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

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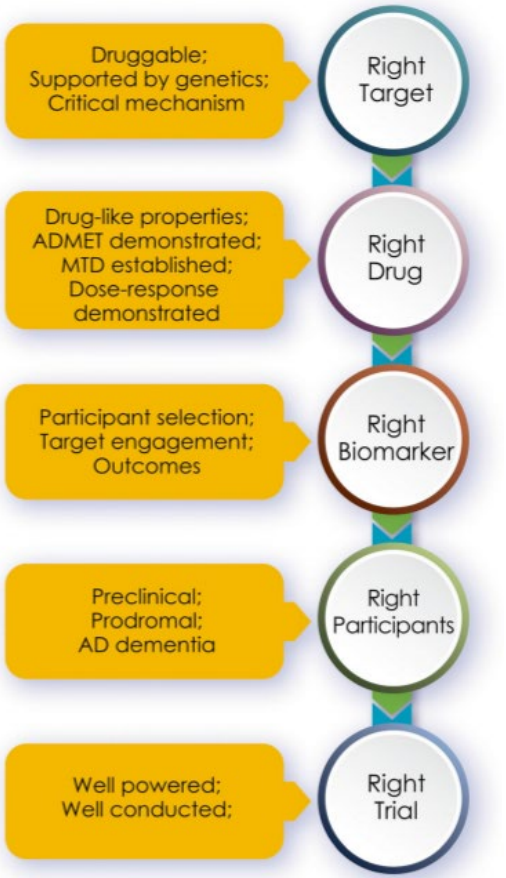
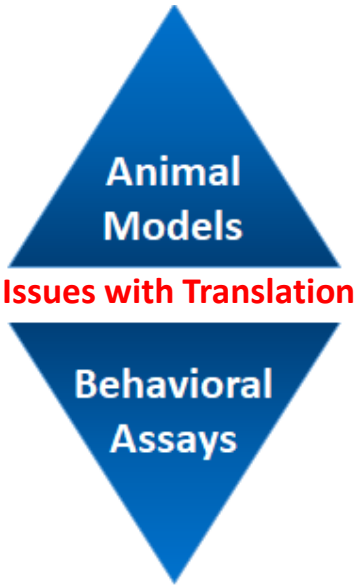
Why Should Clinicians Care About Preclinical Research?



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

Cummings et al. Alzheimer's Research & Therapy (2019) 11:76 https://doi.org/10.1186/s13195-019-0529-5

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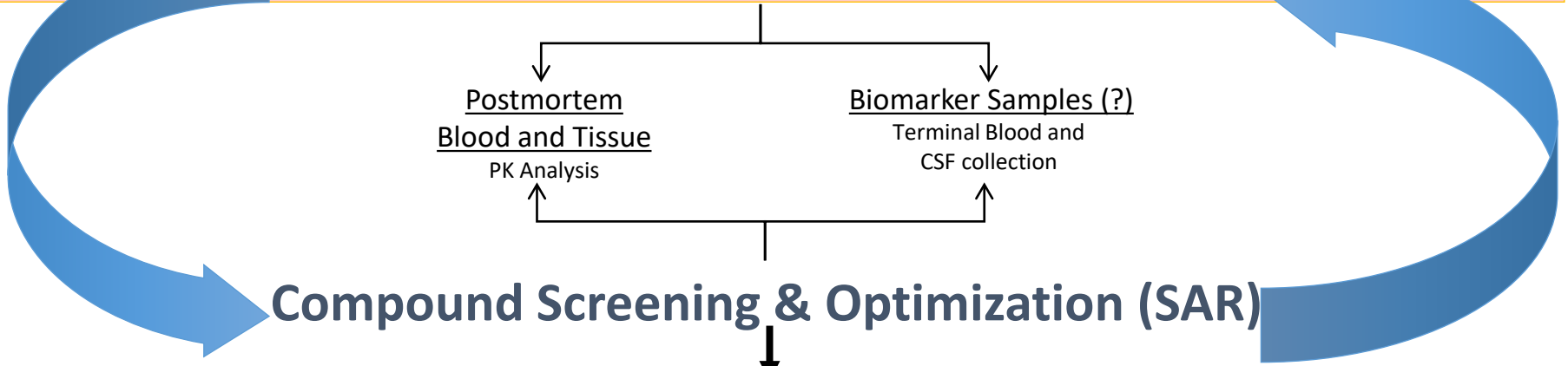
Historical Drug Discovery

Primary Screen: **Behavioral Testing in Rodent Models**

- Reversal of a scopolamine/MK801 induced cognitive deficit in normal *young adults* – *often males only*
- [illegible] "*cognitive deficit*" in *male* Tg mice **– *not correlating with pathology and*** *often only single d[illegible] PK*



PK/PD Modeling (Mouse <---> human)

Young adult male (WT) C57BL/6J male mice for safety/tolerability/toxicology

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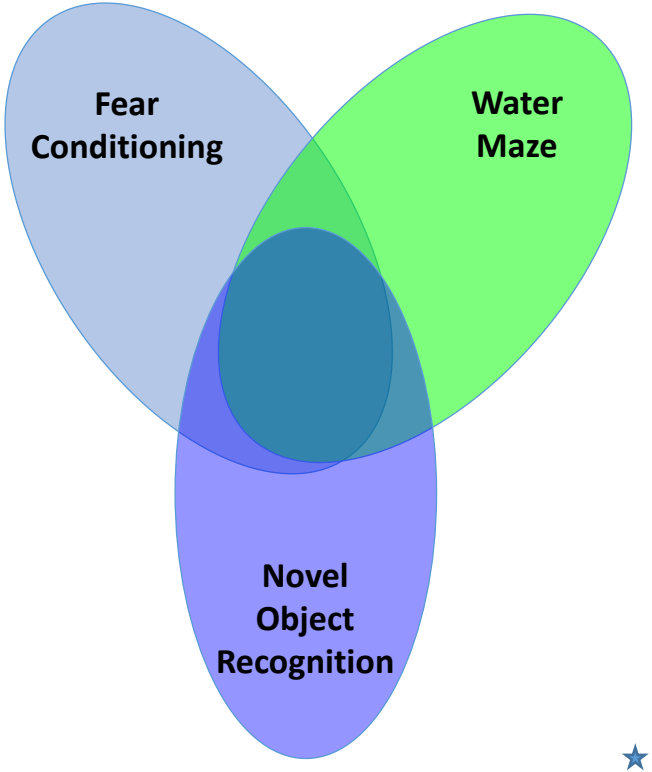
Historical Drug Discovery

Primary Screen: **Behavioral Testing in Rodent Models**

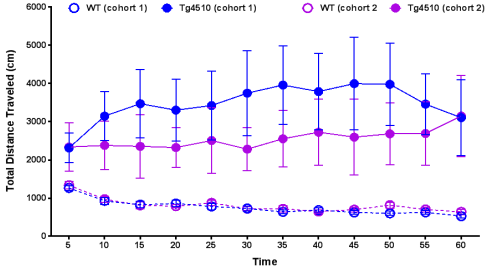
- Reversal of a scopolamine/MK801 induced cognitive deficit in normal *young adults* – *often males only*
- Reversal of a "*cognitive deficit*" in *male* Tg mice ***– not correlating with pathology and*** *often only single dose with no PK*

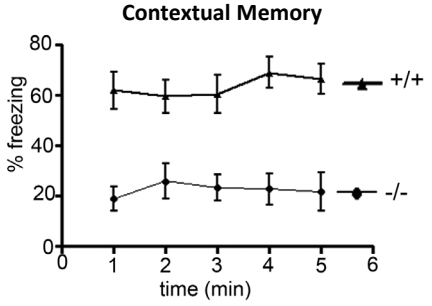
- Assay confounds often resulting in *mis-interpretation of data*
  - Aging dependent visual impairments
  - Hyperactivity –> ability to perform task
  - Background strain effects

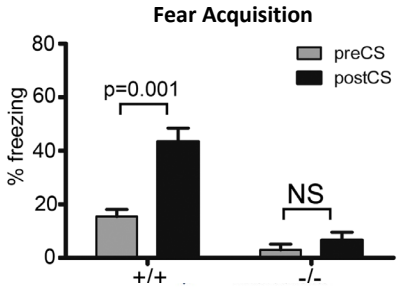


Confounds in Preclinical Efficacy Studies

- **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







Huyn et al 2009

Historical Drug Discovery

Primary Screen: **Behavioral Testing in Rodent Models**

- Reversal of a scopolamine/MK801 induced cognitive deficit in normal *young adults – often males only*
- Reversa… *… no PK*

- Assay confoun…
  - ➢ Aging dep…
  - ➢ Hyperacti…
  - ➢ Backgrou…

Neuroscience and Biobehavioral Reviews 116 (2020) 452–453

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

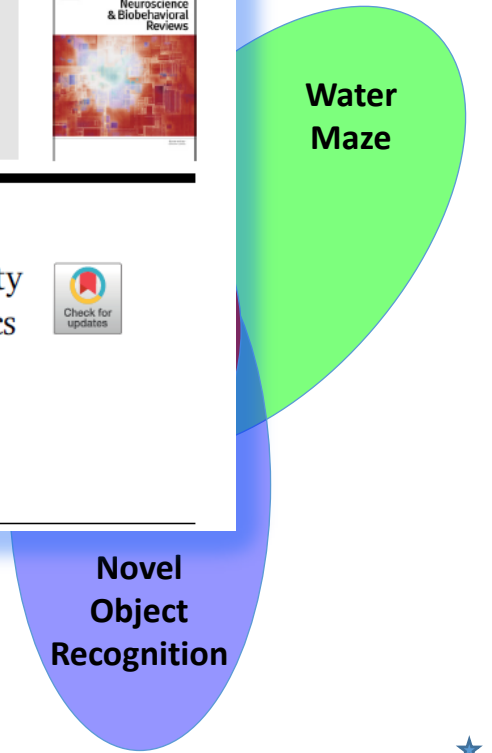
journal homepage: www.elsevier.com/locate/neubiorev

Commentary

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

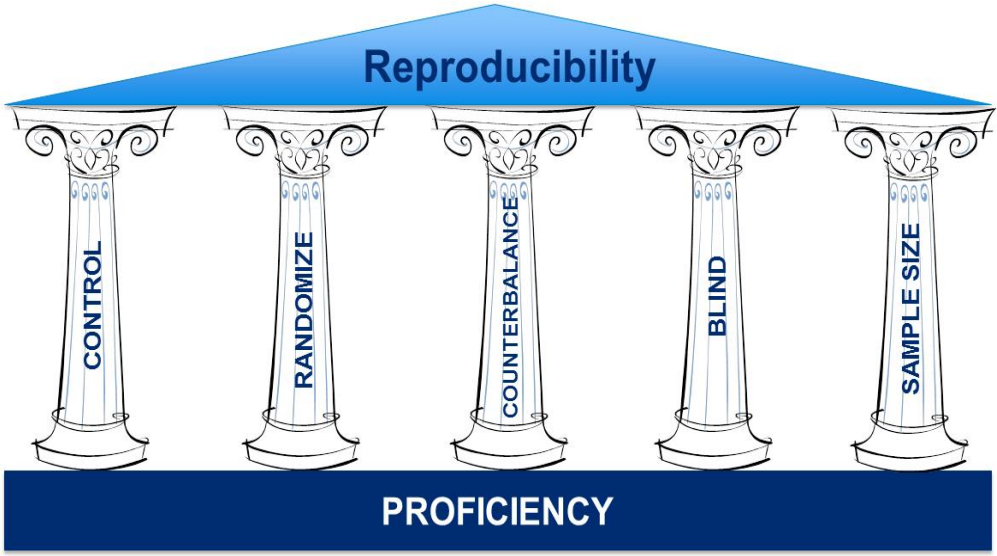
J.L. Silverman[a], J. Nithianantharajah[b], A. Der-Avakian[c], J.W. Young[c], S.J. Sukoff Rizzo[d,*]

[a] University of California, Davis, MIND Institute, School of Medicine, Department of Psychiatry and Behavioral Sciences, Sacramento, CA, USA
[b] The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia
[c] Department of Psychiatry, University of California San Diego, La Jolla, CA, USA
[d] University of Pittsburgh School of Medicine, Department of Medicine, Pittsburgh, PA, USA



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



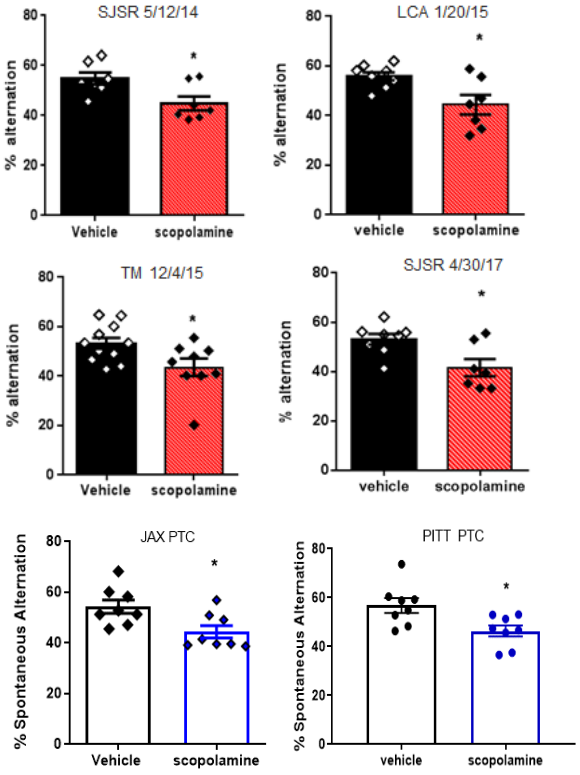
Sukoff Rizzo and Silverman 2016

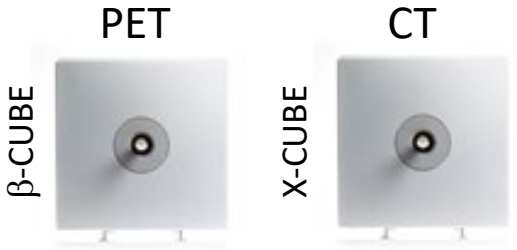
- **Well-designed efficacy studies require rigor comparable to a clinical trial**
  - *Apriori* inclusion and exclusion criteria
  - Pre-determined sample sizes based on power analyses
  - Blinding, randomization, counterbalancing, and appropriate controls
  - Multiple levels of QC under blinded conditions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

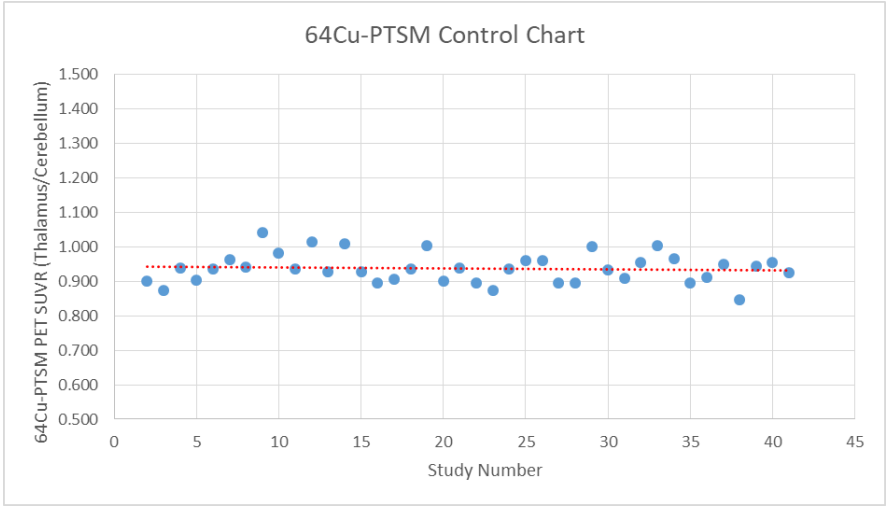
Monitoring Staff Proficiency for Quality

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







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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

NIH National Institute on Aging

NIA Funding Initiative

RFA AG16-04

MODEL-AD Consortium

Model **O**rganism **D**evelopment and **E**valuation for **L**ate-onset **A**lzheimer's **D**isease

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

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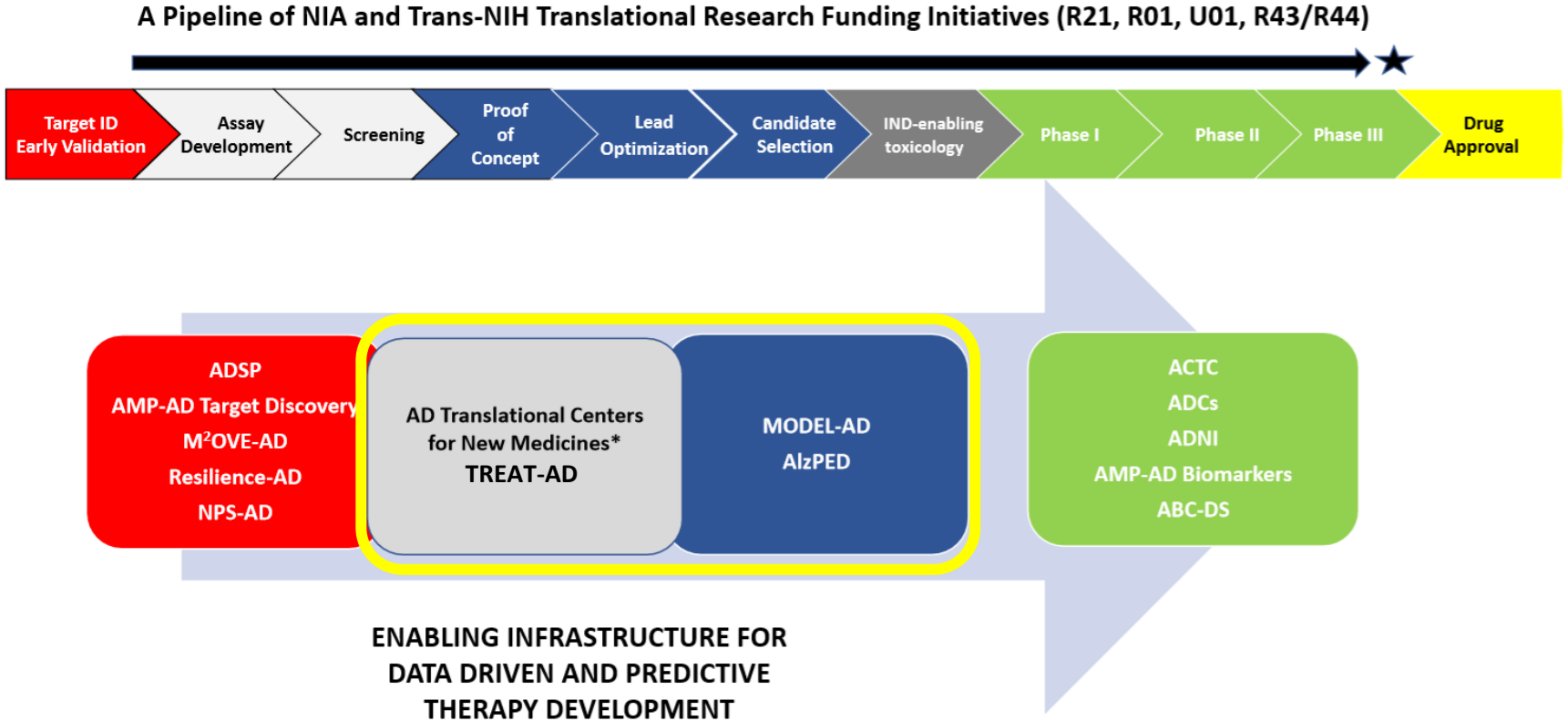
NIA and Trans-NIH translational pipeline for AD and ADRD



Adapted from NIA

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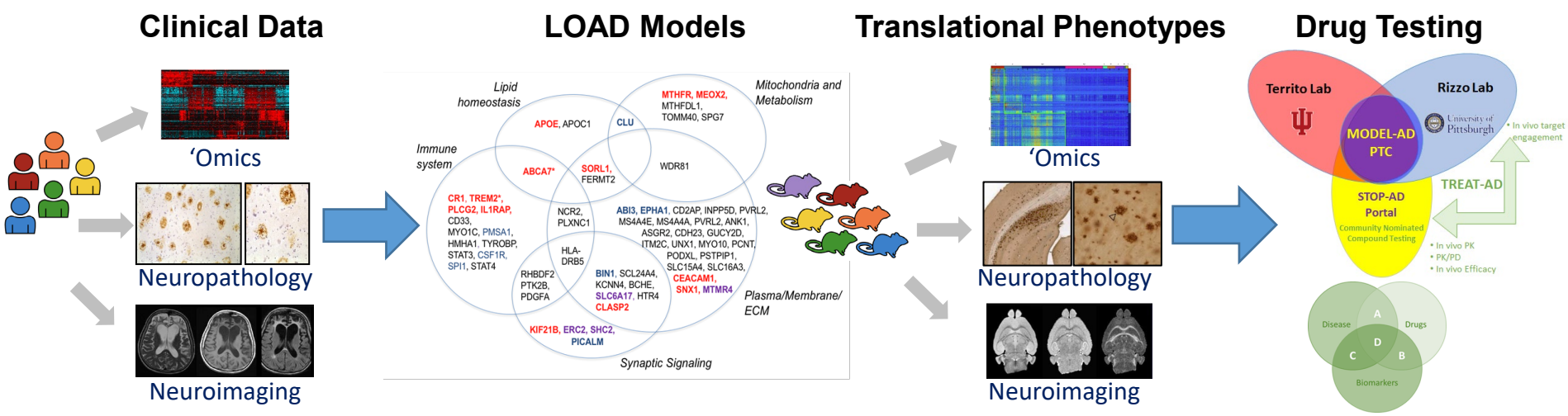
MODEL-AD Infrastructure:
Enabling the NIA and Trans-NIH Translational Pipeline for AD and ADRD



- ✓ 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

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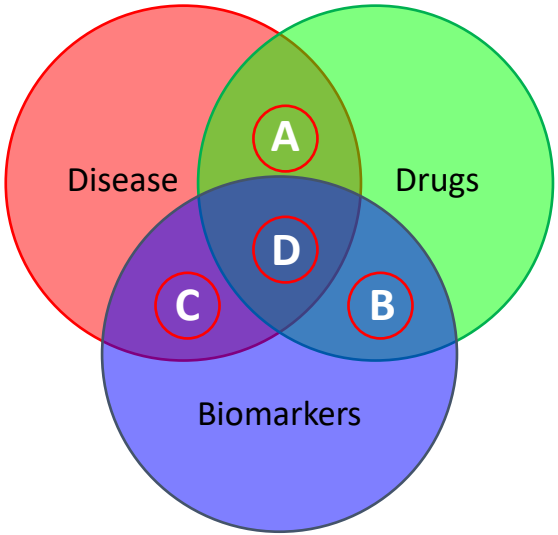
MODEL-AD Preclinical Testing Core (PTC) Aims and Milestones

- **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

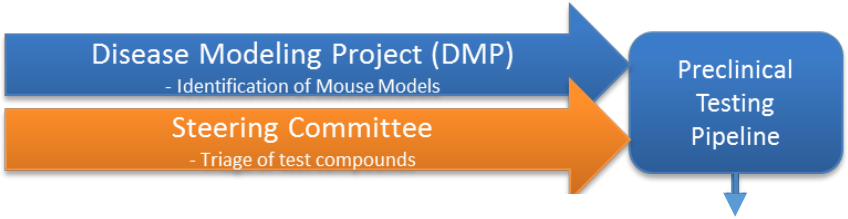
∩	Disease	Drugs
A	−	−
B	−	+
C	+	−
D	+	+



- 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

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.

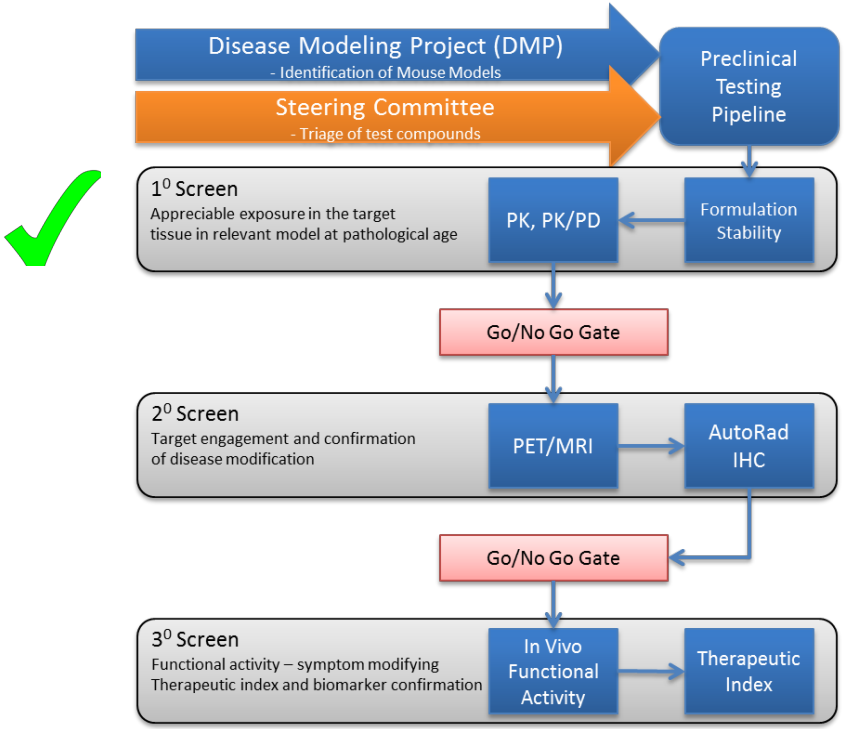
PTC: Building a Preclinical Testing Pipeline



- 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.

Mouse Model	Pathological Hallmark	Drug (Mechanism)	Primary Fluid Biomarker	Primary Biomarker #1	Primary Biomarker #2	Secondary Confirmation	Tertiary Confirmation
			CSF/plasma	PET/MRI	PET/MRI	AutoRad	IHC
5XFAD	Abeta	BACE Inhibitor (Verubecestat)	AB40 AB42	AV45	FDG	AV45 FDG	Abeta
hTau	Tau	Tau Inhibitor	pTau	AV1451	PTSM	AV4151 PTSM	Tau
IL1RAP	Neuro-Inflammation	Anti-Inflammatory	Cytokines	PTSM	FDG	PTSM FDG	IBA1 GFAP

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

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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

Received: 2 April 2020 | Accepted: 11 May 2020 | Published online: 12 June 2020

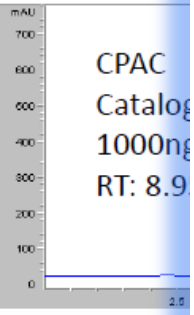
DOI: 10.1002/trc2.12038

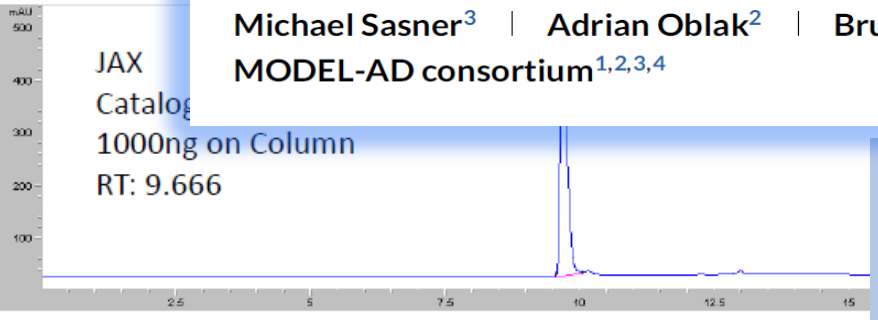
Alzheimer's & Dementia
Translational Research & Clinical Interventions

SHORT REPORT

Improving preclinical to clinical translation in Alzheimer's disease research

Stacey J. Sukoff Rizzo[1] | Andi Masters[2] | Kristen D. Onos[3] | Sara Quinney[2] | Michael Sasner[3] | Adrian Oblak[2] | Bruce T. Lamb[2] | Paul R Territo[2] | for the MODEL-AD consortium[1,2,3,4]





, Batch #01
mn

- **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, $T_{1/2}$, etc

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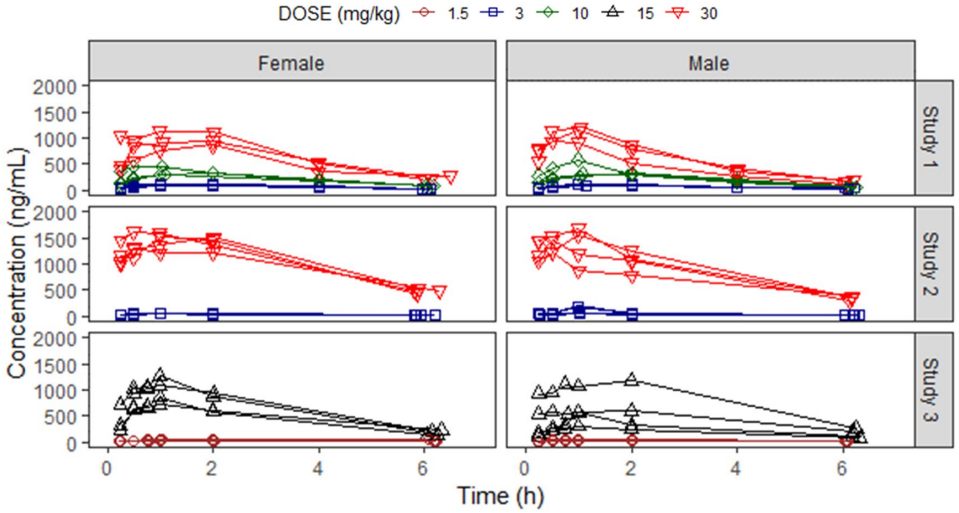
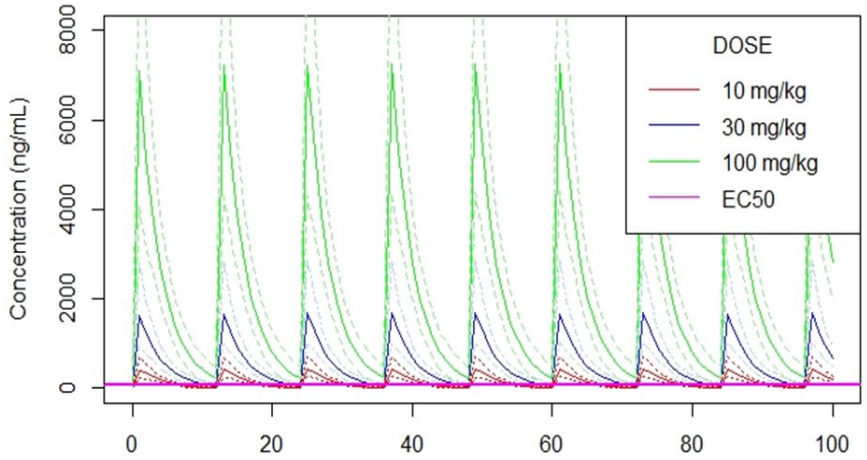
*New Batch** Verubecestat PK Study – Oral Dosing

- 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

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- **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)

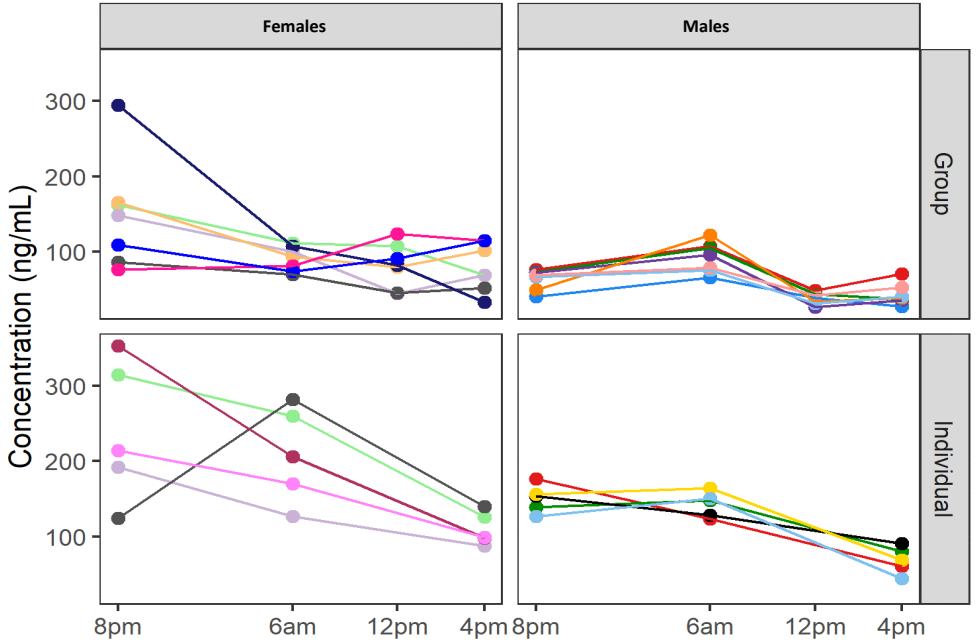
- **Whole Pellets:** Crush with mortar and pestle to fine powder

- **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)

	Observed ng on column	Mean	SD	CV
	Intra-pellet variability			
1a-end slice	0.80	0.81	0.07	8%
1b-middle slice	0.90			
1c-middle slice	0.74			
1d-end slice	0.80			
2a-end slice	0.45	0.63	0.23	36%
2b-middle slice	0.97			
2c-middle slice	0.57			
2d-end slice	0.54			
	Inter-pellet variability			
pellet 1	0.49	0.54	0.17	31%
pellet 2	0.68			
pellet 3	0.51			
pellet 4	0.46			
pellet 5	0.47			
pellet 6	0.40			
pellet 8	0.48			
pellet7	0.35			

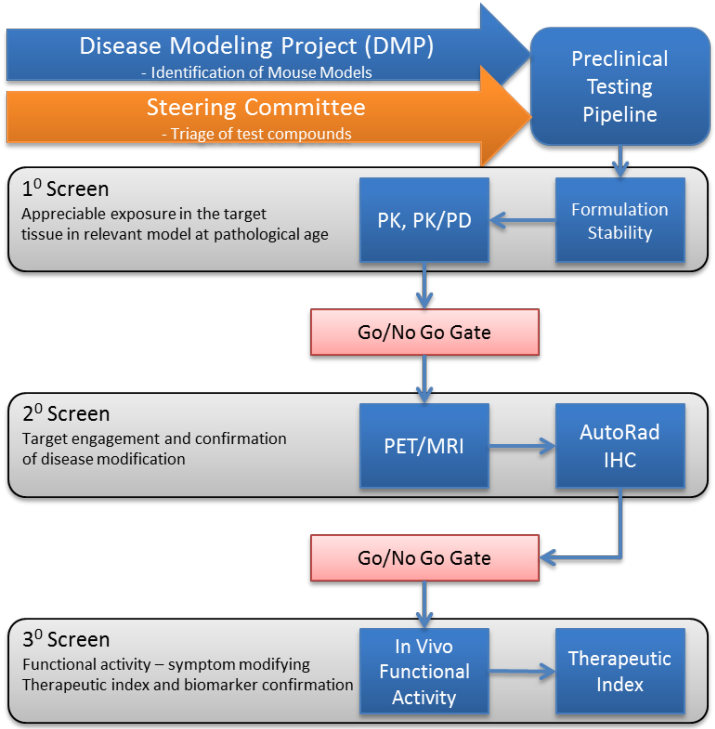
In Vivo PK Protocol for Drug Milled into Chow

- 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)



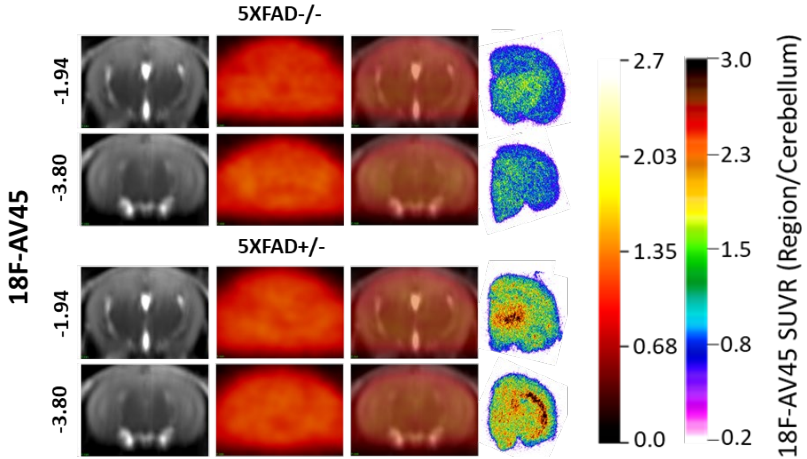
- Significant differences observed for single vs. group housed, male vs. female, and time of day.
  - Concentrations were lower in males than females.
  - Inter-individual variability was greater in females than males.
- PK/PD modeling supported selection of appropriate dose range (10-100 mg/kg/day) 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)



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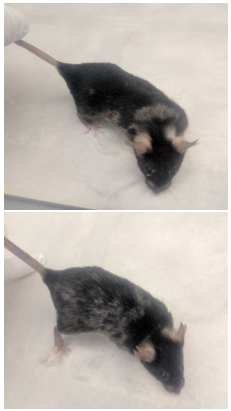
Verubecestat PD Study *In Progress* – Drug in Diet

- Target Engagement!
  - Verubescestat 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.



SCIENTIFIC REPORTS

OPEN

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

Received: 07 October 2015
Accepted: 02 February 2016
Published: 25 February 2016

Derya R. Shimshek[1], Laura H. Jacobson[1,†], Carine Kolly[2], Natasa Zamurovic[2], Kamal Kumar Balavenkatraman[2], Laurent Morawiec[2], Robert Kreutzer[2], Juliane Schelle[5], Mathias Jucker[6], Barbara Bertschi[2], Diethilde Theil[2], Annabelle Heier[2], Karine Bigot[2], Karen Beltz[3], Rainer Machauer[4], Irena Brzak[1], Ludovic Perrot[1] & Ulf Neumann[1]

NEJM April 11, 2019 ORIGINAL ARTICLE

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 Furtek, 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 (5.0%) but not in the placebo group (Table 3).

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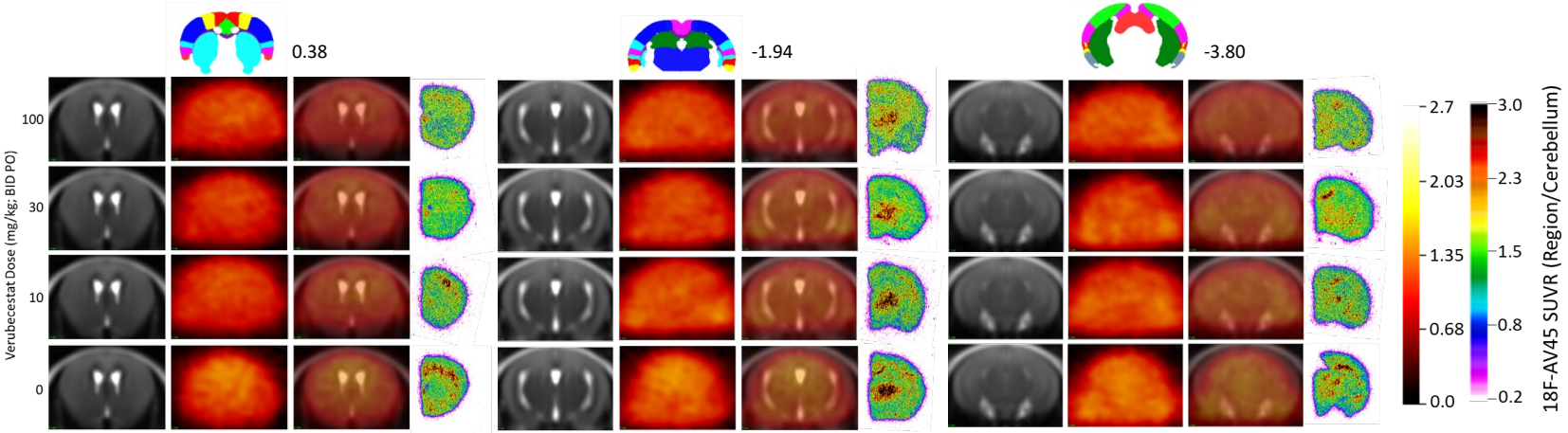
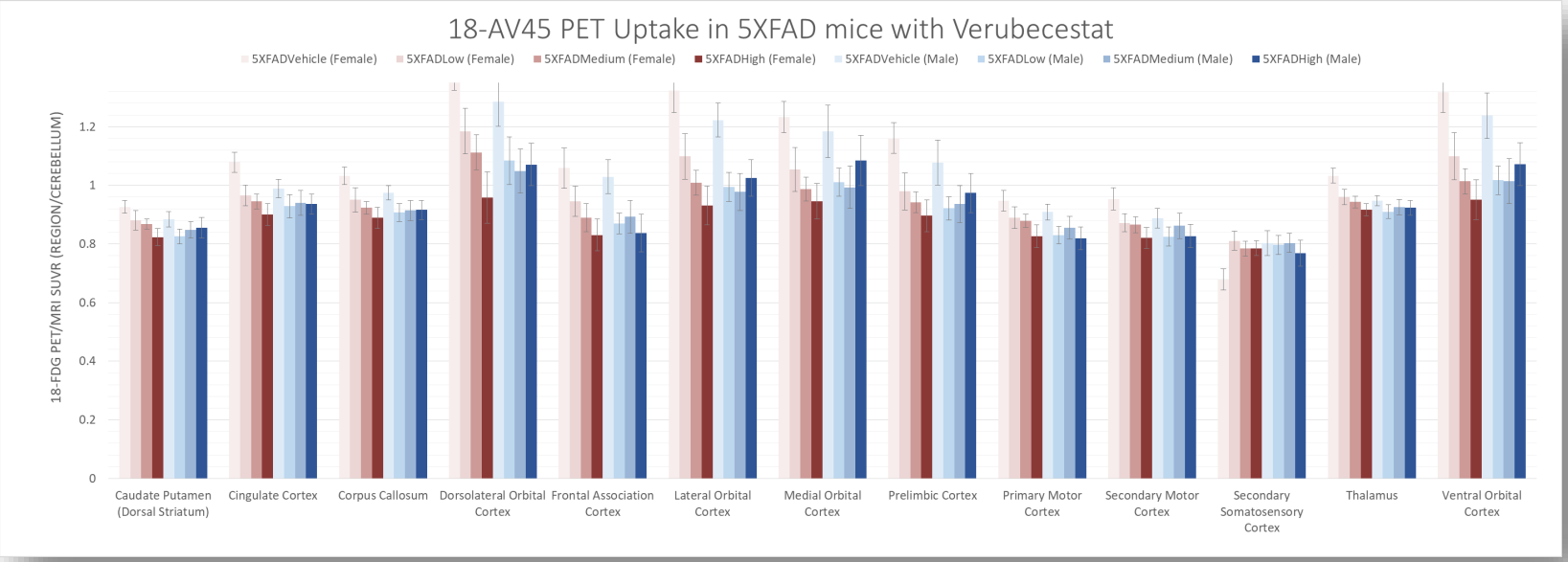
Prophylactic treatment of VER in 6 mo aged 5xFAD mice

- 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



Average MRI (left), PET (center-left), Fused (center-right), and Autoradiography (right) images at three bregma targets (0.38, -1.94, -3.80) as a function of chronic VER dosing (top to bottom). 18F-AV45 PET/MRI images represent an average of 5 randomly selected male and females, while Autoradiography images are representative males or females.

Verubecestat – 18F-AV45 PET/MRI Results



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 manor.

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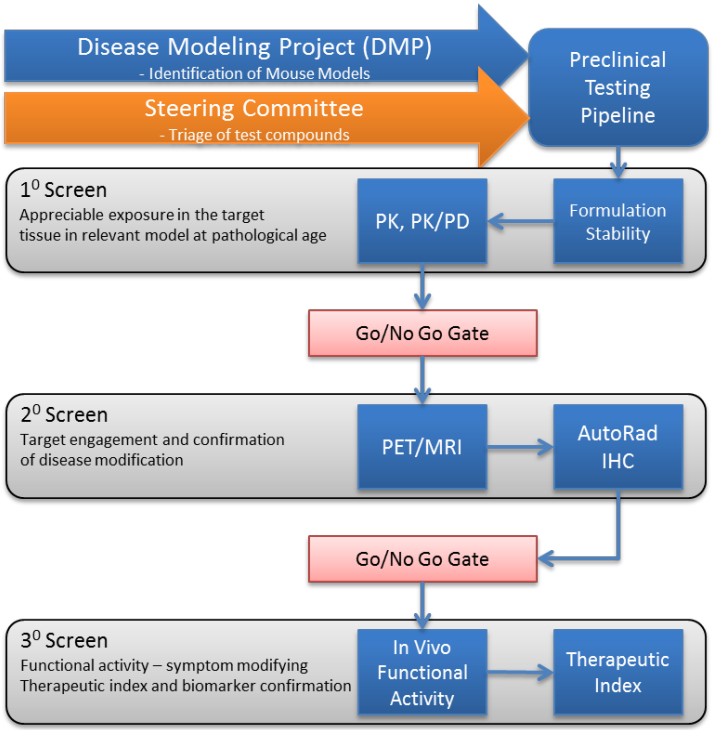
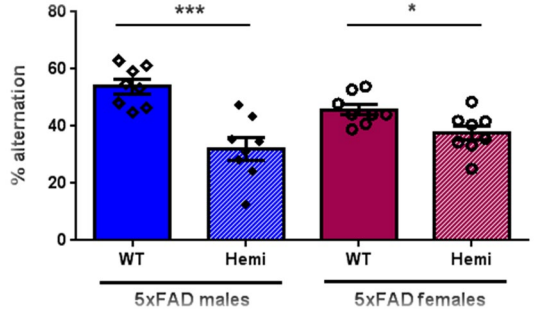
PTC: Building a Preclinical Testing Pipeline



- **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



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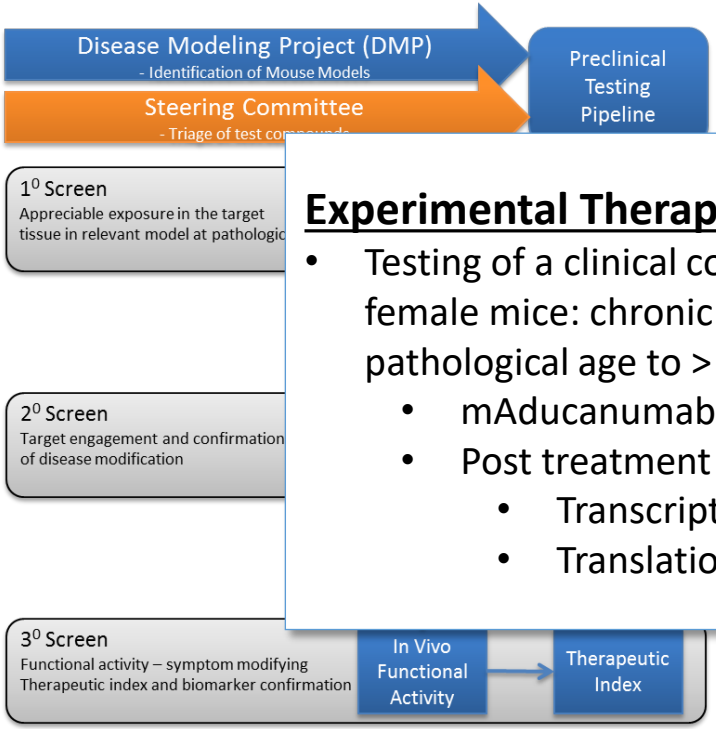

Experimental Therapeutic Approach

- 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

Validation Treatment Strategy

- Dosing initiating before the onset of disease
- female mice chronic
- hs of age
- od, 10, 30, 100 mg/kg)

Approach

- d in 5XFAD male and
- ration from 3-
- 6months of age
  - Levetiracetam (PO, BID, 0, 10, 30, 56 mg/kg)

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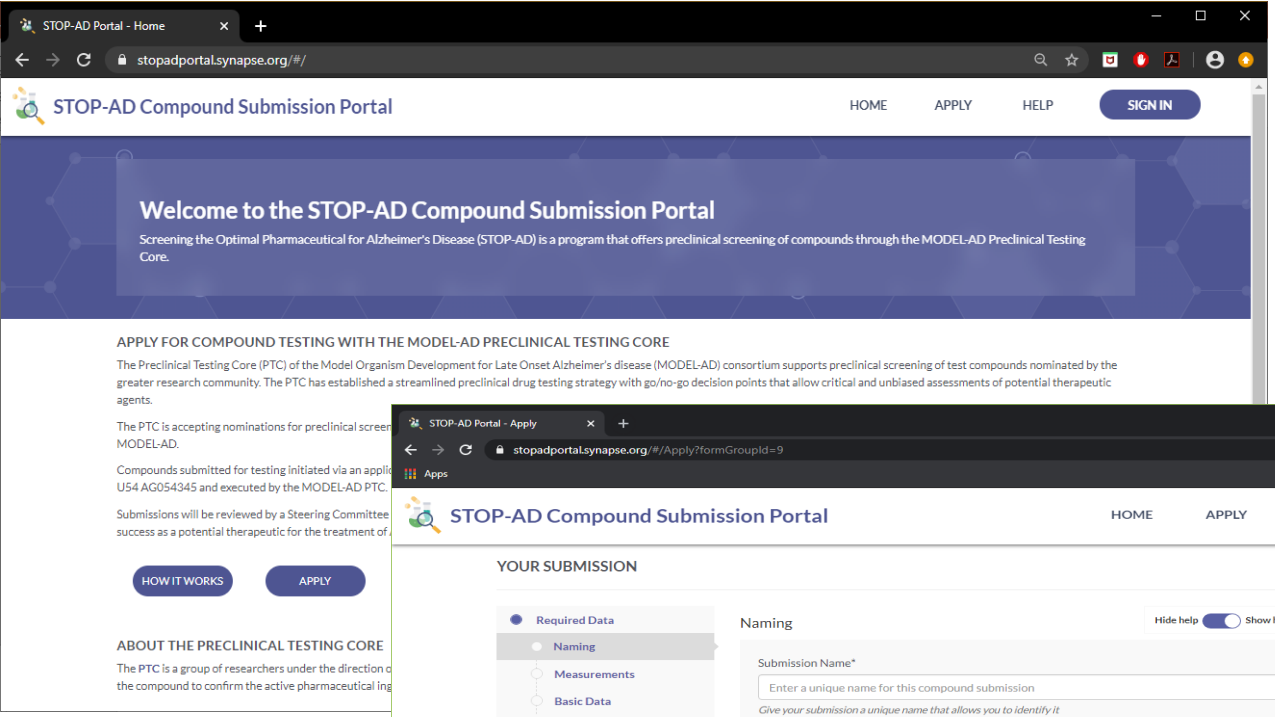

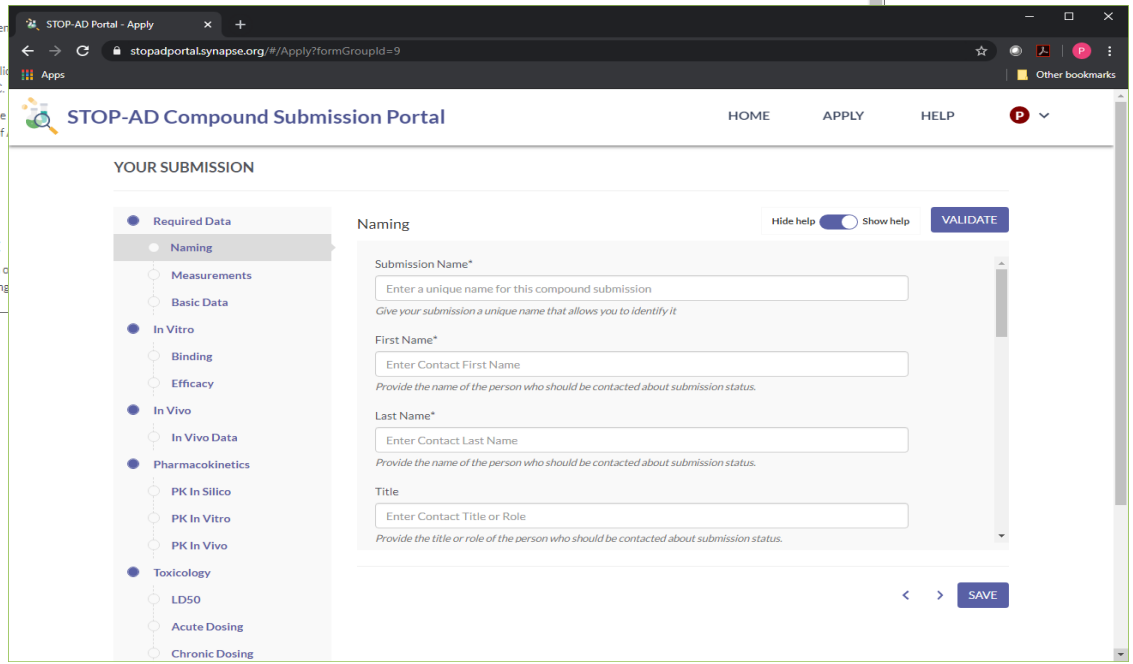

STOP-AD Web Portal

- Required Data
- *In Vitro* Data
- *In Vivo* Data
- Pharmacokinetics
- Pharmacodynamics
- Toxicology
- Clinical Data

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MODEL-AD PTC Educational & Training Resources

Scholarships Available!

- **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

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