THE DEVELOPMENT OF A HUMANIZED ANTIBODY-TARGETED ABO-SPECIFIC PET PROBE FOR EARLY DIAGNOSTIC IMAGING OF ALZHEIMER'S DISEASE

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TOPICS

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BACKGROUND

It’s the only cause of death in the top 10 in America that CANNOT BE PREVENTED, CURED OR SLOWED.

ALMOST TWO THIRDS of Americans with Alzheimer’s disease are women.

1 IN 3 seniors dies with Alzheimer’s or another dementia.

Alzheimer’s disease is the 6TH LEADING CAUSE OF DEATH IN THE UNITED STATES.

Only 45% of people with ALZHEIMER’S disease or their caregivers report BEING TOLD OF THEIR DIAGNOSIS.

More than 90% of people with the four most common types of CANCER have been TOLD OF THEIR DIAGNOSIS.

By 2050, these costs could rise as high as $1.1 TRILLION.

In 2015, Alzheimer’s and other dementias will cost the nation $226 BILLION.
MOLECULAR BASIS

- Amyloid-β plaques
- Neurofibrillary tangles
AMYLOID BETA PLAQUES

• Amyloid Precursor Protein (APP)

• $\alpha\beta_{42}$ vs $\alpha\beta_{40}$

• Peptide $\rightarrow$ oligomer $\rightarrow$ fibril $\rightarrow$ plaque
AMYLOID CASCADE HYPOTHESIS

• Weak correlation between AD and plaques

• Amyloid-β oligomers (AβOs) initiate neurodegeneration
**BACKGROUND**

- **Mild Cognitive Impairment**
  - Duration: 7 years
  - Disease begins in Medial Temporal Lobe
  - Symptoms: Short-term memory loss

- **Mild Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Lateral Temporal & Parietal Lobes
  - Symptoms include:
    - Reading problems
    - Poor object recognition
    - Poor direction sense

- **Moderate Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Frontal Lobe
  - Symptoms include:
    - Poor judgment
    - Impulsivity
    - Short attention

- **Severe Alzheimer’s**
  - Duration: 3 years
  - Disease spreads to Occipital Lobe
  - Symptoms include:
    - Visual problems
ANTIBODIES

• Monoclonal
• NU4 - mouse
• ACU193 - human
PET SYNTHESIS

- PET uses radioactive ligand
- Conjugation of DOTA cage to antibody
- Chelation with radioactive compound/element ($^{64}$Cu)
ABO ARE SEPARATE FROM PLAQUES

• Demonstrate that AβOs are separate from plaques

• Immunostained tg and wt mice with 568-NU4 (AβOs)

• Counterstained with ThioFlavin S (amyloid plaques)
DOTA BINDS TO THE FC REGION OF NU4

- Electrophoresis
- Confirm DOTA binds to constant region
- NU4: 54.091 kDa
- NU4-DOTA: 56.849 kDa
- 6-7 DOTA bound to heavy chain
NU4DOTA RETAINS IMMUNOREACTIVITY TO ABOS

- Dot blots
- EC$_{50}$
- No decrease in immunoreactivity
NU4-DOTA COLOCALIZES WITH ABOS IN VITRO

- NU4DOTA and FAM-ABOs in primary hippocampal neuron culture
- High levels of colocalization
- Retained immunoreactivity in vitro
NU4PET DEMONSTRATE A STRONG AD DEPENDENT SIGNAL IN MICE

- Tg and Wt mice injected
- NU4PET
- IgGPET
- NU4PET demonstrates a strong AD dependent signal
NU4PET DEMONSTRATES SUBSTANTIAL BRAIN UPTAKE

- Blood brain barrier is extremely difficult to cross
- Percent of injected dose retained similar to PiB and Florbetapir

![Graph showing % Injected Dose - Max](image)
PET SIGNAL CORRELATES TO ACU193 IMMUNOFLOUORESCENT INTENSITY

- Immunofluorescently labeled brains with ACU193
- Fluorescent intensity correlates to PET signal
ABOS ARE DETECTABLE AT 2 MONTHS IN 5XFAD MICE

- Target for diagnosis must appear before onset of symptoms
- ERENNA and histology
- AβOs are detectable at 2 months
NU4 demonstrates plaque-like and punctate labeling of pyramidal layer in 5xFAD mouse model

- Identify pathology seen in our 5xFAD mouse model
- Punctate and plaque-like labeling of the dendritic arbors
- AβOs found primarily in hippocampus, dentate gyrus and along the pyramidal layer
ACU193 Distinguishes Between Diseased and Nondiseased Human Brains
ACU193 shows plaque-like, punctate, and diffuse pathology in human brain tissue.
ACU193DOTA distinguishes between 5XFAD and WT tissues down to 2 months (NU4 and ACU193DOTA)
ACU193DOTA BINDS IN AN AGE DEPENDENT MANNER AND DISTINGUISHES BETWEEN TG AND WT MICE

Integrated Density of ACU193DOTA Probe

Integrated Density (Arbitrary Units)

Condition

9M Tg
9M Wt
6M Tg
6M Wt
3M Tg
3M Wt
2M Tg
2M Wt
ACU193 PET SHOWS PROMISE IN DISTINGUISHING BETWEEN DISEASED AND NONDISEASED MICE
DAY 1 RESULTS

Wild Type

5xFAD
DAY 1 RESULTS

Wild Type

5xFAD
CONCLUSION

• ACU193PET demonstrates tremendous potential as an early diagnostic imaging tool for AD

• Future Work:
  • Substantiating AD specific probe signal by autoradiography using mouse and human brain sections
  • Dose curve PET to determine optimal dosing range in mid-stage AD (5xFAD model)
  • Evaluation of gross uptake and clearance in mice in vivo
  • Longitudinal analysis to determine earliest stages at which PET probe detects AβOs
  • Quantitative relationship between PET signals, AβOs detected histologically, AβOs detected biochemically, and memory loss
  • Human trial in 18 months
  • Dual MRI/PET
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