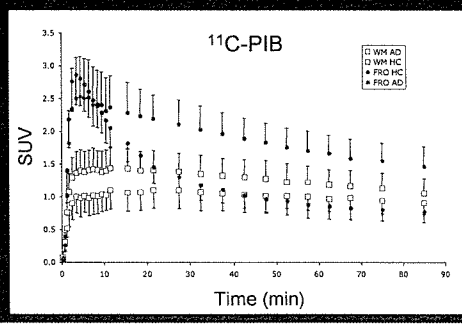


Control AD

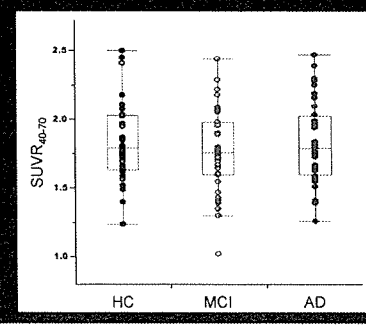
^{11}C-PIB Time-Activity Curves



In vitro Characterisation of PIB binding to white matter

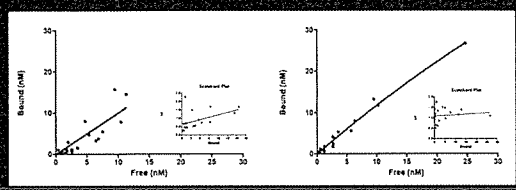


In vitro Characterisation of PIB binding to white matter

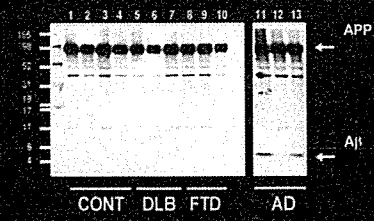


In vitro Characterisation of PIB binding to white matter

AD HC



In vitro Characterisation of PIB binding to white matter



WB (1E8) indicates that binding of ^{3}H-PIB to AD brain homogenate correlates with the presence of Aβ protein

40ug protein per lane
n=4 (CONT), n=3 (DLB, FTD, AD)

Conclusions

In vitro studies indicate that PIB binds weakly and non specifically to both α-synuclein fibrils and DLB brain homogenates.

Therefore, this study suggests that PIB retention within the cortical and subcortical grey matter regions of DLB patients is largely attributable to PIB binding to Aβ plaques and *not* Lewy bodies.

PIB binding to white matter is not different between the groups studied.

PIB binding to white matter is non-saturable indicating non-specific kind of binding.

^{11}C-PIB PET Imaging in Atypical Presentations of AD

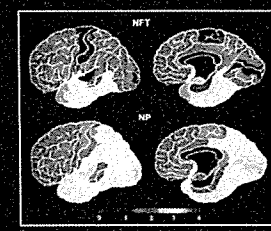
Objective

To examine cerebral PIB uptake in typical and atypical presentation of AD

Stereotypes and Topography of Alzheimer's disease

Four basic types:

- **Amnestic (temporal)**
- **Visuospatial (R>L)**
- **Aphasic (L>R)**
- **Frontal**



After J.Cummings

After G. van Hosen

Background

A progressive decline in memory, affecting activities of daily living is the most common clinical presentation of AD.

However, atypical clinical presentations at onset, has been increasingly recognized in recent years, posing a challenge in the initial differential diagnosis with FTD or DLB.

About 15% of all AD subjects have an atypical presentation, usually with early preservation of memory, attention, executive function and insight.

Background

Primary Progressive Aphasia (PPA)

Characterized by progressive isolated problems in word finding and object naming, sparing other aspects of cognition

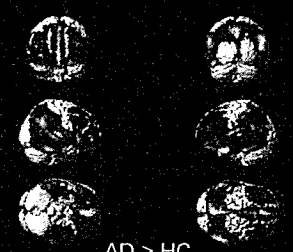
It is consider it a subtype of FTD, though post-mortem shows non-specific findings such as gliosis and spongiosis in most cases, with Pick bodies or AD pathology such as Aβ plaques and neurofibrillary tangles (NFT) in 20% of all cases, respectively

Posterior Cortical Atrophy (PCA)

Characterized by Balint's syndrome (ocular apraxia, optic ataxia and simultanagnosia); visual agnosia and prosopagnosia; ideomotor, constructional and dressing apraxia; environmental disorientation; and left hemi-neglect.

Post-mortem data demonstrated abundant neuritic Aβ plaques and NFTs in parietal and primary visual cortices

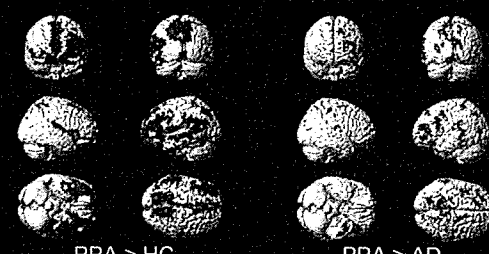
Alzheimer's disease



AD > HC

p = 0.01

Primary Progressive Aphasia

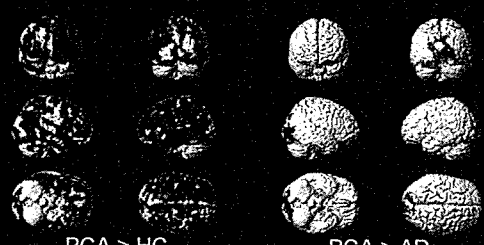


PPA > HC

PPA > AD

p = 0.01

Posterior Cortical Atrophy



PCA > HC

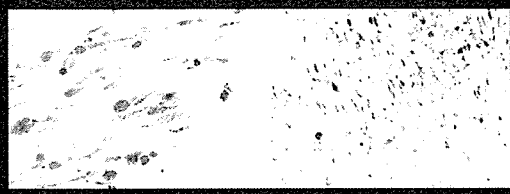
PCA > AD

p = 0.01

Conclusions

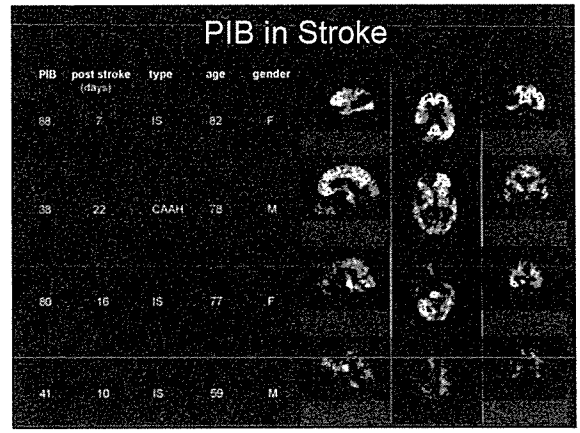
Identification of Aβ in the early stages of the disease will not only contribute to the differential diagnosis of dementia, but as more anti-Aβ treatment options become available it will also allow appropriate therapeutic strategies to be implemented and monitored.

APP is overexpressed in neuronal injury



APP microglia

PIB in Stroke



The AIBL Study of Aging

The Australian Imaging, Biomarkers, and Lifestyle Study of Aging

THE UNIVERSITY OF MELBOURNE

Study Overview

- Funded through CSIRO Flagship initiative
- 3-year prospective longitudinal study of memory and aging
- Large scale cohort study: 1000 participants
- Patients with AD, MCI and healthy volunteers
- Multi-disciplinary study: neuroimaging, psychometrics, biomarkers, assessment of lifestyle
- Focus on early detection, towards lifestyle interventions

Participants

- 1000 Volunteers (minimum age 65 years)

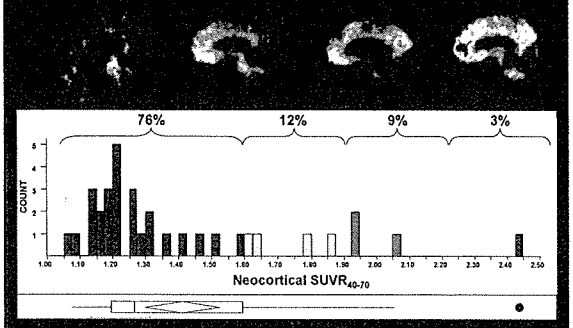
200 participants from 5 population groups:

1. Alzheimer's disease (AD)
2. Mild Cognitive Impairment (MCI)
3. Healthy volunteers (ApoE+)
4. Healthy volunteers (ApoE-)
5. "Memory complainers"

- Cognitive decliners: participants from groups 3, 4 and 5 who develop memory decline over the course of the study

Evaluation of Amyloid Burden in Ageing Subjects with and without Cognitive Decline

Can PIB detect Preclinical AD?



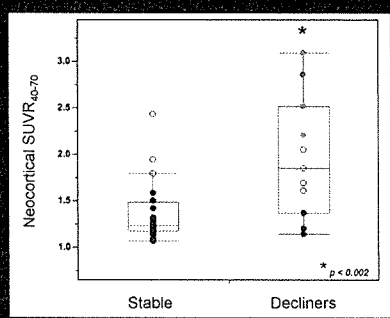
24% PIB positive at mean age of 72 is consistent with post mortem reports that 30% of non-demented persons aged >75 have amyloid plaques

PIB+ve Controls = Preclinical AD?

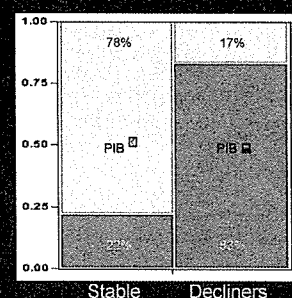
- Studies in Down Syndrome suggest Aβ deposition occurs years before dementia.
- Prevalence of AD at age 85 is 20-30% = PIB +ve rate in elderly controls at age 72.
- In our MCI cases 65% were PIB +ve, consistent with the expected conversion rate to AD.
- 60% of our PIB +ve controls have documented decline in word list recall though still in the normal range.

Longitudinal follow-up is needed but PIB PET shows potential for preclinical diagnosis!

Aβ burden as predictor of cognitive decline

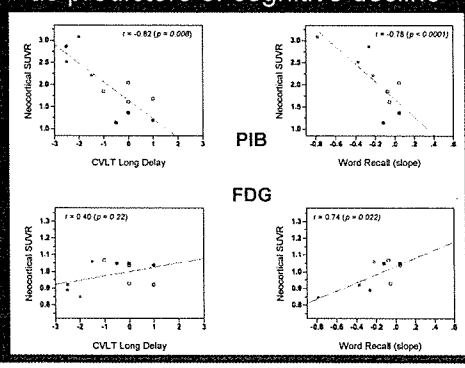


Aβ burden as predictor of cognitive decline



Fisher's exact test (p = 0.002)

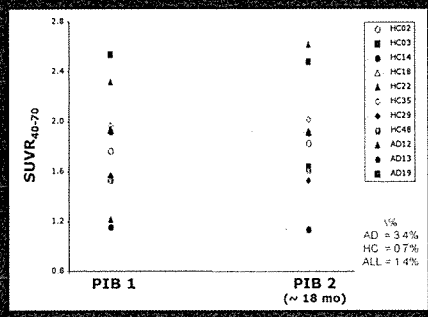
PIB and FDG as predictors of cognitive decline



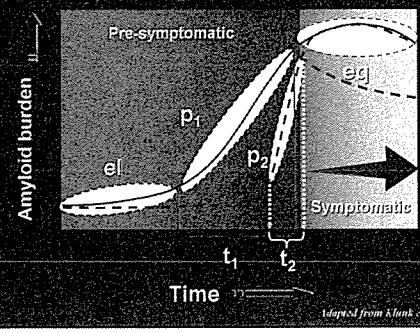
Follow-up of amyloid burden over time

PIB FOLLOW-UP

(n = 11)



Hypothesis on Amyloid Progression

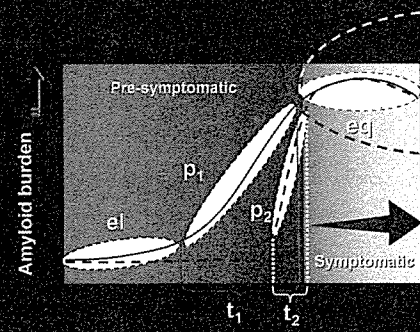


Adapted from Klunk et al, Brain (2006)

Future Directions

- ^{18}F / ^{123}I / ^{99m}Tc labelled ligands
- Longitudinal Studies
- Tau ligands

Hypothesis on Amyloid Progression



Adapted from Klunk et al Brain (2006)

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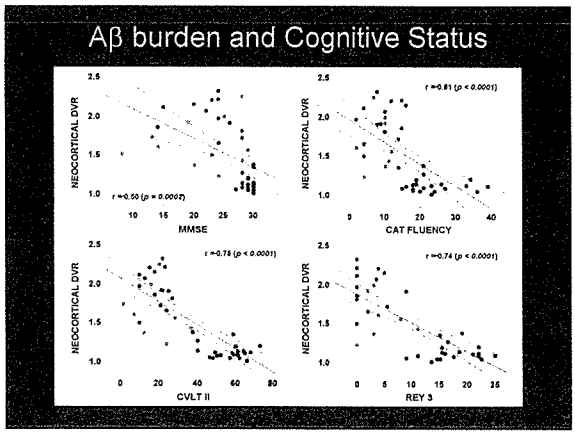
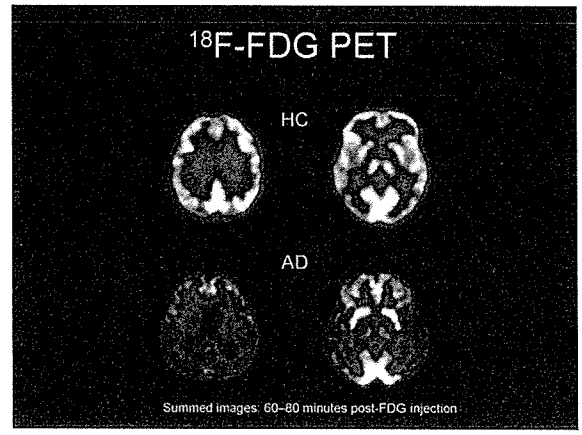
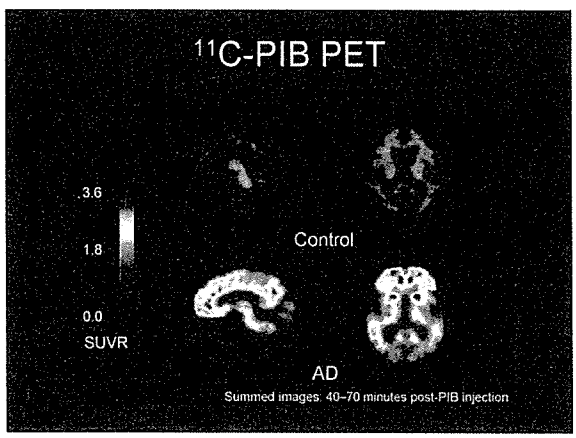

Aβ burden and Cognitive Status
NEOCORTICAL DVR
MMSE
CAT FLUENCY
CVLT II
REY 3


18F-FDG PET
HC
AD
Summed images: 60–80 minutes post-FDG injection


11C-PIB PET
3.6
1.8
0.0
SUVR
Control
AD
Summed images: 40–70 minutes post-PIB injection

Contact:
Alan P. Carpenter, Ph.D., J.D.
Vice President, Business Development & Legal Affairs
Avid Radiopharmaceuticals, Inc.
215.966.6208

AVID ANNOUNCES RESULTS OF AV-1 MOLECULAR IMAGING AGENT FOR ALZHEIMER'S DISEASE PRESENTED AT AD/PD MEETING

First in a series of novel β-amyloid imaging compounds from the University of Pennsylvania

Philadelphia, PA – March 19, 2007 – Avid Radiopharmaceuticals, Inc. (Avid), a product-focused molecular imaging company, today announced the presentation of the first results from a clinical study of ^{18}F-AV-1/ZK (AV-1) a novel radiopharmaceutical for positron emission tomography (PET) imaging of amyloid plaques in patients with Alzheimer's disease. Principal investigator Dr. Christopher Rowe from Austin Hospital of Melbourne, a leading investigator in the field of molecular imaging of Alzheimer's disease, presented the results at the 8th International Conference on AD/PD in Salzburg Austria.

The goal of this first clinical study was to examine whether PET imaging with AV-1 could be used to distinguish patients with Alzheimer's disease from those with normal cognitive function. AV-1 binds avidly to β-amyloid, the chief constituent of amyloid plaques, which accumulates abnormally in the brains of people with Alzheimer's disease.

Dr. Rowe reported that PET imaging with AV-1 clearly distinguishes AD from healthy elderly subjects, and may be used to quantify amyloid burden. AV-1 PET scans showed high levels of signal in the Alzheimer's patients, particularly in areas of the brain known to contain amyloid plaques. In contrast there was no retention of AV-1 in the cerebellar cortex, an area where amyloid plaques do not accumulate.

This is the first scientific report of a clinical trial with an ^{18}F-compound designed for specifically imaging amyloid plaques in AD. The wide availability of ^{18}F allows for the possibility of amyloid imaging at a large number of clinical sites worldwide.

"We are extremely encouraged by the results of this clinical study with Avid's first compound, AV-1. These data have provided the rationale for Avid's next generation compounds for amyloid imaging, which are now in clinical trials in the United States.", said Daniel Skovronsky, MD, PhD, CEO of Avid.

AV-1 is one of a series of novel compounds discovered in the laboratory of Dr. Hank Kung from the University of Pennsylvania and exclusively licensed to Avid for development and

commercialization. The results presented represent a collaborative effort between scientists at Austin Health, the University of Melbourne, Neuroscience Victoria, Avid Radiopharmaceuticals, the University of Pennsylvania, and Bayer Schering Pharma.

New treatment methods for slowing or reversing the deposition of insoluble amyloid in the brains of people with Alzheimer's disease are the subject of intensive clinical research by many large pharmaceutical companies as well as the National Institute of Mental Health (NIMH) (www.nimh.nih.gov/studies/1alzhdis.cfm). Amyloid imaging may help in identifying those patients who will benefit from these emerging treatments.

About Avid

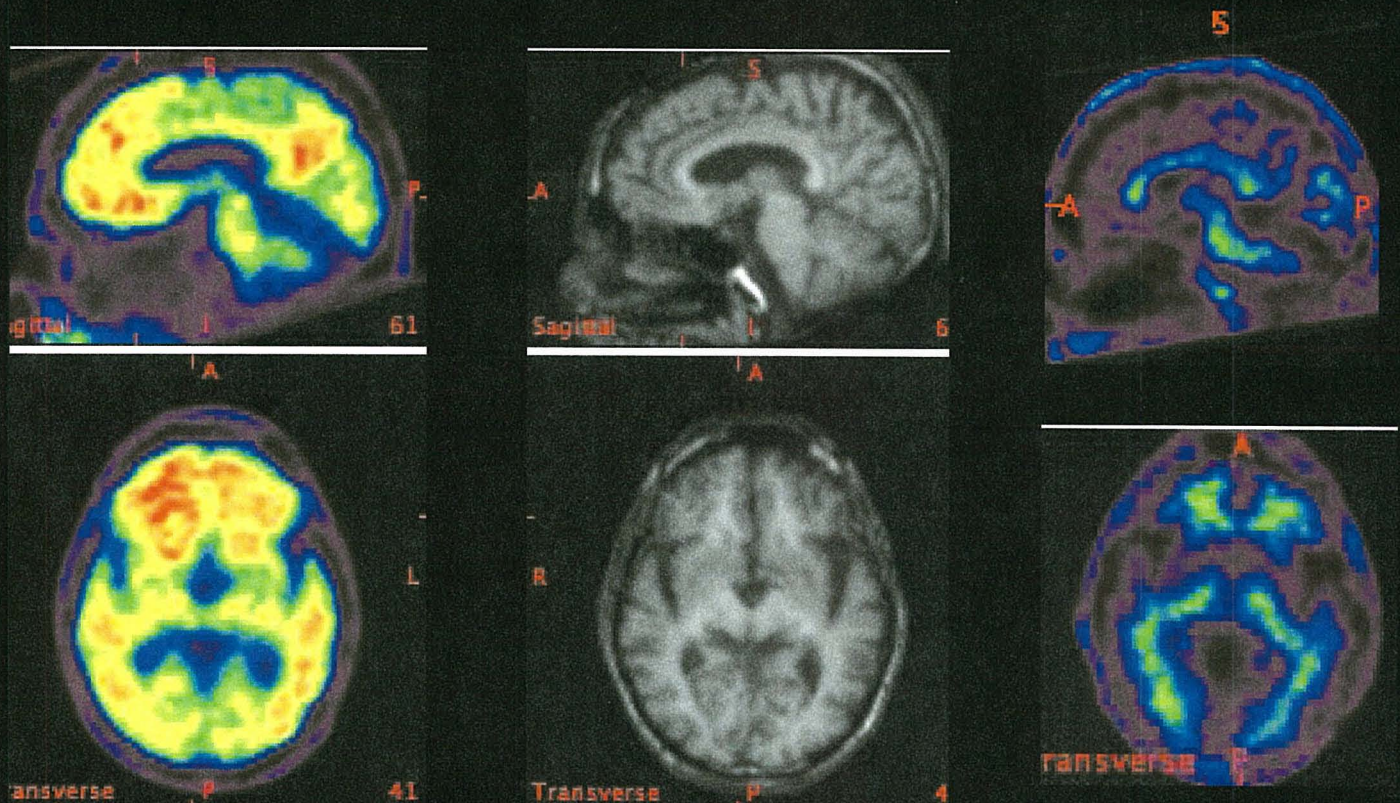
Avid Radiopharmaceuticals, Inc. is developing novel diagnostic imaging agents to enable the early diagnosis, treatment selection and therapeutic monitoring of major medical disorders. The company is a pioneer in the development of molecular imaging agents for Alzheimer's disease. Its lead product candidates are being developed to identify amyloid plaques, which are thought to accumulate in the brain for years before the onset of clinical symptoms of the disease. Avid's compounds may enable the earlier diagnosis of Alzheimer's disease and also allow researchers to better evaluate therapeutic drug candidates for the prevention or reversal of amyloid plaque build-up in the brain. Avid's technology can be used with a variety of imaging technologies such as positron emission tomography (PET) and single photon computed tomography (SPECT) and is currently being tested in a number of pilot human studies. In July 2006 Bayer Schering Pharma AG, Germany, a worldwide leader in specialized pharmaceuticals, and Avid announced a collaboration they have formed through which Bayer Schering Pharma has an exclusive option to develop and market certain of the Avid PET molecular imaging agents known as ^{18}F-stilbenes for Alzheimer's disease. Avid, in collaboration with Dr. Hank Kung of the University of Pennsylvania, also continues its research and development of other new β-amyloid molecular imaging agents; two of which have now entered IND studies. Avid has also initiated an IND study of a new ^{18}F-PET compound for imaging the vesicular monoamine transporter (VMAT-2) which is implicated in diseases involving dopaminergic degeneration such as Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). The VMAT-2 imaging program has grown out of a close collaboration with the University of Michigan as well as the University of Pennsylvania. Avid has also begun a research program on imaging β- cells of the pancreas, as a marker of the onset and progression of diabetes mellitus. For more information, visit www.avidrp.com.

About Alzheimer's Disease

According to the Alzheimer's Association (www.alz.org) there are an estimated 4.5 million people in the United States alone who have Alzheimer's disease. The number of people with Alzheimer's disease is expected to grow – by 2050 the number of individuals with Alzheimer's could range from 11.3 million to 16 million. People with Alzheimer's disease typically experience a progression of symptoms resulting from the underlying nerve cell degeneration that takes place in Alzheimer's disease. Nerve cell damage typically begins with cells involved in learning and memory and later extends to cells that control every aspect of thinking, judgment, and behavior. According to a report commissioned by the Alzheimer's Association (www.alz.org/AboutAD/statistics.asp), Alzheimer's disease costs American businesses $61 billion a year. Of that figure, $24.6 billion covers Alzheimer's health care and $36.5 billion covers costs related to caregivers of individuals with Alzheimer's disease, including lost

productivity, absenteeism and worker replacement. Medicare costs for beneficiaries with Alzheimer's are expected to increase 75 percent, from $91 billion in 2005 to $160 billion in 2010 and Medicaid expenditures on residential dementia care are expected to increase from $21 billion in 2005 to $24 billion in 2010.

[^{18}F]-AV1/ZK



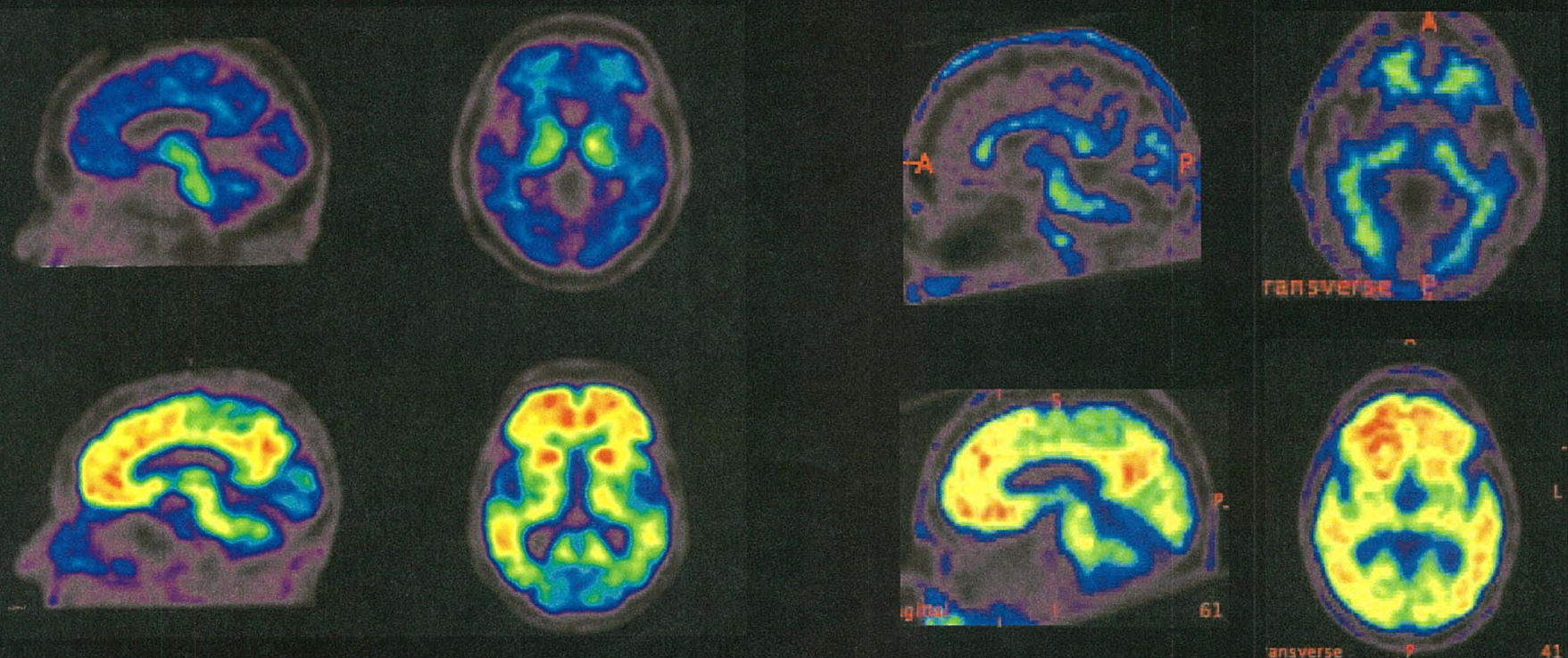
Alzheimer's Disease
80 year old male
MMSE 26

Healthy Elderly Control

Comparison to [^{11}C]-PIB

[^{11}C]-PIB

[^{18}F]-AV1/ZK

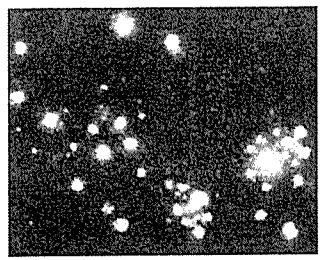


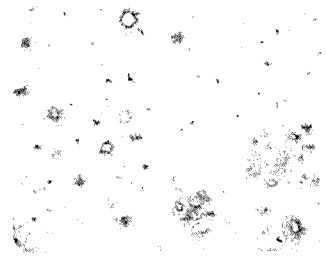
Two Healthy Elderly Subjects – top row
Two Alzheimer's Disease Subjects – bottom row

資料３

アミロイドイメージング用新規^{18}F標識化合物の基礎開発：脳内動態と脱フッ素を指標に標識化合物の最適化

Compound 3
蛍光イメージング

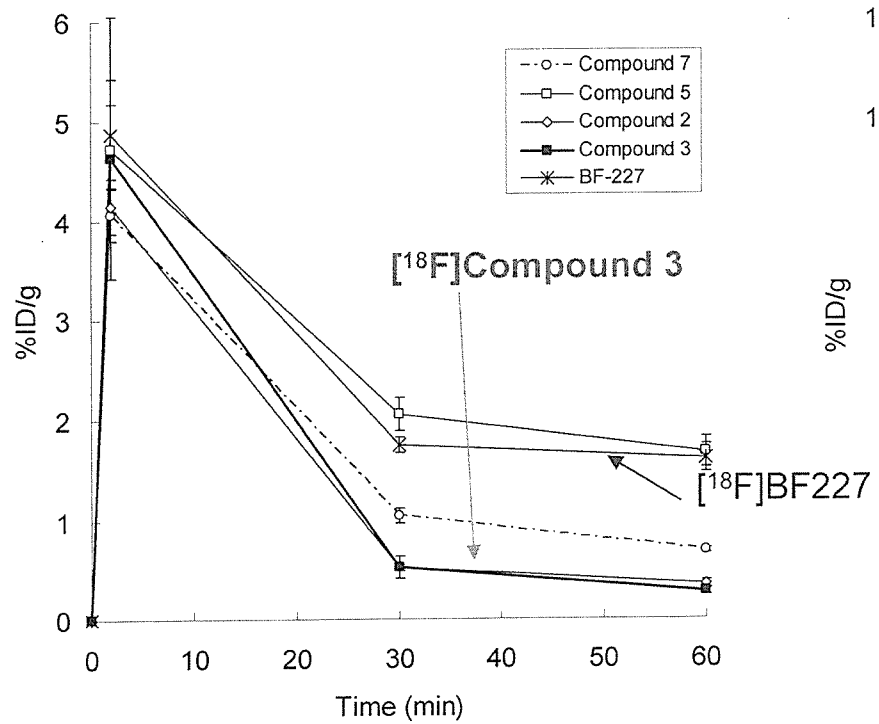




Aβ immunostaining

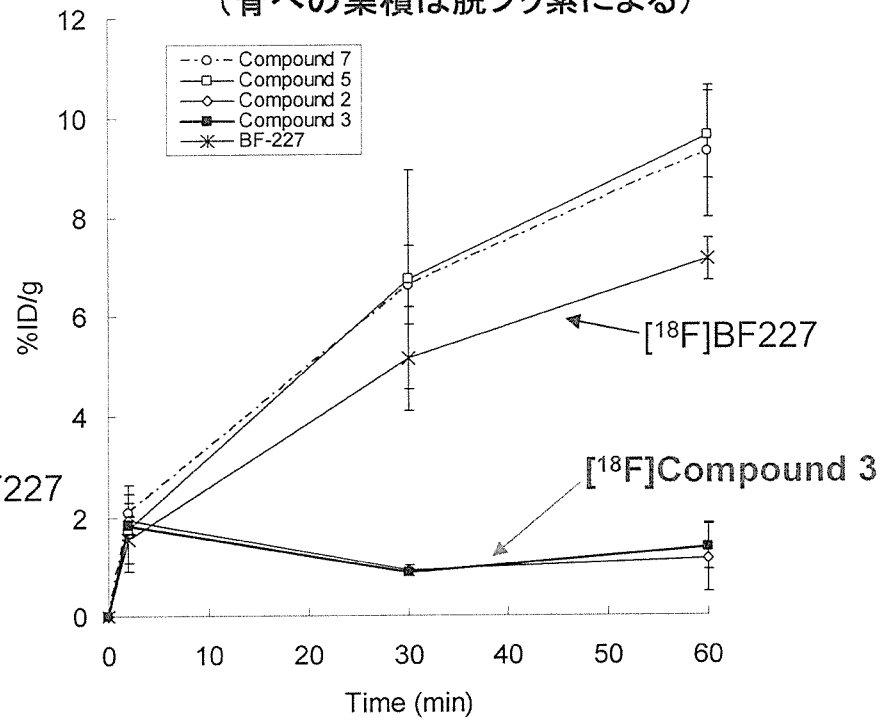
Tg2576 mouse

Brain



Bone

（骨への集積は脱フッ素による）



基礎研究からBF227より優れているCompound 3を見出し、現在急性毒性試験を実施中

分担研究報告

厚生労働科学研究費補助金（萌芽先端医療技術推進事業）
分担研究報告書

国立長寿医療センターにおける[^{11}C]BF227 製造の整備

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[研究要旨]
[^{11}C]BF227 の臨床測定を施行するため、国立長寿医療センターにおける製造環境を整備した。現実的な合成方法を見出した。

A. 研究目的
[^{11}C]BF227[1]の臨床測定を施行するため、国立長寿医療センターにおける製造環境を整備した。

B. 研究方法
既設の ^{11}C-標識薬剤合成装置 CUPID C-11-BII（住友重機械工業）を用い合成を行った。必要に応じ適宜改造を加えた。

C. 研究結果
1．合成装置の整備
既設の ^{11}C-標識薬剤合成装置 CUPID C-11-BII（住友重機械工業）の HPLC 精製部にあらたに高圧６ポートバルブ（model 7000, Rheodyne）を設置した。この改造に伴い、精製 HPLC の分離能が向上した（図１）。

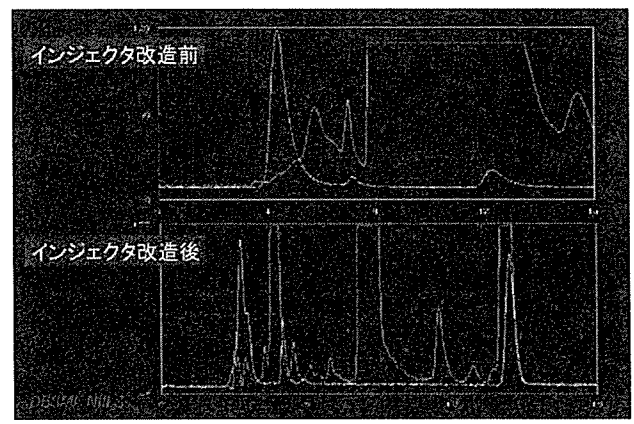

図１インジェクタ改造前後の精製 HPLC の相違

2．合成条件の検討
[^{11}C]メチルトリフレートを用いた *N*-メチル化反応より[^{11}C]BF227 を合成した。反応は室温で３分間、遮光下で行った。８回の合成の結果をまとめると次の通りである。（平均±標準偏差（範囲））放射化学的収率：26.4±15.4% (8.1-47.4)、放射化学的純度：97.6±1.1% (95.5-98.5)、比放射能：42.2±17.9GBq/μmol (25.6-79.3)。

放射化学的収率はバラツキが大きく問題があった。これについて検討を加えた。反応に用いるアルカリ量と放射化学的収率の関係をプロットしたところ図２となり、原料に対し４等量以下の水酸化ナトリウムを用いることで、収率が向上することがわかった。この条件で合成した３例の収率をまとめると 39.5±6.9% となった。放射化学的純度、比放射能について満足すべき結果が得られた。

図２　アルカリ量と放射化学的収率の関係

D. 考察

アルカリ減量による放射化学的収率の向上は水酸イオンによる[11C]メチルトリフレートの分解が成績体の生成と競合するためと考えられる。こうした現象はアセトンなどを溶媒とするときは観察されないことから、DMSO による求核性の向上が原因と考えられる。DMSO は溶解性が高く本合成に最適な溶媒と考えられるので、アルカリ量に注意することで収率の向上を図っていきたい。

E. 結論

[^{11}C]BF227 の臨床測定を施行するため、国立長寿医療センター　における製造環境を整備した。現実的な合成方法を見出した。

[参考文献]

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F. 研究発表

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1. 伊藤健吾：　アルツハイマー病の診療における画像診断の意義－核医学検査を中心に－．第 22 回青森県核医学研究会　青森, 7 月 8 日, 2006 年

G. 知的財産権の出願・登録状況（予定も含む）

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

厚生労働科学研究費補助金（萌芽的先端医療技術推進事業）
分担研究報告書

臨床例での[^{11}C]BF227と[^{11}C]PIBの比較研究

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[研究要旨]
本研究では、[^{11}C]BF227（以下BF227）と現在最も広く臨床研究が行われている[^{11}C]PIB（以下PIB）の２種類のアミロイドプローベによるPET検査を同一被験者で施行し、その違いについて検討した。脳への移行はPIBの方が良好で、脳組織への取り込みと洗い出しもPIBの方が速やかであった。健常者では、BF227、PIB とも視床、脳幹、小脳髄質に非特異的と考えられる集積が見られ、分布は類似していた。アルツハイマー病症例では、BF227とPIBとも大脳皮質で健常者に比べ高い集積が見られたが、PIBでは楔前部や前頭葉に高い集積が見られ、局所差が目立ったが、BF227では脳内の局所差が少なかった。健常者とアルツハイマー病患者の皮質におけるDVRの違いは、BF227では20%程度であるのに対し、PIBでは60%程度であり、PIBの方が感度が高いと考えられた。アミロイドカスケードのステージにおけるそれぞれの集積特性の違いがあれば、病態の進行についての異なった情報を見ることができる可能性がある。

A. 研究目的

PETによるアミロイドイメージングによるアルツハイマー病の超早期診断を目指して、本研究課題のアミロイドプローベ[^{11}C]BF227（以下BF227）と、ピッツバーグ大学で開発され現在最も広く臨床研究に使用されている[^{11}C]PIB（以下PIB）を用いたPET検査を、同一のアルツハイマー病等の認知症患者及び健常者において実施し、その性質の違いを調べることにより、BF227の診断的な特長を明らかにする。

B. 研究方法

１．対象

本年度は健常者４名及びアルツハイマー病等の認知症患者２６名の計３０名を対象とした。初めにB-2.に示すMRI撮影、FDG-PET及びPIB-PETの画像診断を施行し、またアポEフェノタイプ等の髄液バイーマーカーも検討した。それらの結果を説明したうえで、同意された健常者２名とNINCDS-ADRDAとDSM-IVの診断基準で診断したアルツハイマー病患者２名にBF227-PETを施行した。

2．データ収集

３０名の全被験者に、初めに 3D MRI 撮影、FDG-PET 及び PIB-PET を施行した。

3D MRI は SIGNA EXCITE 1.5T（GE 社製）で、3DSPGR プロトコールにより 1.5 mm 間隔の T1 コントラスト矢状断画像を撮影した。

FDG-PET は、約 150 MBq の FDG を静注後 35 分間ベッド上で安静（開眼覚醒、感覚刺激、運動を避ける）を保った後、5 分間の同時収集によるトランスミッションスキャンを施行し、引き続き静注 45 分後より 6 分間のエミッションスキャンを 3D モードで撮影した。

BF227-PET と PIB-PET の撮影プロトコールは全く同様である。5 分間のトランスミッションスキャンの後、約 500 MBq のトレーサを静注後 60 分間のダイナミックスシャン（10 s x 6 F、20 s x 3F、60 s x 2 F、120 s x 1 F、240 s x 1 F、300 s x 10 F）を 3D モードで施行、同時に橈骨動脈に留置したカテーテルから経時的動脈採血（2 分後までは 10 秒間隔、その後間隔を漸増し 25 ポイント）と代謝分析（3 分、10 分、20 分、30 分、40 分、60 分）を行った。PET カメラは SET 2400W（島津製作所）を用いた。

被験者に４名に対し、更に PIB-PET を施行した。PIB-PET の撮影プロトコールは全く同様である。

3．データ解析

BF227-PET と PIB-PET において、代謝物補正を行った血液放射能時間曲線（pTAC）とダイナミック PET データ（脳局所の組織放射能時間曲線、

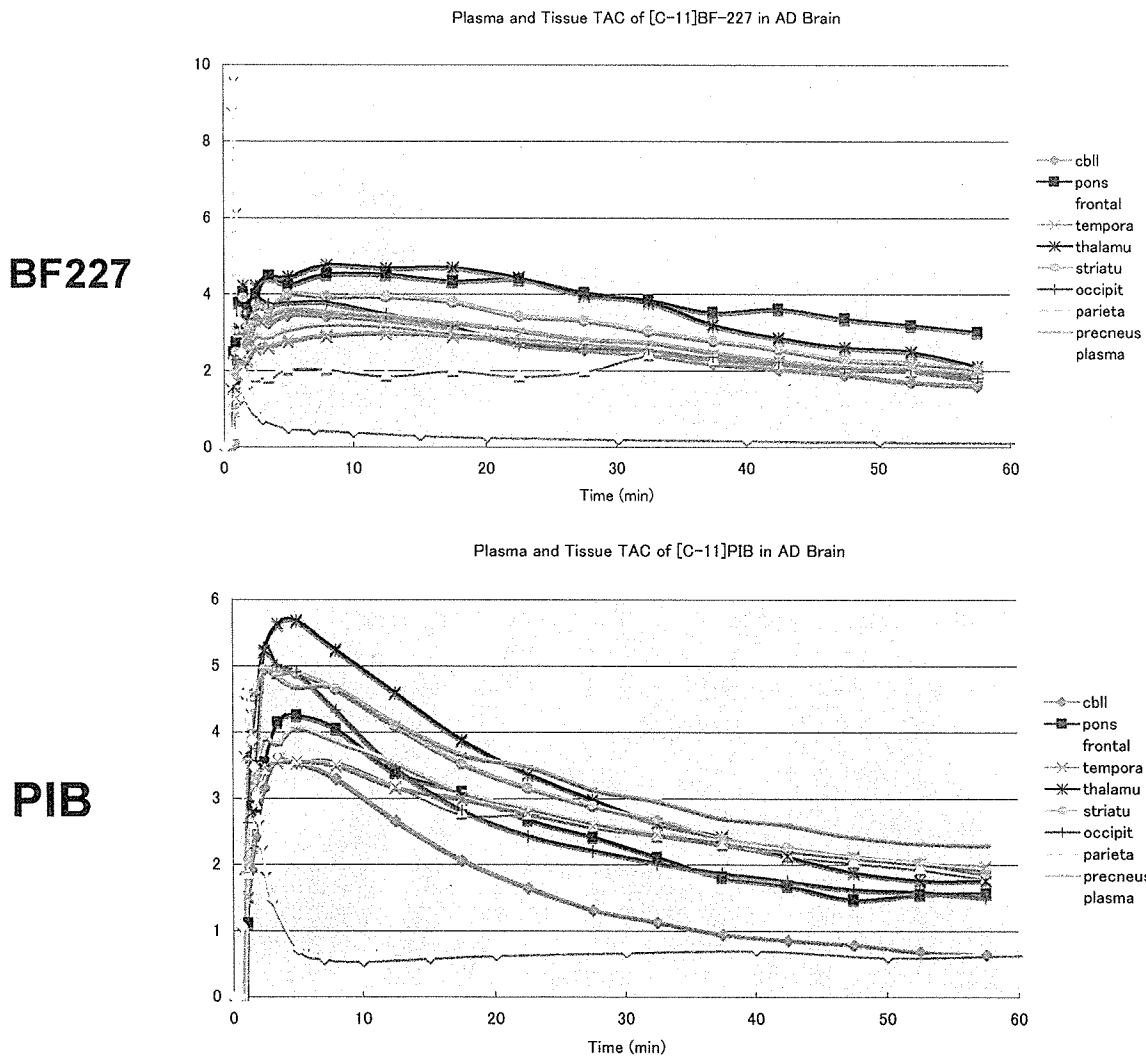

図１　BF227 と PIB の pTAC と tTAC の違い。同一被験者（アルツハイマー病患者　82 歳　男性）における比較。