“Fit-for-purpose” targeted mass spectrometry for quantification of chimeric Aducanumab

Skyline User Meeting, ASMS 2024

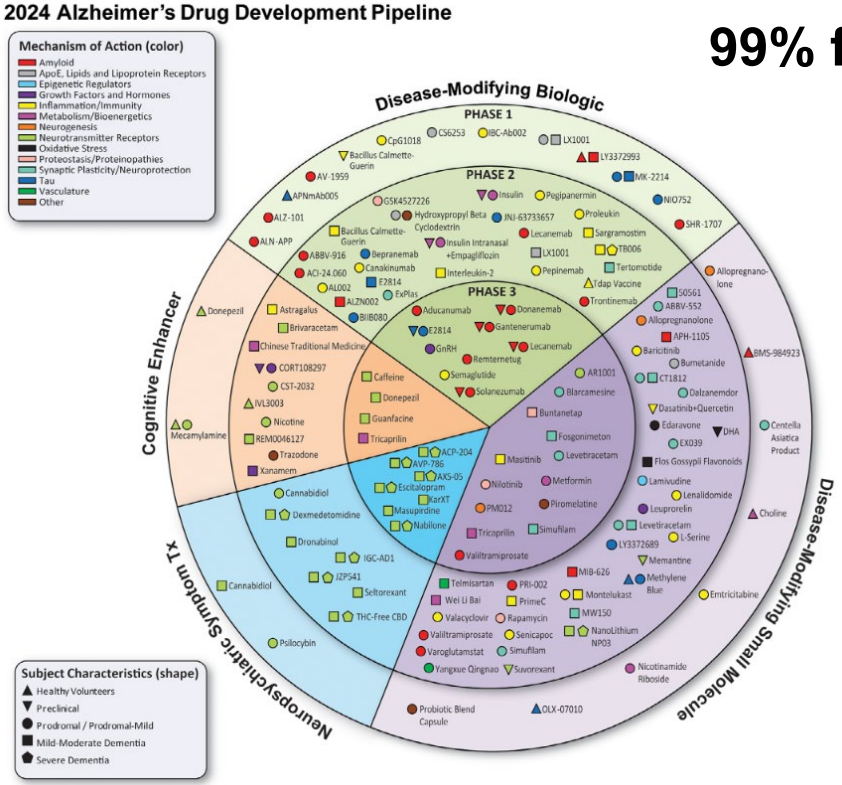
Emma Doud, Ph.D.

CENTER FOR PROTEOME ANALYSIS, INDIANA UNIVERSITY SCHOOL OF MEDICINE

1. Background
2. Study Design
3. Assay Design
4. PRM implementation

Alzheimer’s Disease and MODEL-AD

There are currently no treatments that stop or prevent Alzheimer's Disease



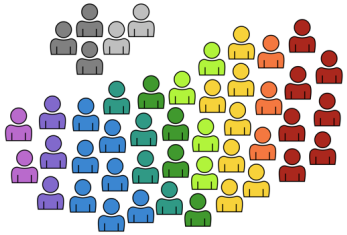
99% failure rate of clinical trials

- ~50 million patients worldwide
- By 2030: 82 million patients
- By 2050: 150 million patients
- Poorly efficacious symptom modifying drugs
- *Modest* efficacy of Aducanumab, and Lecanumab to attenuate disease progression
- Primary focus on Aβ and Tau modulating therapies

Cummings J, et. al, Alzheimers Dement (N Y). 2024 April, 10(2) https://doi.org/10.1002/trc2.12465

Model Organism Development and Evaluation for Late-onset Alzheimer's Disease

Early Onset
AD (< 5%)



Late Onset
AD (> 95%)

- Prioritize LOAD variants for animal modeling
- Create new mouse models with CRISPR (piloting rat models)
- High-capacity screening of all models, deep phenotyping of promising models
- Alignment of mouse and human phenotypes (neuropath, 'omics, imaging)
- Preclinical testing of the most promising models and therapeutics
- **Broad, unrestricted distribution of all data and models**

Existing
Models

New
Models

Bioinformatics and Data Management Core (BDMC)
The Jackson Lab Sage
U Pitt Bionetworks
Indiana U UC Irvine

Disease Modeling Project (DMP)
U Pitt The Jackson Lab
Indiana U UC Irvine

Preclinical Testing Core (PTC)
Indiana U The Jackson Lab
U Pitt Sage Bionetworks

MOD
F

Diseas

1⁰ Screen
Appreciable expos
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rting of *In Vivo* Experiments) guidelines are
search using animals – maximising
unnecessary studies.

lished in **PLOS Biology**, were developed
munity as part of an NC3Rs initiative to
search using animals.

g on the purple box above, or by **following**

ORIGINAL RESEARCH article
Front. Aging Neurosci., 23 July 2021 | https://doi.org/10.3389/fnagi.2021.713726

Check for updates

Comprehensive Evaluation of the 5XFAD Mouse Model for Preclinical Testing Applications: A MODEL-AD Study

 Adrian L. Oblak[1,2*],  Peter B. Lin[2], Kevin P. Kotredes[3], Ravi S. Pandey[3], Dylan Garceau[3] Harriet M. Williams[3] Asli Uyar[3] Rita O'Rourke[3] Sarah O'Rourke[3]

Received: 20 January 2022 | Revised: 6 April 2022 | Accepted: 1 June 2022 | Published online: 25 August 2022
DOI: 10.1002/trc2.12329
RESEARCH ARTICLE
Translational Research & Clinical Interventions

Pharmacokinetic, pharmacodynamic, and transcriptomic analysis of chronic levetiracetam treatment in 5XFAD mice: A MODEL-AD preclinical testing core study

Kristen D. Onos[1] | Sara K. Quinney[2] | David R. Jones[2] | Andrea R. Masters[2] | Ravi Pandey[1] | Kelly J. Keezer[1] | Carla Biesdorf[2] | Ingrid F. Metzger[2] | Jill A. Meyers[2] | Johnathon Peters[2] | Scott C. Persohn[2] | Brian P. McCarthy[2] | Amanda A. Bedwell[2] | Lucas L. Figueiredo[2] | Zackary A. Cope[3] | Michael Sasner[1] | Gareth R. Howell[1] | Harriet M. Williams[1] | Adrian L. Oblak[2] | Bruce T. Lamb[2] | Gregory W. Carter[1] | Stacey J. Sukoff Rizzo[3] | Paul R. Territo[2]

alzheimer's association | Alzheimer's & Dementia Translational Research & Clinical Interventions

RESEARCH ARTICLE | Open Access

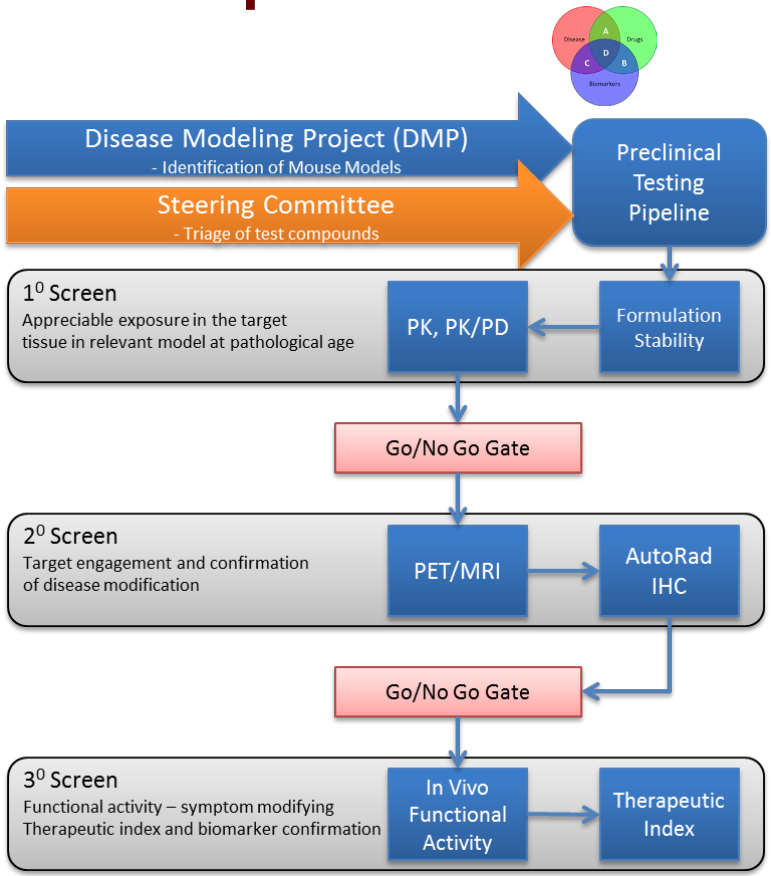
Prophylactic evaluation of verubecestat on disease- and symptom-modifying effects in 5XFAD mice

Adrian L. Oblak, Zackary A. Cope, Sara K. Quinney, Ravi S. Pandey, Carla Biesdorf, Andi R. Masters, Kristen D. Onos, Leslie Haynes, Kelly J. Keezer, Jill A. Meyer, Jonathan S. Peters, Scott A. Persohn, Amanda A. Bedwell, Kierra Eldridge, Rachael Speedy, Gabriela Little, Sean-Paul Williams, Brenda Noarbe, Andre Obenaus, Michael Sasner, Gareth R. Howell, Gregory W. Carter, Harriet Williams, Bruce T. Lamb, Paul R. Territo, Stacey J. Sukoff Rizzo ... See fewer authors

First published: 14 July 2022 | https://doi.org/10.1002/trc2.12317 | Citations: 1

Chimeric aducanumab study design

Validating the pipeline with a biotherapeutic



Prophylactic Treatment 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
  - Verubecestat (PO, In-food, 0, 10, 30, 100 mg/kg)
  - Levetiracetam (PO, BID, 0, 10, 30, 56 mg/kg)

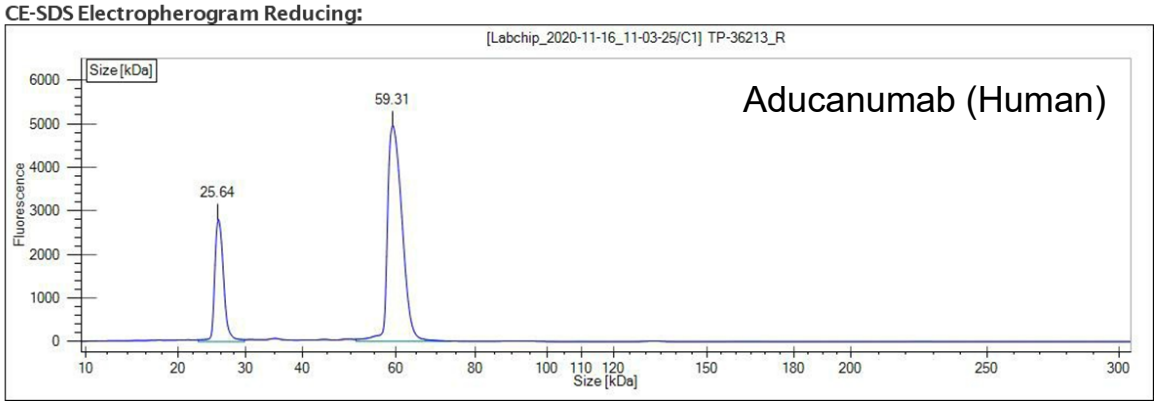
Therapeutic Treatment Strategy

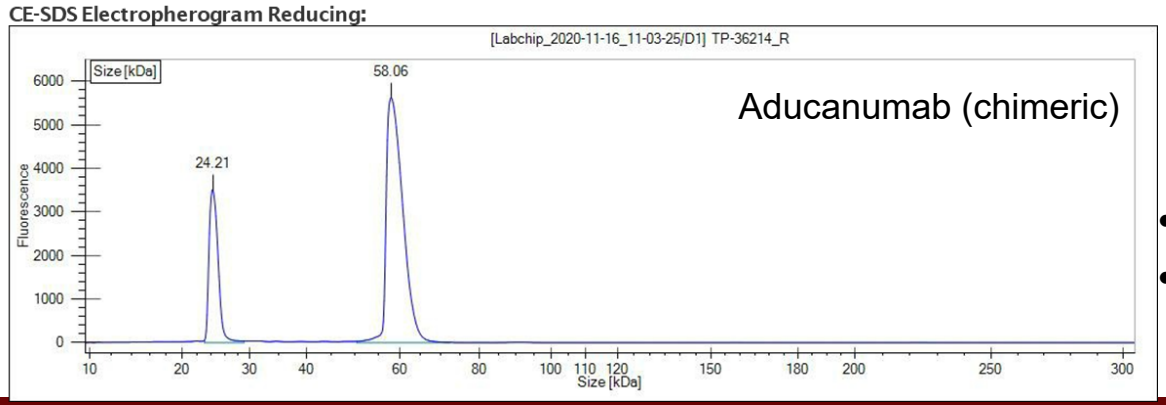
- Dosing initiating AFTER the onset of disease progression
- 5XFAD male and female mice chronic administration from 9 months of age through 12 months of age
  - chAducanumab (SC, Q1W, 0, 1, 3, 10 mg/kg)

Synthesis and Qualification of chAducanumab

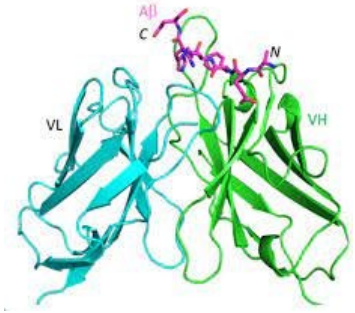
Protein A Affinity Chromatography

Murinized Aducanumab (human variable, mouse IgG2a constant)





LakePharma
The Biologics Company



- Freeze-thaw stability
- 2 batches, SEC and activity testing

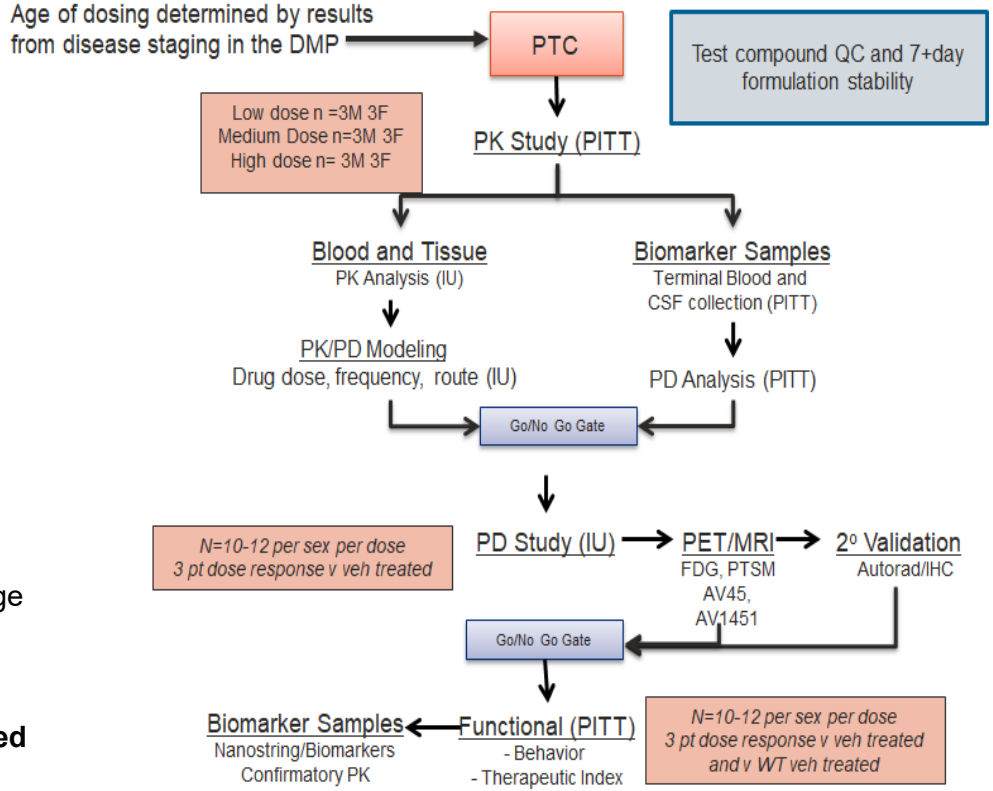
chAducanumab MODEL-AD study

28-day pilot PK study

Male and female 5XFAD and WT mice @ 7+ months of age; determine age of significant Aβ plaque deposition. PK/PD modeling to determine chronic PD dose regimen **1) 2 dose levels chAducanumab and IgG2aκ isotypye control 1x weekly i.p. dosing 2) Single i.p. dose with weekly blood collections**

Chronic PD study (12+ week dosing)

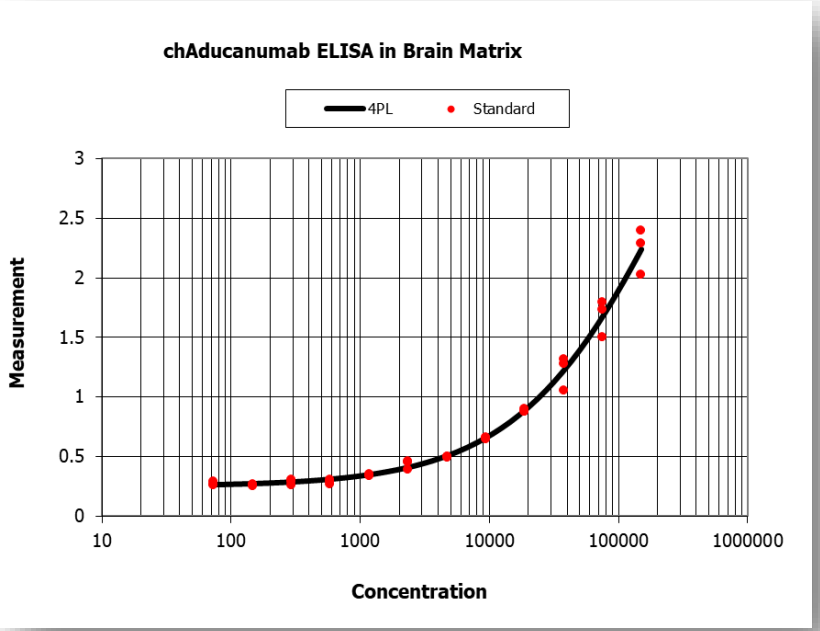
Male and female 5XFAD mice @ 9+ months of age; determine age of significant Aβ plaque deposition required for target engagementWeekly i.p. dosing for 12+ weeks: **1) 3 dose levels chAducanumab in 5XFAD 2) IgG2aκ isotype control treated 5XFAD control 3)Saline treated 5XFAD control 4)Saline treated WT control (negative control for AB ELISA, behavior, omics)**



ELISA for PK/PD of chimeric Aducanumab

Outsourced the development of an ELISA with Jax Assay Services

- Limited dynamic range (2.5 orders of magnitude)
- Interference with endogenous amyloid in the tissues and blood which is genotype specific
- Within and Between genotype variability (i.e. endogenous level of amyloid)



LakePharma Sample Name	Client Sample Name	EC50 (nM)
TP-36213	Human Aducanumab	0.11
TP-36214	Chimeric Aducanumab	0.07
Negative Control	Mouse IgG2aκ Isotype	N/A

What does “fit-for-purpose” mean to MODEL-AD?

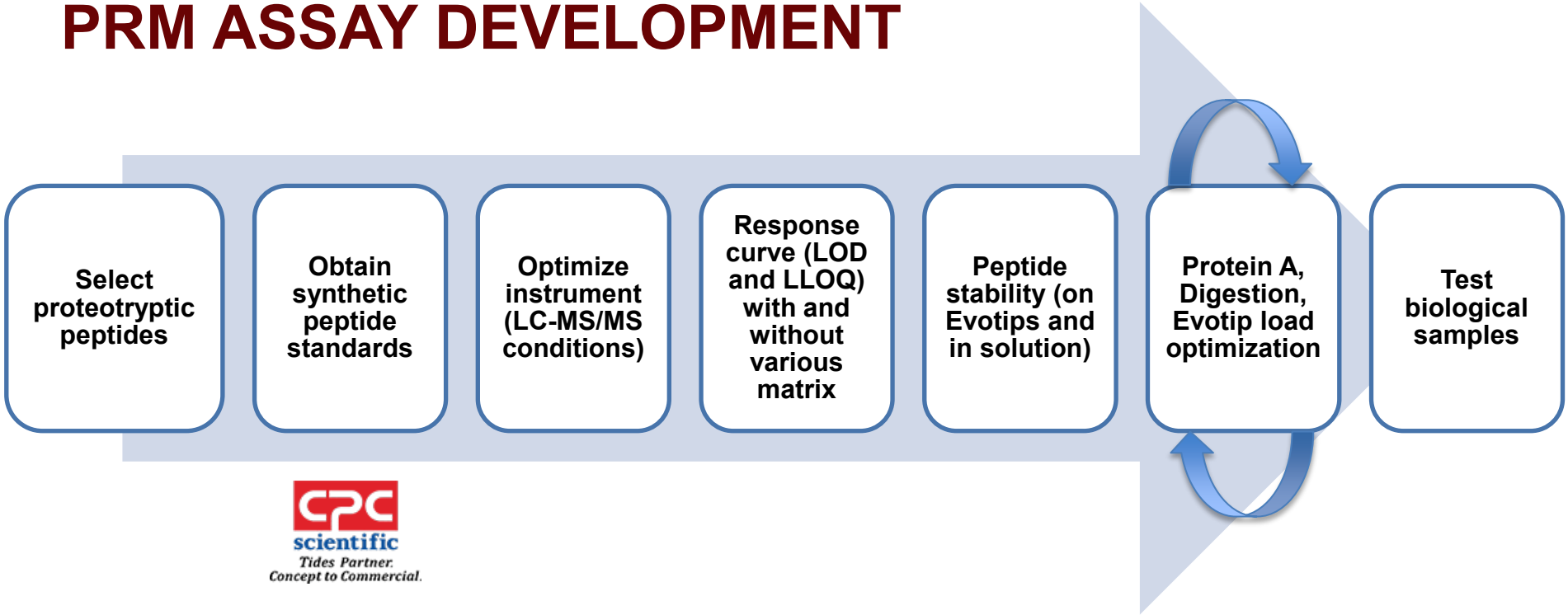
- **Selectively and sensitively** measure chAducanumab concentrations in mouse cortex and plasma

- Adult 5XFAD mice have high levels of Aβ plaques which interfere with ELISA

- Mice treated with IgG controls seem to have phenotypic changes – can we confirm that there were no dosing swaps?

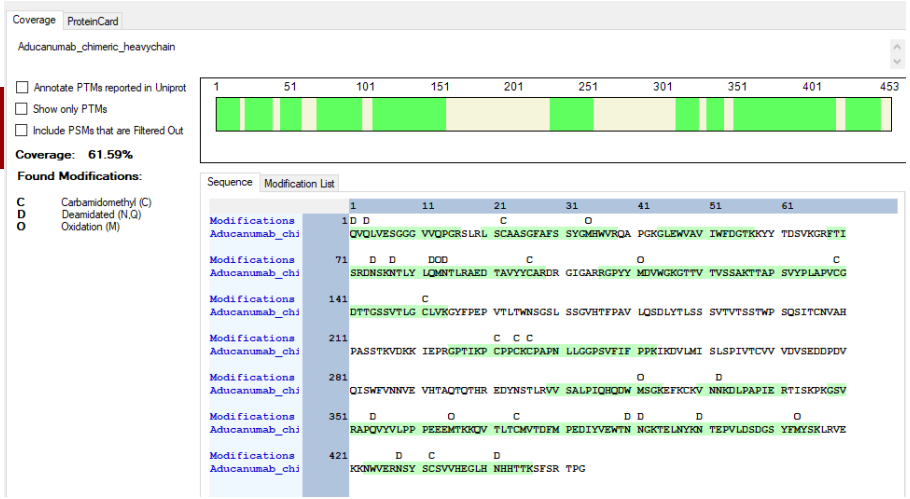
Assay Design

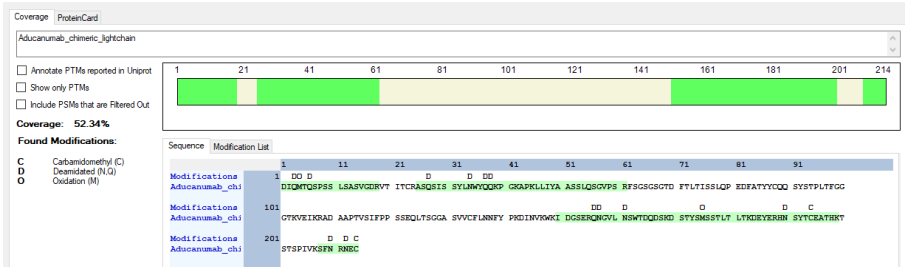
PRM ASSAY DEVELOPMENT



CPC scientific
Tides Partner. Concept to Commercial.

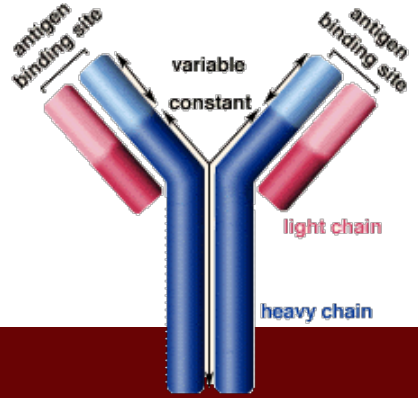
Peptide selection





Accession	Description	Coverage [%]	# Peptides	# PSMs
Aducanumab_chimeric_heavychain	Aducanumab_chimeric_heavychain	62%	24	670
Aducanumab_chimeric_lightchain	Aducanumab_chimeric_lightchain	52%	12	301

- DDA experiment of chAducanumab in mouse plasma, Proteome Discoverer search
- Extensive literature search to determine ‘unique’ peptides
- Humanized variable region and mouse IgG2a constant
- Hedge bets – choose 4 to test



Initial development

Light chain

Light1: -.DIQMTQSPSSLSASVGDR.V [1, 18]

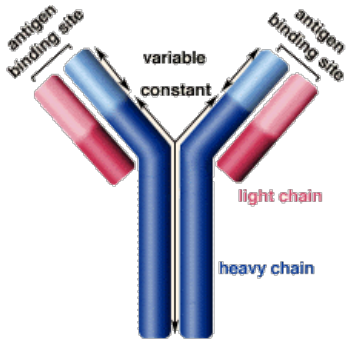
Light2: K.LLIYAASSLQSGVPSR.F [46, 61]

Heavy chain

~~***Heavy1:*** R.LSCAASGFAFSSYGMHWVR.Q [20, 38]~~

Heavy2: R.AEDTAVYYCAR.D [88, 98]

- Heavy1 peptide does not have linear response
- Proceed with 3 peptides (4 including unoxidized DIQ)
- Optimize basic MS settings (precursor charge state, HCD energy, resolution/isolation settings)



Experimental design concerns

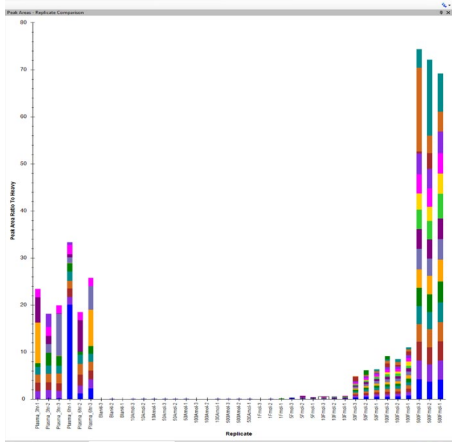
1. Extraction and digestion - need to be consistent in cortex and plasma, 8 M urea utilized

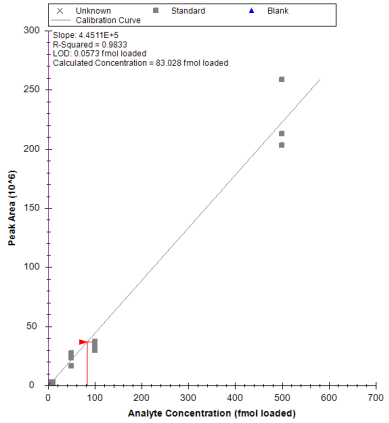
2. Modified peptides –
   a) optimized alkylation procedure until >98% AED peptide alkylated consistently
   b) strong oxidation needed to completely oxidize DIQ peptide, impacts stability of other peptides, monitor both forms of DIQ

3. Throughput – move from EasyNano LC to Evosep 100 SPD for consistency; optimize loading and spike in of SIL on Evotips

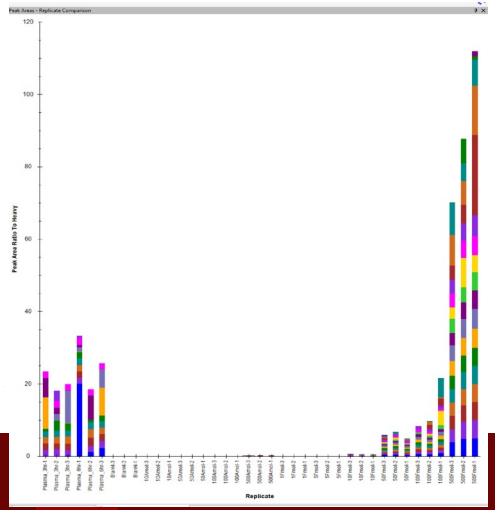
ChAducanumab in cortex homogenate and plasma

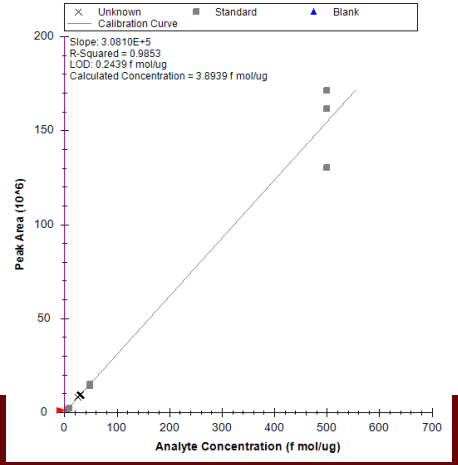
R.AEDTAVYYCAR.D [88, 98]





LOD = 0.06 fmol/µg in cortex





LOD = 0.3 fmol/µg in plasma

But will this LOD/LLOQ work?

4 cortex samples crushed with Cryoprep, resuspended in 8 M Urea, bioruptor, centrifuged, Bradford Assay (about 8-9mg extracted protein)
30 µg digested and 2 µg estimated onto an Evotip Pure

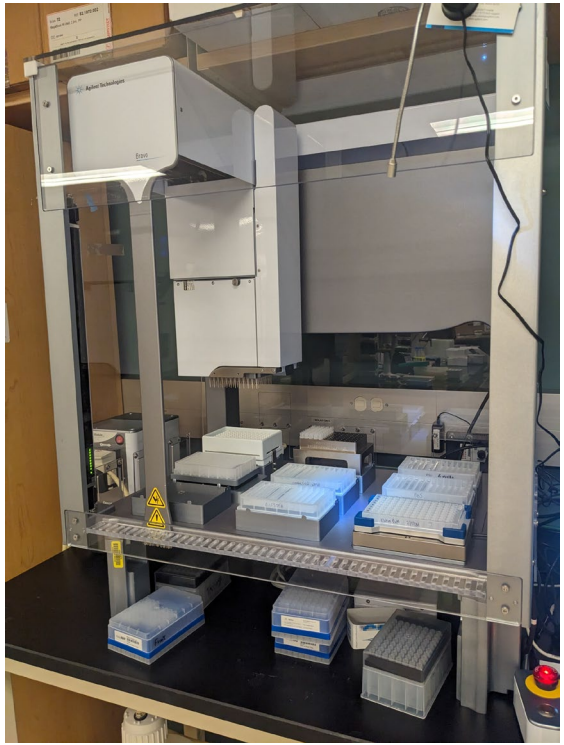
PPC ID	Sex	5xFAD Genotype (transgenic or wildtype)	Dose Concentration (mg/kg)	Drug	Total protein extracted (mg)	Aducanumab PRM assay	
3	M	Tg	1	Aducanumab	9.06	NQ	
4	M	Tg	30	Aducanumab	8.28	NQ	
24	F	Tg	30	Aducanumab	9.42	NQ	
25	F	Tg	30	Aducanumab	8.325	1.5625	fmol peptide/ µg protein

Only one sample with quantifiable chAducanuab….

Add an enrichment step – Protein A on newly acquired Assaymap Bravo

- Change extraction buffer: tested RIPA and HEPES/NaCl/NP-40
- Wash and elution buffers tested
- Automate all steps through Evotip loading

I don’t recommend learning how to use a liquid handler while optimizing a new assay…



Protein A setup

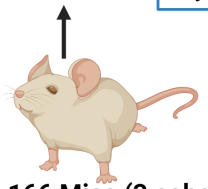
Purchased with funding from IU Simon Comprehensive Cancer Center

More than a few moments later…

- Be confident in your system suitability
- Use syringe washing step on Assaymap
- Run Beta ‘high organic blank’ method between samples to ensure no carryover on Evosep

~166 samples

Cortex



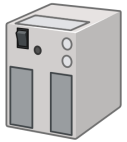
166 Mice (3 cohorts)
5X FAD or WT
Male or Female
Aducanumab or IgG

Plasma
2-9 time points/animal

~1000 samples

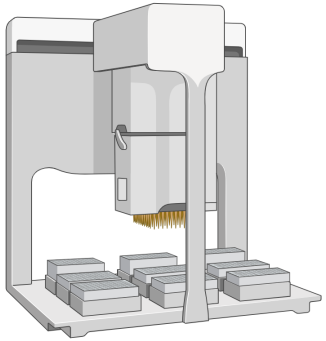
Protein Homogenization and Extraction

Cryo-prep

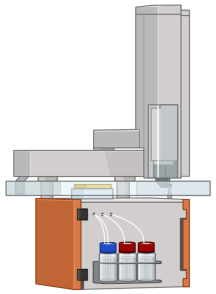


Bioruptor

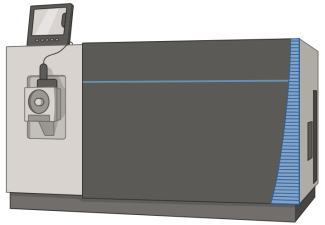
Centrifuge



Assaymap Bravo
1. Protein A enrichment
2. Trypsin Digest
3. Evotip Load



Evosep One
100 SPD



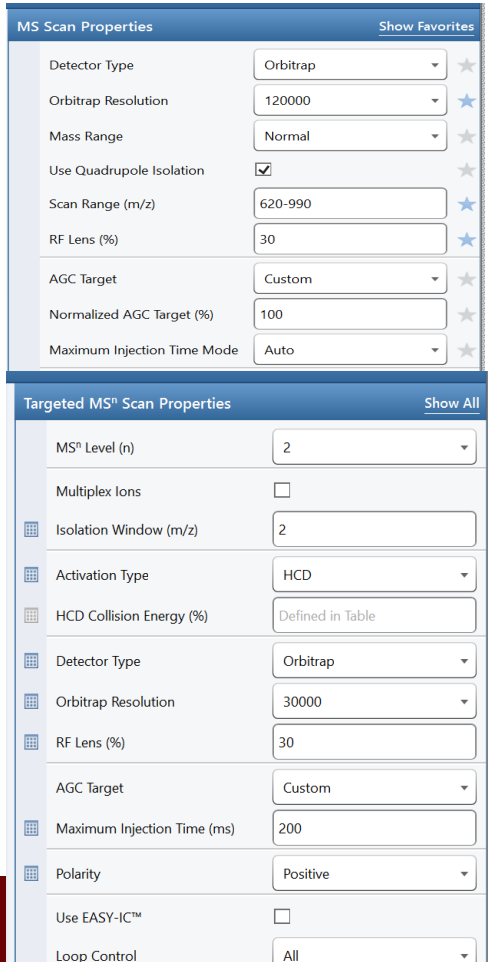
Orbitrap Fusion Lumos
Parallel Reaction Monitoring

Targeted Quantitation
Skyline

LC-MS/MS summary (Evosep 100 SPD-Lumos)

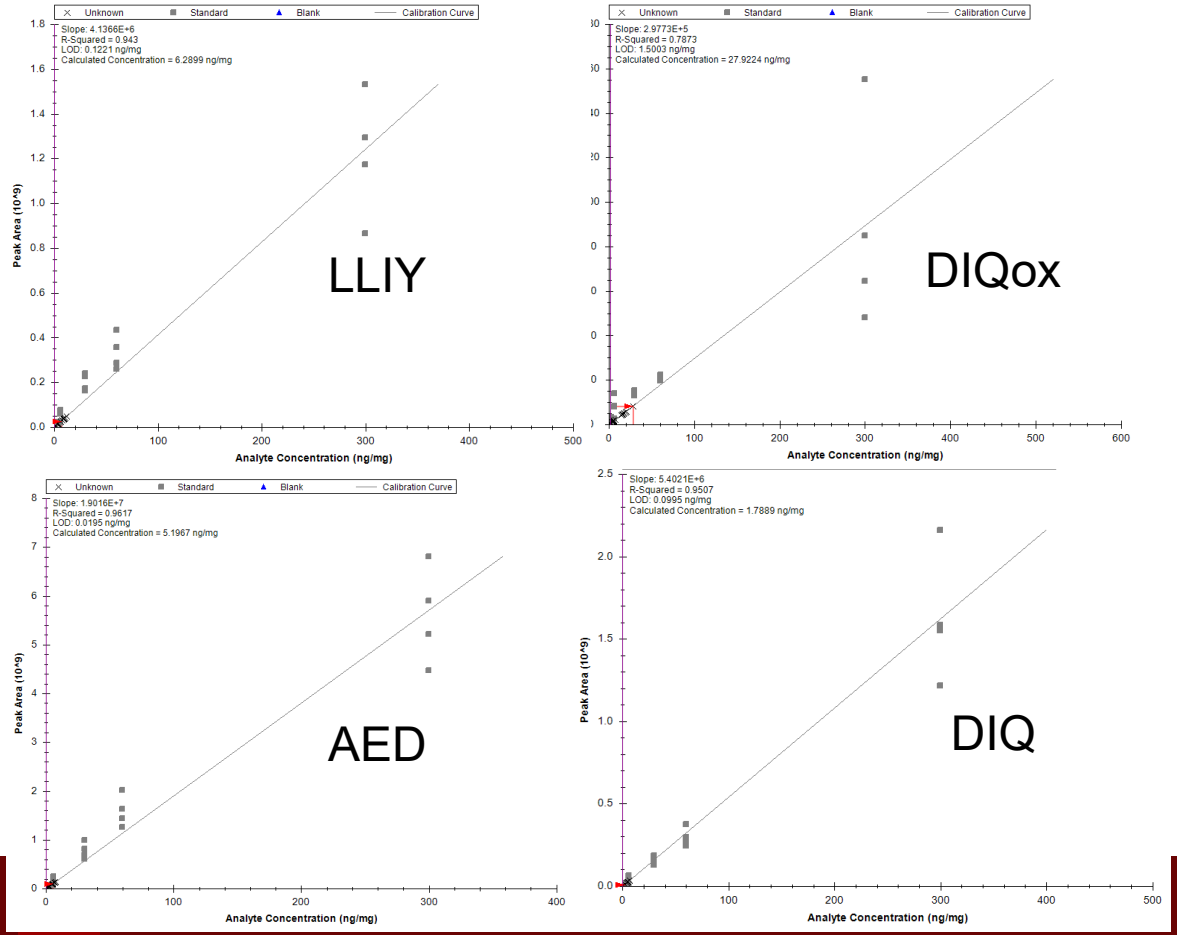
Peptide	Precursor m/z	z	t start	t stop
LLIYAASSLQSGVPSR	831.4647	2	6.3	8.4
LLIYAASSLQSGVPSR^	836.4688	2	6.3	8.4
AEDTAVYYYCAR	659.7903	2	3.18	5.18
AEDTAVYYYCAR^	664.7944	2	3.18	5.18
DIQMTQSPSSLSASVGDR	947.9442	2	3.68	5.68
DIQMTQSPSSLSASVGDR^	952.9483	2	3.68	5.68
DIQMTQSPSSLSASVGDR	939.9467	2	4.76	6.76
DIQMTQSPSSLSASVGDR^	944.9509	2	4.76	6.76

- Evosep 100 SPD 5cm endurance column (EV1064)
- Top 5 transitions with minimal interference monitored and MS2 sum used for quantification



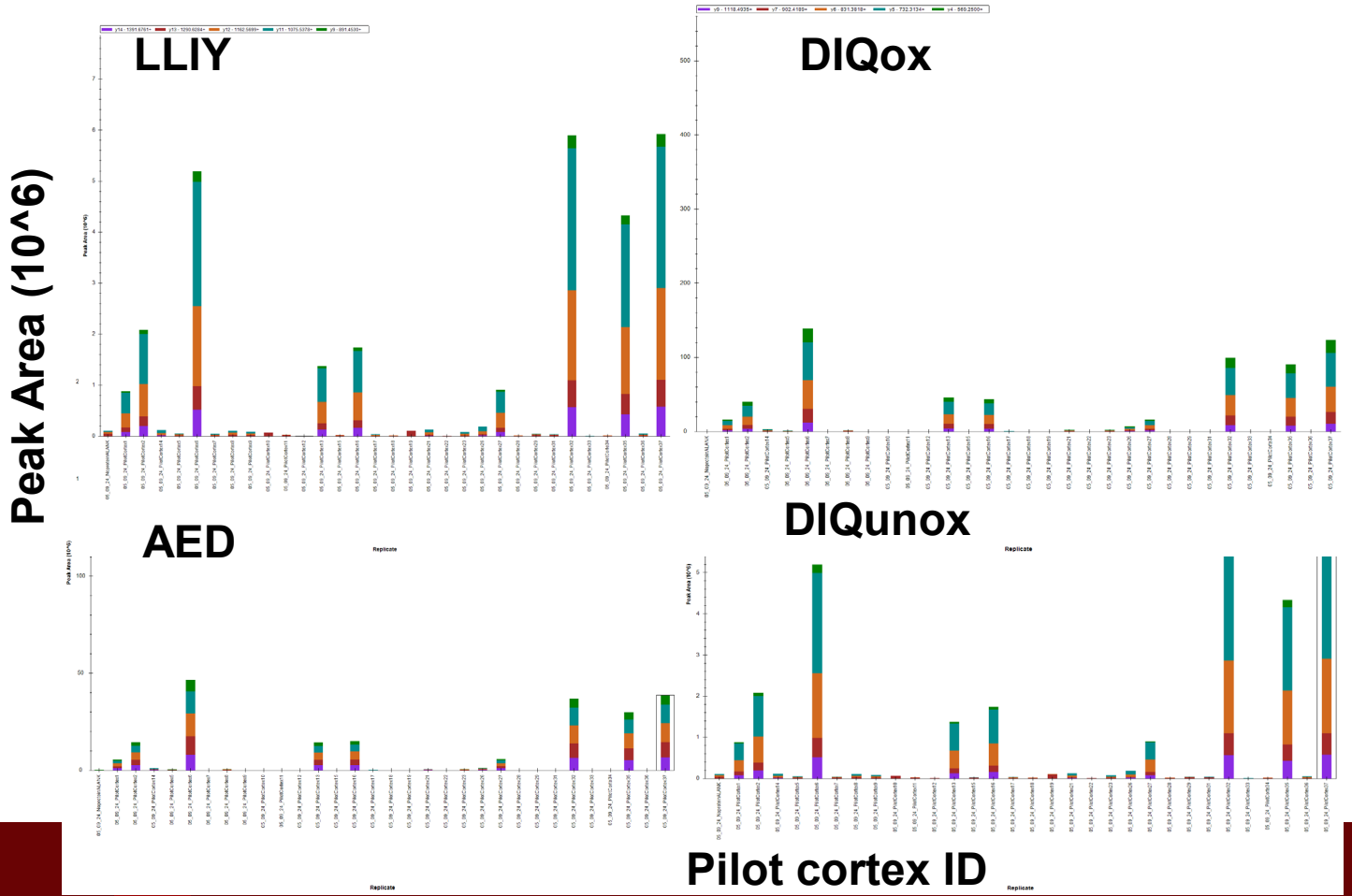
Assay Implementation

Final workflow Cortex LOD/LLOQ



- Process and technical duplicate
- Spread in 300 ng/mg standard/max

4 peptides across 31 pilot cortex samples:



- All track each other; LLIY and AED track with quantitative precision

Example Cortex 6 quant:

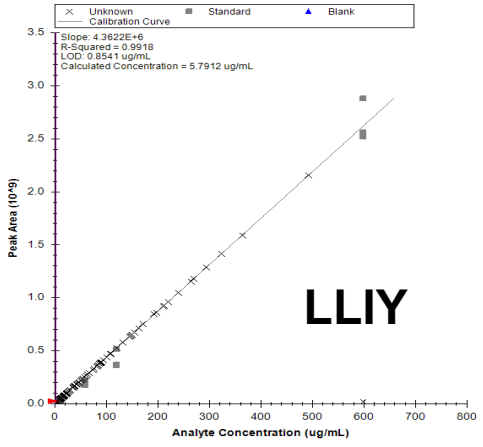
- LLIY: 11.44 ng/mg
- AED: 7.5 ng/mg

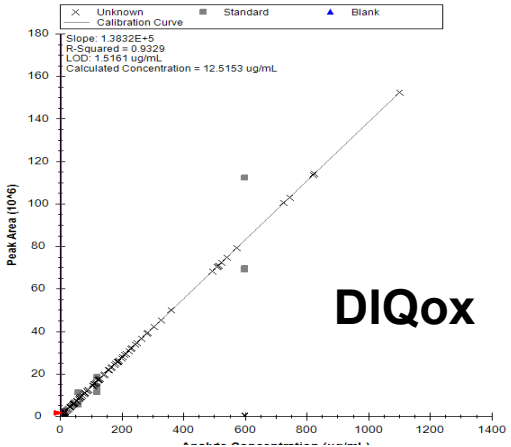
Pilot Cortex concentrations

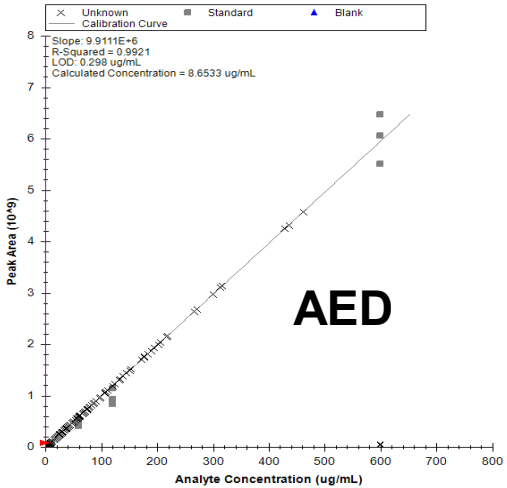
Cortex ID	ratio:heavy	Value		Sex	Genotype	Dose	Drug	Frequency
6	15.3468	7.5181	ng/mg	F	Tg	30	Aducanumab	Single
37	15.4673	6.6858	ng/mg	M	Tg	30	Aducanumab	Weekly
32	12.9199	5.3502	ng/mg	F	Tg	30	Aducanumab	Single
35	9.7646	4.9079	ng/mg	M	Tg	30	Aducanumab	Weekly
13	6.0483	2.4634	ng/mg	F	Tg	30	Aducanumab	Weekly
16	5.7426	2.3784	ng/mg	F	Tg	30	Aducanumab	Weekly
2	4.5312	2.1149	ng/mg	M	Tg	30	Aducanumab	Single
27	2.043	0.8851	ng/mg	F	Tg	30	Aducanumab	Single
1	1.8525	0.8642	ng/mg	M	Tg	30	Aducanumab	Single
26	0.9602	0.3746	ng/mg	F	Tg	30	Aducanumab	Weekly
14	0.3546	0.1795	ng/mg	F	Tg	1	Aducanumab	Weekly
23	0.2495	0.1036	ng/mg	M	Tg	1	Aducanumab	Weekly
21	0.2569	0.1006	ng/mg	M	Tg	1	Aducanumab	Weekly
8	0.1912	0.0944	ng/mg	F	Tg	1	Aducanumab	Weekly
5	0.1324	0.0627	ng/mg	F	Tg	1	Aducanumab	Weekly
17	0.1094	Below LOD	ng/mg	F	WT	30	IgG	Weekly
36	0.0305	Below LOD	ng/mg	M	Tg	30	IgG	Weekly
33	0.0278	Below LOD	ng/mg	F	Tg	30	IgG	Weekly
28	0.0177	Below LOD	ng/mg	F	WT	30	IgG	Weekly
9	0.0103	Below LOD	ng/mg	M	WT	30	IgG	Weekly
7	0.0162	Below LOD	ng/mg	F	Tg	30	IgG	Weekly
18	0.0223	Below LOD	ng/mg	F	WT	30	IgG	Weekly
15	0.0131	Below LOD	ng/mg	F	Tg	30	IgG	Weekly
29	0.0068	Below LOD	ng/mg	F	WT	30	IgG	Weekly
34	0.0134	Below LOD	ng/mg	M	Tg	30	IgG	Weekly
19	0.0177	Below LOD	ng/mg	F	WT	30	IgG	Weekly
22	0.0056	Below LOD	ng/mg	M	Tg	30	IgG	Weekly
12	0.0015	Below LOD	ng/mg	M	WT	30	IgG	Weekly
10	0.0002	Below LOD	ng/mg	M	WT	30	IgG	Weekly
11	0.0101	Below LOD	ng/mg	M	WT	30	IgG	Weekly

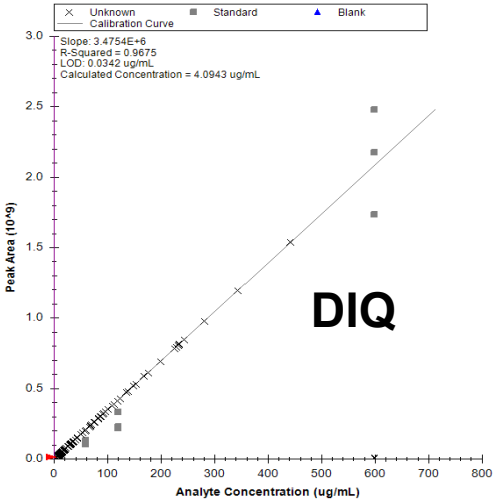
- Assay can confidently quantify chAducanumab extracted from 5X FAD cortex
- Can confirm no chAducanumab in IgG control cortex samples

Final workflow plasma LOD/LLOQ





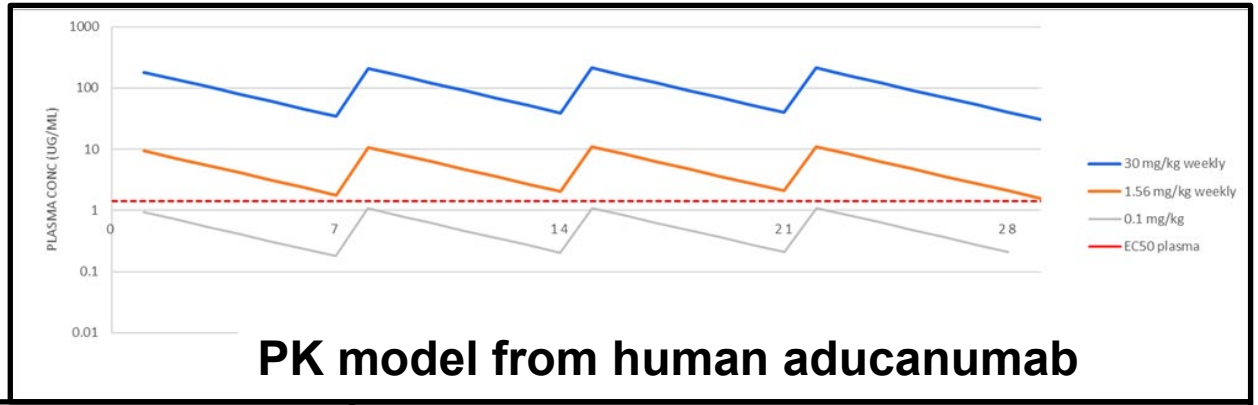




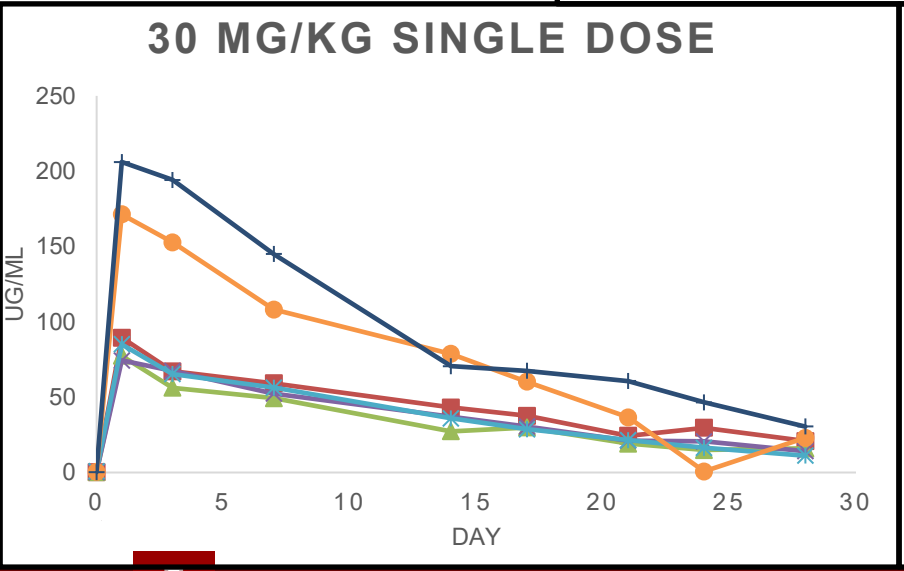
Process triplicate

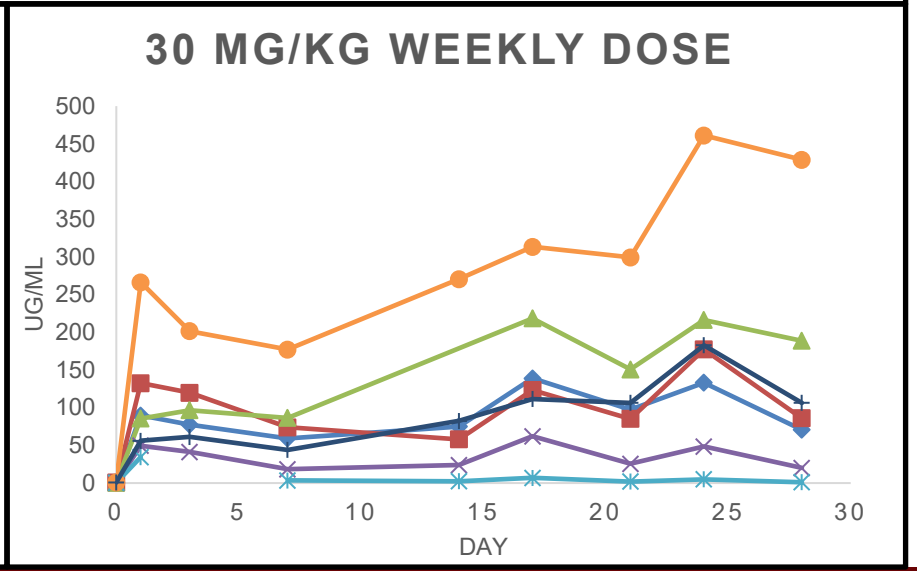
4-week Pilot PK plasma study:

Model versus measured concentration profiles following IP dosing



PK model from human aducanumab

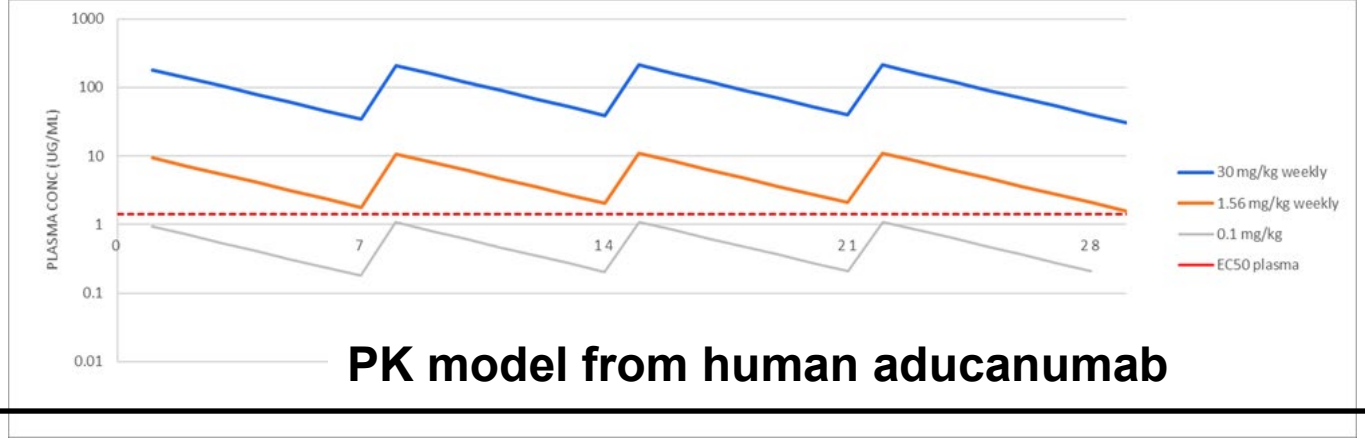




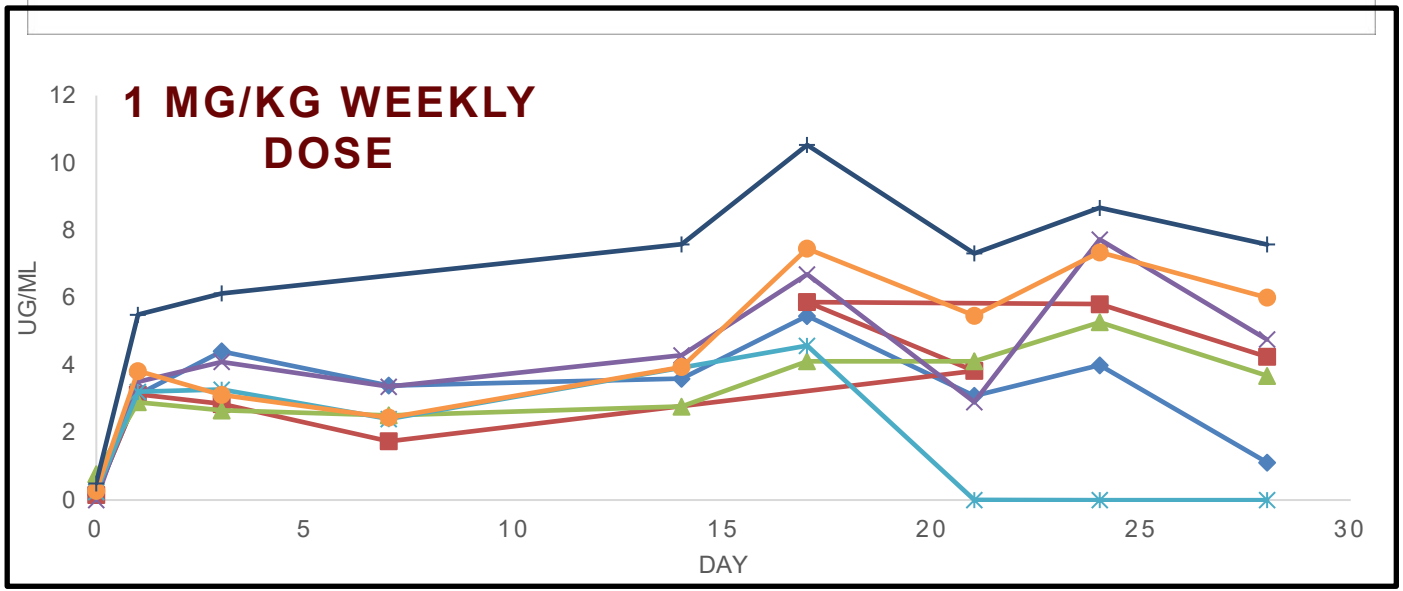
PK model from Sevigny et al 2016 Sep 1;537(7618):50-6. doi: 10.1038/nature19323.

4-week Pilot PK plasma study:

Model versus measured concentration profiles following IP dosing



PK model from human aducanumab



Next steps:

- Finish running cohort samples from Pitt and IUSM
- Integrate with Aβ plaque quantitation, behavior, and imaging data (U-Pitt and IU labs)
- Be on the lookout for bioRxiv and Panorama uploads –coming this summer

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Emma Doud
Chunna Guo
Whitney Smith-Kinnaman
Kasi Hansen
Jaison Arrivalagan (former)

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Mette Peters
Anna Greenwood

The Jackson Laboratory

Mike Sasner
Harriet Williams
Kristen Onos
Kelly Keezer
Leslie Haynes
Martha Abbott, Administrator

Check out other posters/science from IUSM and the CPA!

Monday:

MP 122 Evaluation of potential spike-in proteins for normalization of TPP quantitation

10:30am-2:30pm Jun 3 (Pacific) — Chunna Guo

MP 711 Analysis of protein complex stability and missense protein sequence variants associated with neurodegenerative disease

10:30am-2:30pm Jun 3 (Pacific) — Avery Runnebohm

Wednesday:

WP 149 Protein biomarker discovery across stages of pancreatic neoplasms using mass-spectrometry based proteomics of pancreatic cyst fluid

10:30am-2:30pm Jun 5 (Pacific) — Gina Chang

WP 593 Fit for purpose high-throughput absolute quantitation of chimeric aducanumab in mouse cortex and plasma

10:30am-2:30pm Jun 5 (Pacific) — Emma H Doud

WP 553 Case study emphasizing the need for proteomics in clinical genetics: RNA-protein discordance in rare neurodegeneration-associated var…

10:30am-2:30pm Jun 5 (Pacific) — HR Sagara Wijeratne

WP 673 Quantitative analysis of non-histone lysine methylation sites and lysine demethylases in breast cancer cell lines

10:30am-2:30pm Jun 5 (Pacific) — Christine Berryhill

INDIANA UNIVERSITY