MODEL-AD
Preclinical Efficacy Testing Pipeline and Training Resources

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NIH AD Research Summits 2012/2015 Recommendations Aimed at:
Increasing the Predictive Value of AD Animal Models and Enabling Transparent and Reproducible Preclinical Efficacy Testing

- Establish and implement guidelines for rigorous preclinical testing in AD Tg models with the standards/rigor comparable to clinical trials in humans
- Provide a resource/facility for standardized therapeutic efficacy testing of preclinical drug candidates that prioritizes biochemical and physiological endpoints
- Preclinical efficacy testing of selected candidate AD therapeutics using standardized best practices
- Develop a database of preclinical studies that would be available to the AD scientific community and incorporating experimental details as well as unpublished negative and positive data

NIA Funding Initiative RFA AG16-04

MODEL-AD Consortium
Model Organism Development and Evaluation for Late-onset Alzheimer’s Disease
PTC Aims and Milestones

• **Years 1-2**
  - **Establish and validate processes and procedures**
    - Testing protocols (SOPs), exclusion/inclusion criterion, subject identification, logistics (e.g. sample shipment JAX to IU)
  - **Recruit and Train staff**
    - Staff are required to reproduce data validation sets under blinded conditions
    - Develop training protocols and provide this resource to the community
  - **Establish preclinical testing pipeline**
    - Validate pipeline with BACE inhibitor in well characterized mouse model (5xFAD)
    - Determine Go/NoGo criterion
    - Refine processes and procedures
    - Test preclinical pipeline with drug currently in clinic
  - **Develop and implement process for vetting potential drug candidates nominated by the greater AD research community**
    - Establish a publically accessible web mechanism to submit drug candidates

• **Years 3-5: Evaluate 2 novel compounds per year in MODEL-AD LOAD mouse models**

All raw data, methods, and analyses published via Synapse/Sage portal and AlzPED for community access
### Primary Screen: Behavioral Testing in Rodent Models

- Reversal of a scopolamine/MK801 induced cognitive deficit in normal young adult males
- Reversal of a “cognitive deficit” in Tg mice (often limited to males only)
- Under-reported: dose response relationship; misinterpretation of behavioral confounds; ARRIVE guidelines?

### Compound Screening & Optimization (SAR)

- PK/PD Modeling (Mouse <-> human)
  Young adult male (WT) C57BL/6J male mice for safety/tolerability/toxicology
MODEL-AD Preclinical Testing Core (PTC)

- Emphasis on prioritizing pharmacokinetic and translational pharmacodynamics over behavior as a primary screen for preclinical efficacy

ARRIVE Guidelines and Best Practices
- Drug QC & formulation stability
- N=10-12 per sex per dose
- Age-matched vehicle controls
- Blinded technicians
- Blinded data analysis
- Subjects randomized and counterbalanced for order of testing
- Raw data and SOPs to Sage/Synapse

Disease Modeling Project:
Selection of mouse model for drug testing has been previously evaluated for disease staging

Steering Committee:
- Triage of Test Compounds

Preclinical Testing Pipeline

1º Screen
Appreciable exposure levels in target tissues in Biologically Relevant Model at Pathological Age

Go/No Go Gate

2º Screen
Target Engagement Disease Modifying Effect

PET/MRI
- Autoradiography
- Immunohistochemistry

Go/No Go Gate

3º Screen
Functional Activity – Symptom Modifying Therapeutic Index

In Vivo Functional Activity

Therapeutic Index
Disease Modeling Project: Selection of mouse model for drug testing has been previously evaluated for disease staging

Steering Committee: - Triage of Test Compounds

Preclinical Testing Pipeline

• Mouse models will be best matched to the compound of interest being evaluated in the screening pipeline based on both disease pathology and compound mechanism of action.

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Pathological Hallmark</th>
<th>Drug (Mechanism)</th>
<th>Primary Fluid Biomarker</th>
<th>Primary Biomarker</th>
<th>Secondary Biomarker</th>
<th>Primary Confirmation</th>
<th>Secondary Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXFAD</td>
<td>Abeta</td>
<td>BACE Inhibitor (verubecestat)</td>
<td>CSF/plasma</td>
<td>PET/MRI</td>
<td>PET/MRI</td>
<td>AutoRad</td>
<td>IHC</td>
</tr>
<tr>
<td>hTau</td>
<td>Tau</td>
<td>Tau Inhibitor</td>
<td>AB42</td>
<td>AV45</td>
<td>FDG</td>
<td>AV45 FDG</td>
<td>Abeta</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pTau</td>
<td>AV1451</td>
<td>PTSM</td>
<td>AV1451 PTSM</td>
<td>Tau</td>
</tr>
</tbody>
</table>
Age of dosing determined by results from disease staging in the DMP

PK Study (JAX)
- Low dose n = 3M 3F
- Medium Dose n = 3M 3F
- High dose n = 3M 3F

Blood and Tissue
- PK Analysis (IU)
- PK/PD Modeling
  - Drug dose, frequency, route (IU)

Biomarker Samples
- Terminal Blood and CSF collection (JAX)

Go/No Go Gate

PD Study (IU)

PET/MRI
- FDG, PTSM
- AV45, AV1451

2° Validation
- Autorad/IHC

Go/No Go Gate

Biomarker Samples
- RNAseq/Histopathology
- Confirmatory PK

Functional (JAX)
- Behavior
- Therapeutic Index

*N=10-12 per sex per dose
3 pt dose response v veh treated

N=10-12 per sex per dose
3 pt dose response v veh treated and v WT veh treated
PTC: Therapeutic Strategy

• Our goal is to develop a testing strategy that maximizes the therapeutic potential of all drug candidates by initiating the dosing strategy prior to the onset of disease relevant biomarker readouts.

• To do this, our strategy in the 5XFAD mouse is to initiate dosing at 3mo with a duration of 3mo, thus maximizing the neuroprotective effects of the candidate.
Evaluation of Verubecestat in 5xFAD

PK Study (JAX)
- 3 mg/kg PO n = 3M 3F
- 10 mg/kg PO n = 3M 3F
- 30 mg/kg PO n = 3M 3F

Biomarker Samples
Terminal Blood and CSF collection (JAX)

Blood and Tissue PK Analysis (IU)
Serial Blood Samples: 0.25, 0.5, 1, 2, 4, 8, 24hr(T)

Graphs:
- CPAC
  Catalog #S8564, Lot#S856401
  1000ng on Column
  RT: 8.953
- JAX
  Catalog #S8173, Batch #01
  1000ng on Column
  RT: 9.666
QC process for confirming test compounds prior to initiating studies is a critical component of the PTC

Both compounds injected into one vial

<table>
<thead>
<tr>
<th>Compound</th>
<th>Catalog #</th>
<th>Lot/Batch</th>
<th>Concentration</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPAC</td>
<td>S8564</td>
<td>S856401</td>
<td>1000ng</td>
<td>9.013</td>
</tr>
<tr>
<td>JAX</td>
<td>S8173</td>
<td>01</td>
<td>1000ng</td>
<td>9.722</td>
</tr>
</tbody>
</table>

• PTC Bioanalytical Team (CPAC @ IU)
  • LC/MS/MS analysis of Standards + Test compound
  • Compound is not Verubecestat
  • Vendor replacement of drug lot
  • Verubecestat PK and Imaging studies were swapped with the Levetiracetam studies to conserve time and resources.

These data highlight the importance of validating the test compound prior to full study conduct, and as a result saved the PTC, MODEL-AD, and NIA $$$$$
Evaluation of Levetiracetam in 5xFAD

PK Study (JAX)
- 10 mg/kg PO n=3M 3F
- 30 mg/kg PO n=3M 3F
- 100 mg/kg PO n=3M 3F

Test compound QC and 7+day formulation stability

Serial Blood Samples: 0.25, 0.5, 1, 2, 4, 6 hr (T)

Blood and Tissue PK Analysis (IU)

Levetiracetam (mg/L) vs Time (hr)

- Males: 10 mg/kg, 30 mg/kg, 100 mg/kg
- Females: 10 mg/kg, 30 mg/kg, 100 mg/kg

Serial blood samples collected at 0.25, 0.5, 1, 2, 4, 6 hr (T).

Drug dose, frequency, route (IU)

Blood and Tissue PK Analysis (IU)
Evaluation of Levetiracetam in 5xFAD

<table>
<thead>
<tr>
<th>Dosing Schedule</th>
<th>Cmax</th>
<th>Cmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q12 hours</td>
<td>7.16</td>
<td>0.18</td>
</tr>
<tr>
<td>10 hours</td>
<td>7.1</td>
<td>0.1</td>
</tr>
<tr>
<td>14 hours</td>
<td>7.3</td>
<td>0.1</td>
</tr>
<tr>
<td>8 hours</td>
<td>7.05</td>
<td>0.55</td>
</tr>
<tr>
<td>16 hours</td>
<td>7.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>
MODEL-AD Preclinical Testing Core (PTC)

**Prophylactic Strategy**
- Dosing initiating before the onset of disease progression
- 5XFAD male and female mice chronic administration from 3 months of age through 6 months of age
  - Levetiracetam (PO, BID, 0, 10, 30, 56 mg/kg)
  - Verubecestat (TBD – PK in progress)

**PD Readout**
- PET/MRI/AutoRad as a PD readout of cerebral changes in:
  - Regional Metabolism (18F-FDG)
  - Beta Amyloid deposition (18F-AV45)

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**1º Screen**
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**Go/No Go Gate**

**2º Screen**
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**In Vivo Functional Activity**

**Therapeutic Index**

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T2W MRI  
18F-FDG PET

**18F-FDG PET/MRI**
**18F-FDG Autorad**

*IndyPET3 scanner and Siemens 3T Prisma scanner co-registered to Paxinos-Franklin atlas*
18F-AV45 PET/MRI/Autoradiography: Prophylactic treatment of LEV in 6 mo aged 5xFAD mice

- No alteration of amyloid deposition as measured at 6 mo of age in male and female 5xFAD treated prophylactically with LEV
  - N=73 6mo old 5xFAD mice (n=32 female; n=41 male; N=7-11 per sex per dose level) with 56 brain regions per subject (N=4088 total regions; 1792 females, 2296 males) extracted from co-registered to Paxinos-Franklin atlas
  - Post mortem 18F-AV45 autoradiography in 16 brain regions per subject (N=7008 total; 3936 males, 3072 females) at 3 bregma targets according to Paxinos-Franklin.

**FDG-PET and behavioral cohorts prophylactic dosing in progress**
PTC: Candidate Drug Submission Portal – In Development

Process Model – PTC DSC

1. DSC Portal
2. System Core
3. Database
4. Email Submitter
5. Email PTC Team
6. Decision Queue
7. Team Review
8. Team Review
9. System Core
10. PTC Team
11. Submitter

MODEL-AD PTC Screening Criteria

MODEL-AD PTC Screening Forms

Enter Basic Compound Information

What is the compound name?
- True
- False

How much compound is available?

What is the therapeutic indication?
- True
- False

Is this drug phosphoryl or symptomatic?
- phospholytic
- symptomatic

What is the mechanism of action?
- absorbed
- active
- effector
- other
- no
- unknown

What is the molecular weight of the compound (Da)?

MODEL-AD
MODEL ORGANISM DEVELOPMENT AND EVALUATION FOR LATE-ONSET ALZHEIMER’S DISEASE

Life Sciences
NIH
National Heart, Lung, and Blood Institute
ALFRED P. SIDAN FOUNDATION
NIH
PTC: Drug Selection Criteria – In Development

Process Model – PTC DSC

1. DSC Portal
2. System Core
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4. Email Submitter
5. Email PTC Team
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7. Team Review
8. PTC Team
9. Data Base

PTC: Drug Selection Criteria

- Enter the drug name/compound information along with source above.
- For each factor that the drug contains, select a fuzzy term from the list for the gamma value.
- Once completed for all factors review weight below (see M119).

4. Interpretation:
   a) \( W < 0.45 \), drug failed to meet minimum threshold
   b) \( 0.45 > W < 0.69 \), drug may need more information to proceed
   c) \( W > 0.7 \), drug meets minimum threshold

<table>
<thead>
<tr>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>Fair</td>
<td>Excellent</td>
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Legend:
- Alpha value can only be 0 or 1 as integer (automatically set when selecting Gamma weights)

Process Model:
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MODEL-AD PTC Educational & Training Resources

IMPROVING PRECLINICAL TRANSLATION OF ALZHEIMER’S DISEASE RESEARCH
Location: Bar Harbor ME

We invite you to join us for an immersion workshop focusing on the improvement of preclinical translation in Alzheimer’s Disease research. This workshop will leverage the expertise and facilities of the Indiana University (IU)/JAX Model Organism Development for Evaluation of Late Onset Alzheimer’s Disease (MODEL-AD) Precision Medicine consortium.

**Lecture Topics**
- Drug Discovery and Development Process
- Pharmacokinetics and Bioanalytical
- Pharmacodynamics and PD endpoints for AD
- PK/PD Modeling
- Behavioral Phenotyping for AD mouse models
- Translational Pharmacology (PET/MR)
- Intersection of Clinical and Preclinical Genetics
- MODEL-AD Consortium Resources and new AD mouse model Resources
- Preclinical Biostatistics

**Hands On Training & Practicums**
- *in vivo* PK studies
- drug formulation
- routes of administration (PO, IP, SC, etc)
- serial blood sample and terminal CSF and tissue collections
- Executing experiments in line with ARRIVE guidelines
  - Blinding
  - Randomization
  - Counterbalancing
  - Controls
  - Sample size Analyses

**MODEL-AD Consortium Resources and new AD mouse model Resources**

**Preclinical Biostatistics**

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**MODEL-AD Consortium Resources and new AD mouse model Resources**

**Preclinical Biostatistics**
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