Preclinical drug screening in new generation Alzheimer's disease mouse models: The MODEL-AD Consortium Strategy

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

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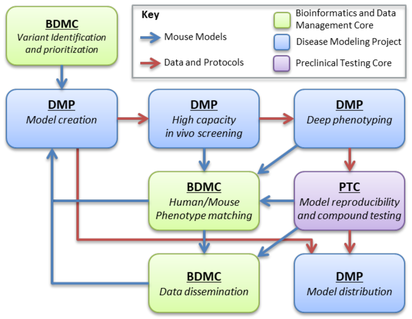
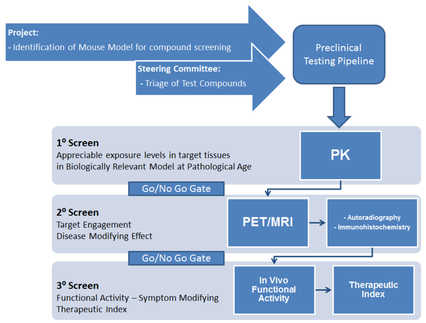
INTRODUCTION

- Historically, preclinical screening of test compounds for Alzheimer's Disease (AD) employed behavioral endpoints in rodent models as the primary screen. Often the rodent models used did not necessarily have construct validity for AD, and experiments often evaluated the ability of a test compound to reverse an acute pharmacological deficit (e.g. scopolamine induced memory deficit) in wild-type or normal animals. Other screens evaluated the ability of the test compound to normalize a behavioral phenotype, and these studies rarely used biomarkers or other clinically translational endpoints. Young or naïve wild-type animals were often used in place of aging animals, and critically pharmacokinetic (PK) and pharmacodynamic (PD) data in the AD model at the biologically and pathologically relevant ages had not been evaluated.
- The Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) program has been established with the goals of: 1) identifying novel genetic variants, genes and biomarkers from late onset AD (LOAD) patient data; 2) generating and validating mouse models with construct and face validity for LOAD; and 3) developing a preclinical testing strategy to evaluate potential therapeutic agents for the treatment of AD in these new models.



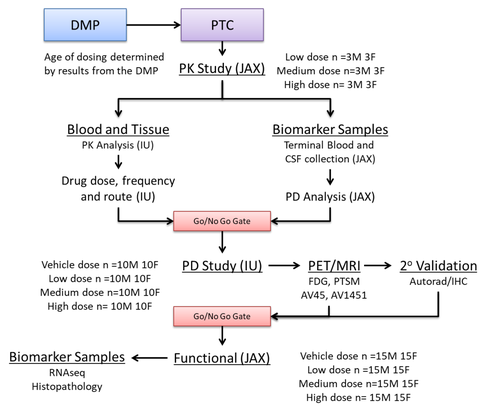
- The MODEL-AD process workflow schematic illustrates the disease variants, models, screening schemes, phenotyping modes, drug testing, data integration and modeling, and finally model distribution to the scientific community. In the diagram the workflow starts in the upper left quadrant with the prioritization of variants by the Bioinformatics and Data Management Core (BDMC). These variants are then created via CRISPR-Cas9, screened, and phenotyped by the Disease Modeling Project (DMP). Models which show disease-relevant phenotypes at defined ages are then selected to be moved on for therapeutics testing by the Preclinical Testing Core (PTC). Within the PTC pipeline, models will be evaluated for drug disposition, pharmacodynamics, and functional testing (see below). In all cases, data generated by each team is then funneled back to the DMP and BDMC for combined mathematical modeling and dissemination to the community.

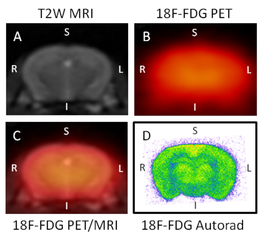


- The PTC has established a streamlined preclinical strategy with go/no-go decision points that allow critical and unbiased assessments of potential therapeutic agents while matching each test compounds' specific mechanism of action to the animal model best suited to interrogate its symptom and/or disease modifying activity.
- The PTC screening strategy includes an initial primary screen to determine appreciable multi-dose PK and target tissue activity in the disease model at the pathologically relevant age followed by predictive PK/PD modeling. A secondary screen evaluating target engagement and disease modifying activity of the test compound utilizing non-invasive PET/MRI as a pharmacodynamic readout of cerebral changes in metabolism (18F-FDG), cerebral blood flow (64Cu-PTSM), beta amyloid deposition (18F-AV45), and tau deposition (18F-AV1451). Provided the compound meets the *a priori* criteria for success in the primary and secondary screens, the tertiary screen will evaluate symptom modifying effects of the test compound to normalize a disease-related phenotype in cognition tests, relative to the age- and sex-matched littermate WT controls. All raw data, standard operating procedures, methods and protocols will be made publicly accessible via the external facing Sage Synapse portal to the AD research community at large.

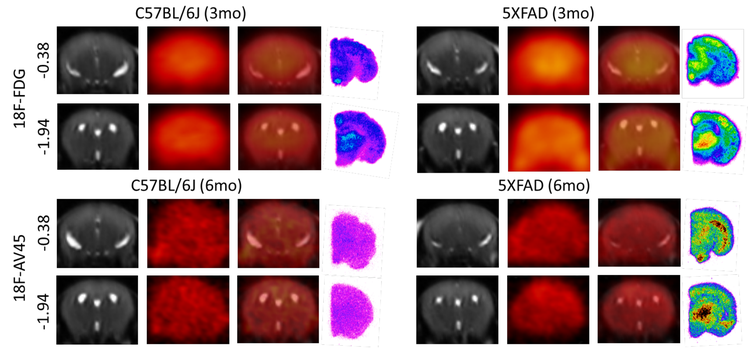
PRECLINICAL TESTING CORE SCREENING STRATEGY

The flowchart below depicts the preclinical testing screening strategy. Only models that have been phenotypically validated to demonstrate LOAD-like endo-phenotypes (as characterized in the DMP) will enter the primary screen. Each phase has a pre-determined Go/No Go criteria that must be met before moving to the next phase.



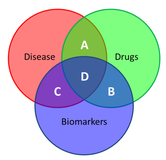


Representative mouse 3T T2W MRI (A), 18F-FDG PET (B), Fused 18F-FDG PET/MRI (C), and ex vivo 18F-FDG Autoradiography (D). The effective in-plane resolution for PET, MRI, and Autoradiography are 1mm^3, 0.25mm^3, and 0.025mm^3, respectively. Symbols, S, I, L, and R are superior, inferior, left and right, respectively.



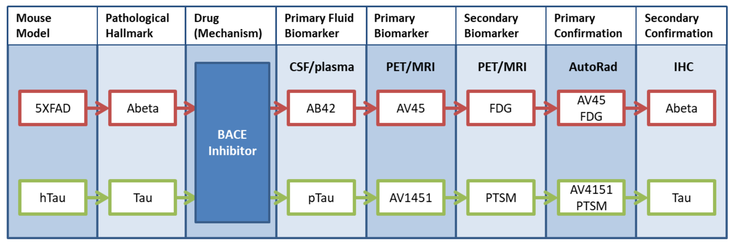
TRANSLATIONAL MEASURES FOR COMPOUND SCREENING

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. Drug testing will be conducted at the age in the model that demonstrates the expected pathology (as identified within the DMP). Determining the optimal readout for a biomarker is based on the intersection(s) of the disease, drug mechanism of action, and biomarker properties. Each region (A-C) represents potential false negative/positive readouts, while region D provides the optimal measure of drug's action on a disease process.



∩	Disease	Drugs
A	–	–
B	–	+
C	+	–
D	+	+

The diagram below depicts an example of key primary and secondary biomarkers and outcome measures that are disease relevant and translational and will be specific for each model system/test compound combination. Behavioral testing aimed to demonstrate symptom-modifying effects relative to age- and sex-matched vehicle treated WT littermate controls will only be conducted as tertiary outcome measures provided the test compound meets its primary and secondary endpoints.



DRUG SELECTION CRITERIA & TEST COMPOUND PRIORITIZATION

The community is encouraged to nominate compounds for testing in the PTC. To ensure unbiased selection and testing prioritization, the PTC has developed selection criteria based on a cumulative weighting scheme that takes into consideration all known biophysical properties of the compound as well as known preclinical and/or clinical data for efficacy and safety, which can be described as follows:

$$W(j) = \frac{1}{m}\sum_{i=1}^{n} \alpha(i)\beta(i,j)\gamma(i,j) \qquad \gamma(i,j) = (a_1/b_1),(a_2/b_2),+\ldots+,(a_k/b_k)$$

$$\alpha = \begin{cases} 0, & if\ \gamma = None \\ 1, & if\ \gamma > None \end{cases} \qquad a = \{none, poor, fair, good, excellent\}$$

$$\beta \rightarrow [0.0,1.0] \qquad (a,b), \text{where } b \text{ is a set}$$

$$a: b \rightarrow [0,1] \text{ as a sigmoid function}$$

The information provided about the submitted compounds availability, mode of action, in vitro and in vivo efficacy, in vitro and in vivo pharmacokinetics, and any toxicology which may exist in either the target or non-target species can be used to help rank the likelihood of a candidate compound having bio-pharmaceutical properties suitable for testing in the PTC pipeline. Shown below are categorical information that may be provided for each candidate compound. Values in parentheses are the β weights.

Biophysical Characteristics

1. Must not have IP restrictions which would prevent dissemination of data (0.0 or 1.0)
2. Must have a defined TID (0.0 or 1.0)
3. Must have a well characterized MOA (0.0 or 1.0)
4. Drugs have known modifying functionality
   a. Drug is disease modifying (DMOD) (0.67)
   b. Drug is symptom modifying (SMOD) (0.34)
5. For studies where compounds are used, data are weighted as follows
   a. Clinical (Phase 1-4 or OLU) compounds (1.0)
      i. Within species (i.e. mouse data within a mouse model) *(nested values carry 0.67 of total weight)*
         1. Disease model *(nested values carry 0.45 of total weight)*
            a. ED_{50} or EC_{50} (0.33)
            b. K_I (K_D or K_i or K_m) (0.22)
            c. IC_{50} (0.11)
         2. Control model *(nested values carry 0.22 of total weight)*
            a. ED_{50} (0.17)
            b. K_I (K_D or K_i or K_m) (0.12)
            c. IC_{50} (0.06)
      ii. Across species (i.e. rat data within a mouse model) *(nested values carry 0.33 of total weight)*
         1. Disease model *(nested values carry 0.22 of total weight)*
            a. ED_{50} or EC_{50} (0.17)
            b. K_I (K_D or K_i or K_m) (0.12)
            c. IC_{50} (0.06)
         2. Control model *(nested values carry 0.11 of total weight)*
            a. ED_{50} or EC_{50} (0.08)
            b. K_I (K_D or K_i or K_m) (0.06)
            c. IC_{50} (0.03)
   b. Preclinical compounds (0.5)
      i. Within species (i.e. mouse data within a mouse model) *(nested values carry 0.33 of total weight)*
         1. Disease model *(nested values carry 0.22 of total weight)*
            a. ED_{50} or EC_{50} (0.17)
            b. K_I (K_D or K_i or K_m) (0.12)
            c. IC_{50} (0.06)
         2. Control model *(nested values carry 0.11 of total weight)*
            a. ED_{50} or EC_{50} (0.05)
            b. K_I (K_D or K_i or K_m) (0.04)
            c. IC_{50} (0.02)
      ii. Across species (i.e. rat data within a mouse model) *(nested values carry 0.17 of total weight)*
         1. Disease model *(nested values carry 0.11 of total weight)*
            a. ED_{50} or EC_{50} (0.08)
            b. K_I (K_D or K_i or K_m) (0.06)
            c. IC_{50} (0.03)
         2. Control model *(nested values carry 0.06 of total weight)*
            a. ED_{50} or EC_{50} (0.04)
            b. K_I (K_D or K_i or K_m) (0.03)
            c. IC_{50} (0.01)

Pharmacokinetic Characteristics

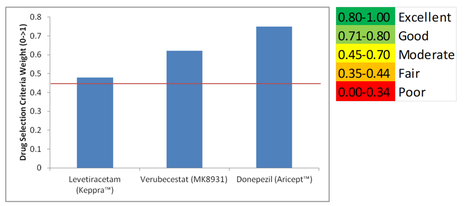
6. For studies where PK data exists, the data are weighted in the following order
   a. In vivo PK (values below are nested and carry 0.75 of total weight)
      i. Within species (i.e. mouse data within a mouse model) *(nested values carry 0.67 of total weight)*
         1. Disease model *(nested values carry 0.50 of total weight)*
            a. Cl_u(0.44)
            b. $T_{1/2}$ (0.38)
            c. [x] (0.33)
            d. B_{max} (0.27)
            e. M_t (0.22)
            f. F (0.16)
            g. C_{max} (0.11)
            h. V_d (0.06)
         2. Control model *(nested values carry 0.25 of total weight)*
            a. Cl_u(0.22)
            b. $T_{1/2}$ (0.19)
            c. [x] (0.17)
            d. B_{max} (0.14)
            e. M_t (0.11)
            f. F (0.08)
            g. C_{max} (0.06)
            h. V_d (0.03)
      ii. Across species (i.e. rat data within a mouse model) *(nested values carry 0.33 of total weight)*
         1. Disease model *(nested values carry 0.25 of total weight)*
            a. Cl_u(0.22)
            b. $T_{1/2}$ (0.19)
            c. [x] (0.17)
            d. B_{max} (0.14)
            e. M_t (0.11)
            f. F (0.08)
            g. C_{max} (0.06)
            h. V_d (0.03)
         2. Control model *(nested values carry 0.13 of total weight)*
            a. Cl_u(0.12)
            b. $T_{1/2}$ (0.10)
            c. [x] (0.09)
            d. B_{max} (0.07)
            e. M_t (0.06)
            f. F (0.04)
            g. C_{max} (0.03)
            h. V_d (0.01)
   b. In vitro PK (values below are nested and carry 0.5 of total weight)
      i. Caco2 (0.4)
      ii. LogD (0.3)
      iii. ADMET (0.1)
   c. In silico PK (values below are nested and carry 0.25 of total weights)
      i. LogP (0.4)
      ii. pKa (0.3)
      iii. MW (0.2)
      iv. Class (0.1)

Toxicology Characteristics (No nesting as these are typically performed in control animals)

7. For studies where toxicology data exists, the data are weighted in the following order
   a. LD_{50} (0.8)
   b. Chronic Toxicology Dosing (≥3 Day) (0.6)
   c. Acute Toxicology Dosing (1 Day) (0.4)
   d. Low Teratogenicity (0.2)

Using the above categories, the PTC analyzed Levetiracetam, Verubecestat, and Donepezil to determine if the current approach provided a reasonable means to stratify the candidate compounds which would be submitted the Drug Selection Criteria (DSC).

The rank order of the drugs were based on the composite weight DSC and were used to "pressure test" the model for prioritizing novel test articles. These drugs were selected to provide a wide range of approved and experimental compounds that are undergoing clinical evaluation.



Shown to the left are the results of an extensive literature evaluation of the current FDA approved drugs in use or in clinical evaluation for the treatment of Alzheimer's Disease. As observed from the chart, Levetiracetam, Verubecestat, and Donepezil all show a high Drug Selection Criteria weight. In the current model, a criterion cutoff of 0.45 (horizontal red line) was selected as the minimum weight needed for a compound to progress into the PTC's testing pipeline.

Based on the aforementioned strategies, the PTC has begun the process of validation of the pipeline with two known pharmaceuticals, Verubecestat, which is currently in phase 3 trials for disease modifications of beta-amyloid, and Levetiracetam, which is being evaluated for both symptomatic and disease modifying properties, but at levels which are 10-15x lower than indicated for seizures.

FURTHER INFORMATION

- MODEL AD: www.modelad.org
- AMP-AD Knowledge Portal: www.synapse.org/ampad
- JAX AD models: https://www.jax.org/alzheimers

Scan the QR code to visit the MODEL-AD website, which provides information on how to obtain mouse models, links to access validation data along with accompanying protocols, and news and updates.

ACKNOWLEDGEMENT

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