Bridging the Preclinical to Clinical Translation Gap in Alzheimer’s Disease

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Why Should Clinicians Care About Preclinical Research?

Relevance of Animals as Models for Human Diseases
(Genetics, Anatomy, Physiology, Function, Pathology)

Challenges Translating Behavioral Changes Observed in Animal Models to Human Behaviors

7 ‘R’s
Right Target
Right Drug
Right Biomarker
Right Model System
Right Translational Assays
Rigor
Reproducibility
**Primary Screen:** Behavioral Testing in Rodent Models
- Reversal of a scopolamine/MK801 induced cognitive deficit in normal young adults – *often males only*
- Reversal of a "cognitive deficit" in male Tg mice – *not correlating with pathology and often only single dose with no PK*

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**Compound Screening & Optimization (SAR)**

- PK/PD Modeling *(Mouse <--- human)*
  Young adult male (WT) C57BL/6J male mice for safety/tolerability/toxicology

**Historical Drug Discovery**
- Biomarker Samples (?)
  - Terminal Blood and CSF collection
- Postmortem Blood and Tissue
  - PK Analysis
Primary Screen: Behavioral Testing in Rodent Models

- Reversal of a scopolamine/MK801 induced cognitive deficit in normal young adults – *often males only*
- Reversal of a “cognitive deficit” in male Tg mice – *not correlating with pathology* and often only single dose with no PK

• Assay confounds often resulting in *mis-interpretation of data*
  - Aging dependent visual impairments
  - Hyperactivity → ability to perform task
  - Background strain effects

Historical Drug Discovery
Confounds in Preclinical Efficacy Studies

• **Locomotor Activity**
  - Hyperactivity confounds ability of mice to perform tasks
  - AD mouse models with hyperactivity relative to WT include: 5XFAD, 3XTg, Tg4510, Tg2576, APP/PS1, TgCRND8....

• **Vision & Hearing**
  - Several background strains of AD mouse models carry retinal degeneration alleles: FVB, C3H
  - Mice demonstrate age-dependent visual and hearing impairments

• **Competing Behaviors**
  - Stereotypy
  - Shock sensitivity
    - Hyperactive mice may not respond to shock which confounds ability to learn the task
    - If the animal has not learned the task, memory *cannot* be tested

Huynh et al 2009
Primary Screen: Behavioral Testing in Rodent Models

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Assay confounds often resulting in mis-interpretation of data

- Aging dependent visual impairments
- Hyperactivity –> ability to perform task
- Background strain effects

Lack of consideration for responses to stimuli that vary across sex/age/genotype (e.g. shock intensity)

High inter-subject variability and inconsistent responses across sex

"state versus trait" phenomena

Inconsistencies in expected aging dependent impairments and often in baseline responses in controls

Limited application of the ARRIVE guidelines and/or thorough validation of assay protocols/environment/technicians

Ignorance of single dose "efficacy" in the absence of dose response and/or supporting PK; lack of knowledge of therapeutic index

Commentary

Lost in translation: At the crossroads of face validity and translational utility of behavioral assays in animal models for the development of therapeutics


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Historical Drug Discovery

Poor Face & Predictive Validity
Enabling Reproducibility

• Pilot studies are exploratory studies aimed at testing a hypothesis
  • Data can be used to inform:
    • A go/nogo decision for an independent confirmatory study
    • A power analysis for an independent study designed to assess efficacy
  • It is inappropriate to add samples to a pilot study to achieve “statistical significance” and qualify it as an efficacy study

• Well-designed efficacy studies require rigor comparable to a clinical trial
  • *Apriori* inclusion and exclusion criteria
  • Pre-determined sample sizes based on power analyses
  • Blinding, randomization, counterbalancing, and appropriate controls
  • Multiple levels of QC under blinded conditions
  • Dose–response relationships that correlate with in vivo target engagement/pharmacodynamics
  • Both sexes at pathophysiologically relevant age (demonstration of disease biomarker/target)
Monitoring Staff Proficiency for Quality

Proficiency Metrics for Executing Behavioral Studies

Staff are required to reproduce a positive control under blinded conditions prior to being assigned to run experimental studies.
Recommendations from 2015 NIA AD Summit

**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 LOAD 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 translational biochemical and physiological endpoints (e.g. PET/MR) over behavioral measures using 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.

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**MODEL-AD Consortium**

- Model Organism Development and Evaluation for Late-onset Alzheimer’s Disease
- U54 AG054345 (IU/JAX), U54 AG054349 (UCI)

**Expand animal model resources for basic research and preclinical testing of candidate therapeutics with 50 new mouse models of AD.**
NIA and Trans-NIH translational pipeline for AD and ADRD

A Pipeline of NIA and Trans-NIH Translational Research Funding Initiatives (R21, R01, U01, R43/R44)

- Target ID Early Validation
- Assay Development
- Screening
- Proof of Concept
- Lead Optimization
- Candidate Selection
- IND-enabling toxicology
- Phase I
- Phase II
- Phase III
- Drug Approval

ADSP
AMP-AD Target Discovery
M²OVE-AD
Resilience-AD
NPS-AD

AD Translational Centers for New Medicines*
TREAT-AD

MODEL-AD
AlzPED

ACTC
ADCs
ADNI
AMP-AD Biomarkers
ABC-DS

ENABLING INFRASTRUCTURE FOR DATA DRIVEN AND PREDICTIVE THERAPY DEVELOPMENT

Adapted from NIA
MODEL-AD Infrastructure: Enabling the NIA and Trans-NIH Translational Pipeline for AD and ADRD

- Establish and implement guidelines for rigorous preclinical testing in LOAD models with the standards/rigor comparable to clinical trials
- Provide a resource for standardized therapeutic efficacy testing of preclinical drug candidates that prioritizes translational biochemical and physiological endpoints (e.g. PET/MR) over behavioral measures using 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
MODEL-AD Preclinical Testing Core (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 logistics 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: STOP-AD
    • 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
### Disease/Drug/Biomarker Optimization

Intersection of the disease, drug mechanism of action, and biomarker properties yields region (A-C) represents potential false negative (-) or positive (+) readouts. Region D provides the optimal measure of drugs action on a disease process.

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<thead>
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<tr>
<td>D</td>
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- The **Disease ∩ Drug ∩ Biomarkers** (D)
- An MOA relevant and translatable biomarker is available
- PET Biomarkers provide clinically relevant information on disease endpoints
- PET Biomarkers provide rapid clinical translation based on current clinical use
- Secondary confirmation via AutoRad ensures reliability of PET Biomarkers at higher resolution
- Tertiary confirmation via Immunopathology ensures target engagement independent of PET or AutoRad
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.
PTC: Building a Preclinical Testing Pipeline

ARRIVE: Highlights

Model Systems
- Construct validity of models
- Face validity of models

Animal Care
- Housing conditions (single vs. multiple)
- Husbandry (food, water, lighting, bedding)

Study Conduct
- Subject randomization/allocation
- Blinding of study personnel (techs, PI)
- Counter balancing for groups, sex, age
- Sample sizes yielding well powered studies (n=10-12 per sex per dose level per tracer)
- Inclusion/Exclusion criteria
Evaluation of Verubecestat in 5xFAD – In vivo PK

Age of dosing determined by results from disease staging in the DMP

PTC

Test compound QC and 7+day formulation stability

SHORT REPORT

Improving preclinical to clinical translation in Alzheimer’s disease research

Stacey J. Sukoff Rizzo¹ | Andi Masters² | Kristen D. Onos³ | Sara Quinney² | Michael Sasner³ | Adrian Oblak² | Bruce T. Lamb² | Paul R Territo² for the
MODEL-AD consortium¹,²,³,⁴

Critical need to test PK first and confirm
- Drug is the active and correct compound
- There is exposure in the target tissue (brain)
- Identify optimal dosing regimen
  - Based on Cmax, Tmax, T1/2, etc
**New Batch** Verubecestat PK Study – Oral Dosing

- 6 mo aged 5xFAD males and females (n=3-4 per dose level)

PK analysis revealed a short half-life (~2.4h) and effect of dose and formulation on absorption rate constant. Cmax:Cmin PK/PD modeling indicate administration required every 4-6 hrs to maintain exposure levels.
• Alternative to 4x Daily PO dosing – Verubecestat milled into Chow

• Quality Control
  • Ensure drug is the active and correct compound
  • Ensure concentrations as expected in chow

**Drug Milled into Diet (Commercial Vendor)**

- **Whole Pellets**: Crush with mortar and pestle to fine powder

- **Pellet Slices**: Using a clean razor blade divide pellet into 4 sections (A and D are the end pieces of the pellet, B and C are the middle pieces)

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<td>2b-middle slice</td>
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<table>
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<td>pellet 7</td>
<td>0.35</td>
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• In Vivo PK Protocol for Drug Milled into Chow
  • At least 1 week ad libitum (daily weights to evaluate taste aversion/adverse effects)
  • Within subject sampling- 1x with a 3 night inter-sample interval
  • Sample times: @ lights off (6pm), @ peak feeding (8pm), midnight, @ lights on (6am), midday (timed to PD)

- Significant differences observed for single vs. group housed, male vs. female, and time of day.
  • Concentrations were lower in males than females.
  • Inter-individual variability was greater in females than males.

- PK/PD modeling supported selection of appropriate dose range (10-100 mg/kg/day) for chow formulation to cover pellet variability and sex differences in exposure levels for long term PD studies in progress.
PTC: Building a Preclinical Testing Pipeline

PET/MRI/AutoRad as a PD biomarker of:

- Glucose Metabolism (18F-FDG)
- Tissue Perfusion (64Cu-PTSM)
- Beta Amyloid Deposition (18F-AV45)
- Tau (3R/4R) Deposition (18F-AV1451)
Verubecestat PD Study In Progress – Drug in Diet

• Target Engagement!
  • Verubecestat produced coat color changes beginning at approximately 4 weeks of age in treated mice in the current study.
  • Verubecestat is a small-molecule inhibitor of BACE1
  • Fur hypopigmentation was observed in rabbits and mice but not in monkeys (Kennedy et al. 2016)
  • PMEL (Pigment cell-specific melanocyte protein also known as PMEL17, Silver, SILV, gp100) is a transmembrane glycoprotein solely expressed in melanocytes and is involved in eumelanin synthesis.

Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice

Derya R. Shimshack1, Laure H. Jacobson2, Carine Kolly2, Natalae Zamorovici2, Kamal Kumar Belaverketsman2, Laurent Morewietz2, Robert Kreutz2, Juliane Schelle1, Mathias Jucker1, Barbara Bertels1, Dietlind Theil1, Annabelle Heier1, Karine Egoz1, Karen Delitz1, Rainer Mackensen1, Irena Brizak1, Ludovic Perrot1 & Ulf Neumann1

Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer’s Disease

Michael F. Egan, M.D., James Kost, Ph.D., Pierre N. Tariot, M.D., Paul S. Aisen, M.D., Jeffrey L. Cummings, M.D., Sc.D., Bruno Vellas, M.D., Ph.D., Cyrille Sur, Ph.D., Yuki Mukai, M.D., Tiffini Voss, M.D., Christine Furtak, B.S., Erin Mahoney, B.A., Lyn Harper Mozley, Ph.D., Rik Vandenberghe, M.D., Ph.D., Yi Mo, Ph.D., and David Michelson, M.D.

A change in hair color was observed in both the 12-mg group (2.5%) and the 40-mg group (0.0%) but not in the placebo group (Table 3).
Prophylactic treatment of VER in 6 mo aged 5xFAD mice

- VER (10-100 mg/kg, BID, PO) reduced amyloid deposition as measured at 6 months of age in male and female 5xFAD treated prophylactically for 3 months.
Quantitative analysis of 18F-AV45 PET/MRI uptake in male and female 5XFAD mice as a function of VER dose. Data are presented as means ± 1 SEM, and analyzed with a 2-way ANOVA, with sex and treatment as factors. As predicted, VER altered 18F-AV45 uptake in a dose and region dependent manner.
PTC: Building a Preclinical Testing Pipeline

- **4 Step QC Process for Behavior**
  - Confirm genotypes from terminal tail tips
  - Blinded groups (e.g. A, B, C, D, E) evaluated for *a priori* inclusion/exclusion criteria (e.g. technical issues – *not mathematical*)
  - *Individual responses* correlated with PK and PD (e.g. Aβ levels)
    - “Assigned” treatment group is not reflective of actual dose (via ad libitum drug/feeding paradigm); exposure relevant measures of PK and PD are required
    - Exclusion criteria: control subjects received drug erroneously
  - Data unblinded and interpreted with all raw data uploaded to Synapse
PTC: Building a Preclinical Testing Pipeline

Validation Treatment Strategy
- Dosing initiating before the onset of disease progression
- Validation in 5XFAD male and female mice chronic administration from 3-6 months of age
- Verubecestat (PO, In-food, 10, 30, 100 mg/kg)

Experimental Prophylactic Approach
- Testing of a clinical compound in 5XFAD male and female mice chronic administration from 3-6 months of age
- Levetiracetam (PO, BID, 0, 10, 30, 56 mg/kg)

Experimental Therapeutic Approach
- Testing of a clinical compound in 5XFAD male and female mice: chronic administration from peak pathological age to > 3 months
  - mAducanumab (IP, Frequency TBD)
- Post treatment analysis:
  - Transcriptomics, Proteomics, Metabolomics
  - Translational PK/PD
STOP-AD Web Portal

- Required Data
- *In Vitro* Data
- *In Vivo* Data
- Pharmacokinetics
- Pharmacodynamics
- Toxicology
- Clinical Data
MODEL-AD PTC Educational & Training Resources

• 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

Scholarships Available!

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