Skyline & Panorama Case Study: Targeted Proteomics Enables Alzheimer's Disease Biomarker Development

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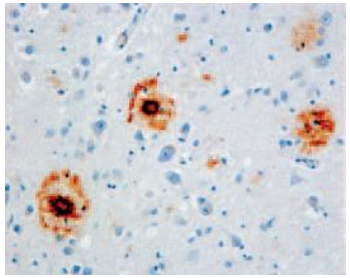
ASMS Skyline Users Meeting
June 15, 2014

Alzheimer's Disease

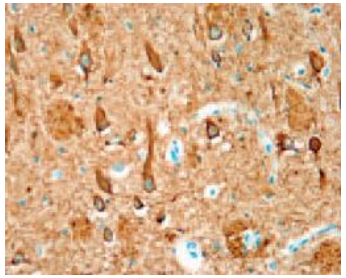
- Leading cause of dementia (26 million affected)
- Protein aggregation disease
  - Amyloid beta Plaques
  - Tau (hyperphosphorylated) Tangles
- Genetic risk
  - Early onset (1%) autosomal dominant
  - Late onset Apoε4 increases risk
- No disease modifying therapies available

Pathology Hallmarks

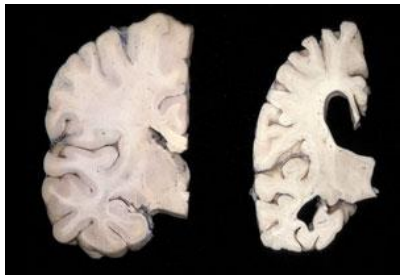
Amyloid Plaques - Aβ



Neurofibrillary tangles - tau

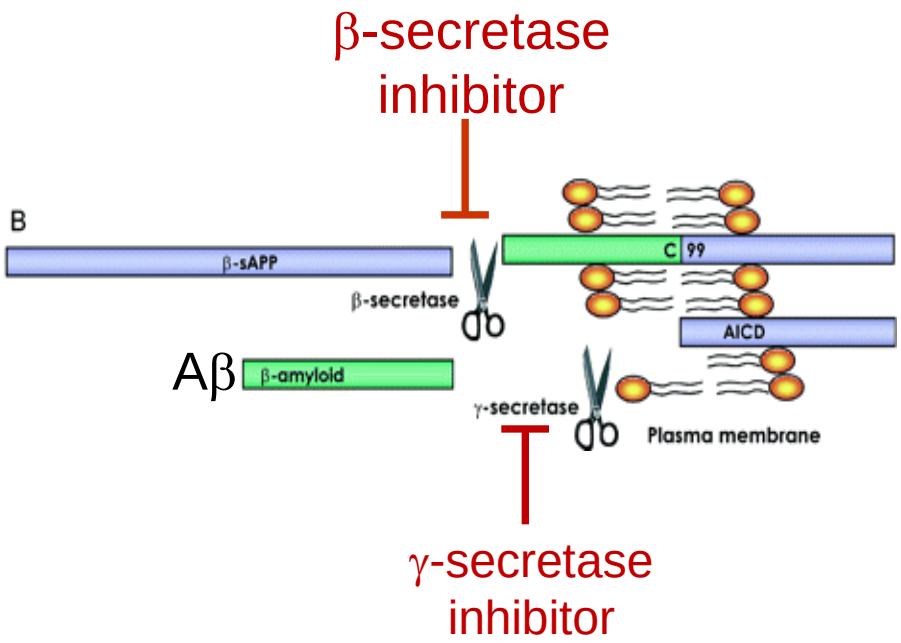


Atrophy

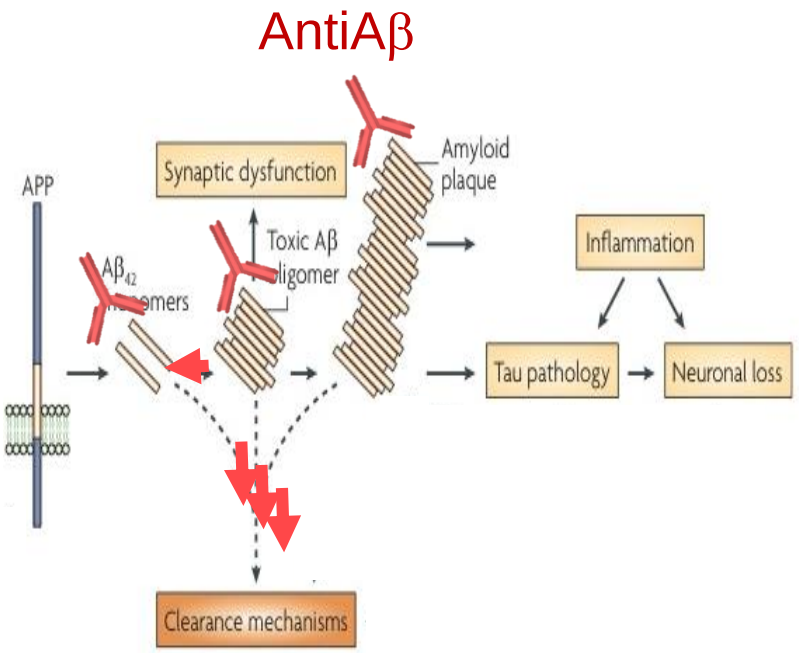


Therapeutic strategies test the Aβ hypothesis

Decrease Aβ production

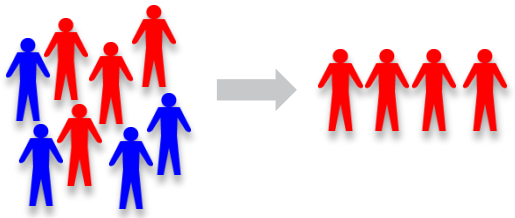


Enhance Aβ clearance



Roles of biomarkers in Alzheimer's trials

1. Increase diagnostic confidence



AD CSF Signature = high tau / low Aβ

Drug

↓

Target

2. Demonstrate target-engagement

↓

Biological Response

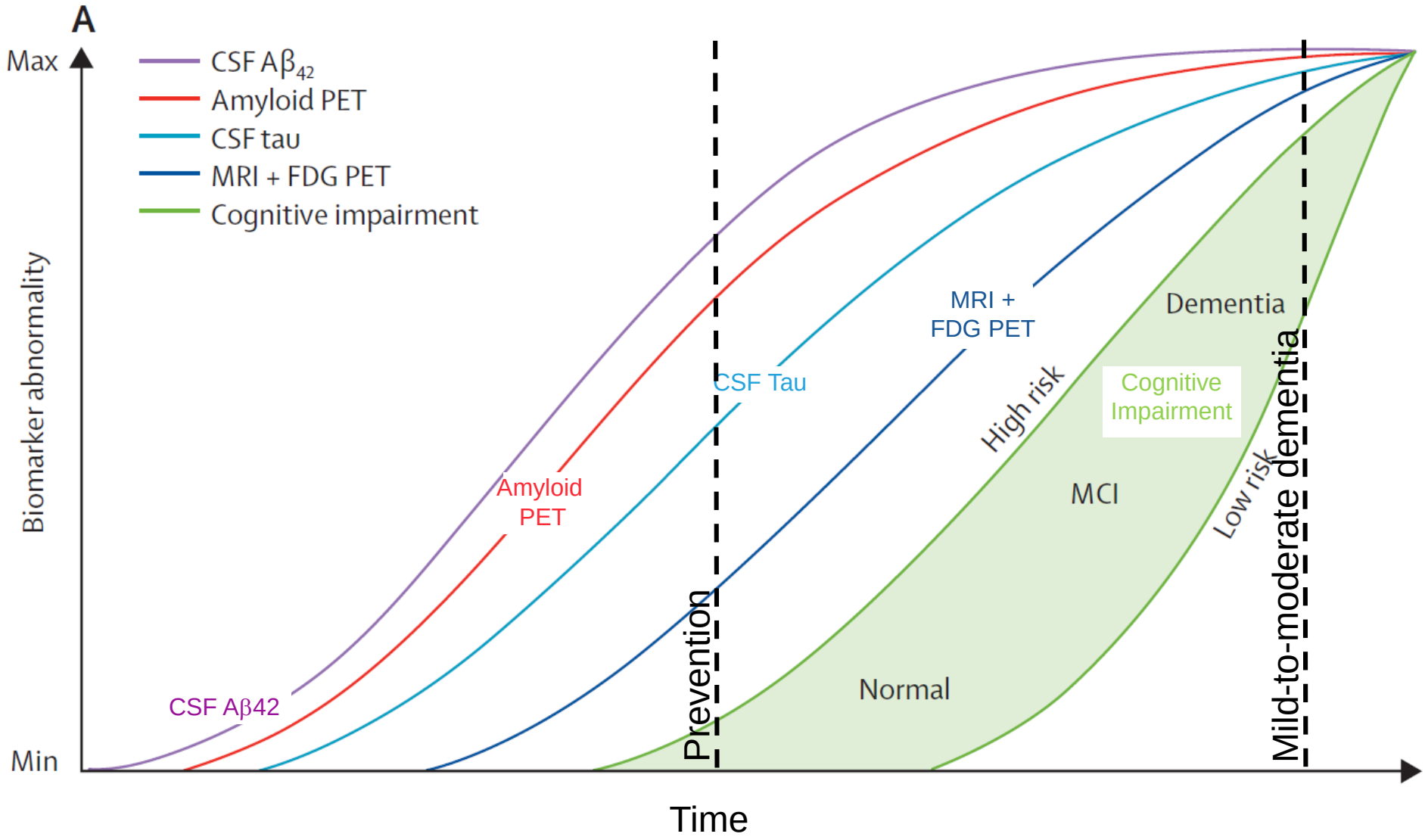
3. Demonstrate disease-modification

↓

Efficacy

4. Biomarker linked to clinical outcome

The utility of biomarkers in clinical trials changes with disease progression



C.Jack 2013 Lancet Neurol. 12:207

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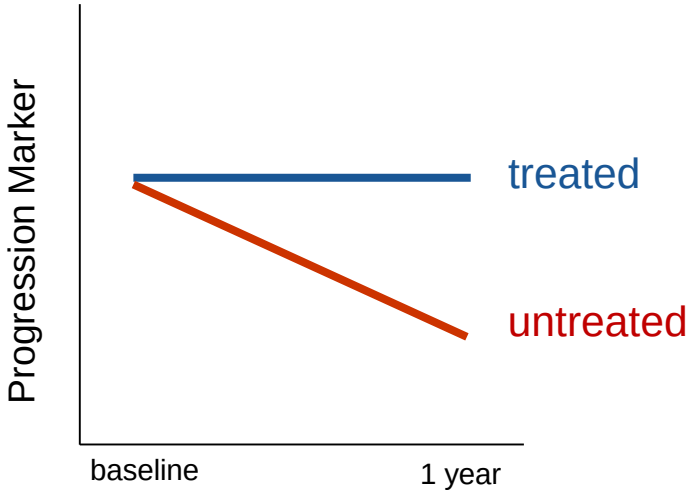
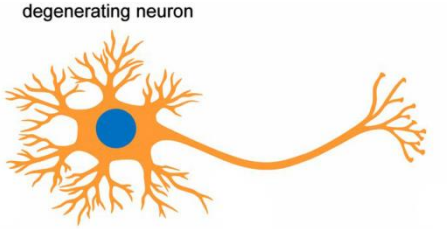
Novel biomarkers needed to provide evidence of disease-modification in clinical trials

- Discover progression markers in established Alzheimer's patients



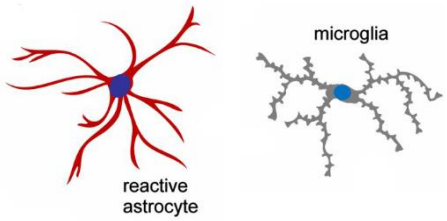
- Discover novel pathway biomarkers beyond the classic Alzheimer's biomarkers Aβ and tau
  - Inflammation
  - Synaptic dysfunction
  - Oxidative Stress
  - Mitochondial dysfunction

Candidate biomarkers selected for targeted proteomics



Neurodegeneration
Chromogranin A
Contactin 1
Contactin 2
Neuronal Pentraxin Receptor
NrCAM
Tetranectin
Visinin-like protein 1

Aβ pathway
Albumin
Amyloid precursor protein
Amyloid precursor like protein 1
ApoE
ApoE4
β-2-microglobulin
ApoJ (Clusterin)
Cystatin C
Plasminogen
Prion protein
Prostaglandin-d2-synthase
Transthyretin



Neuroinflammation
α-1-antitrypsin
CH3L1 (YKL-40)
Complement C3
Complement C4

Antioxidant, other
α-1-β-glycoprotein
ApoH (β-2-glycoprotein)
Ceruloplasmin
Retinol binding protein
Superoxide dismutase
Transferrin

Multiplexed MRM assay developed for candidates

Candidate proteins in CSF

protease digestion

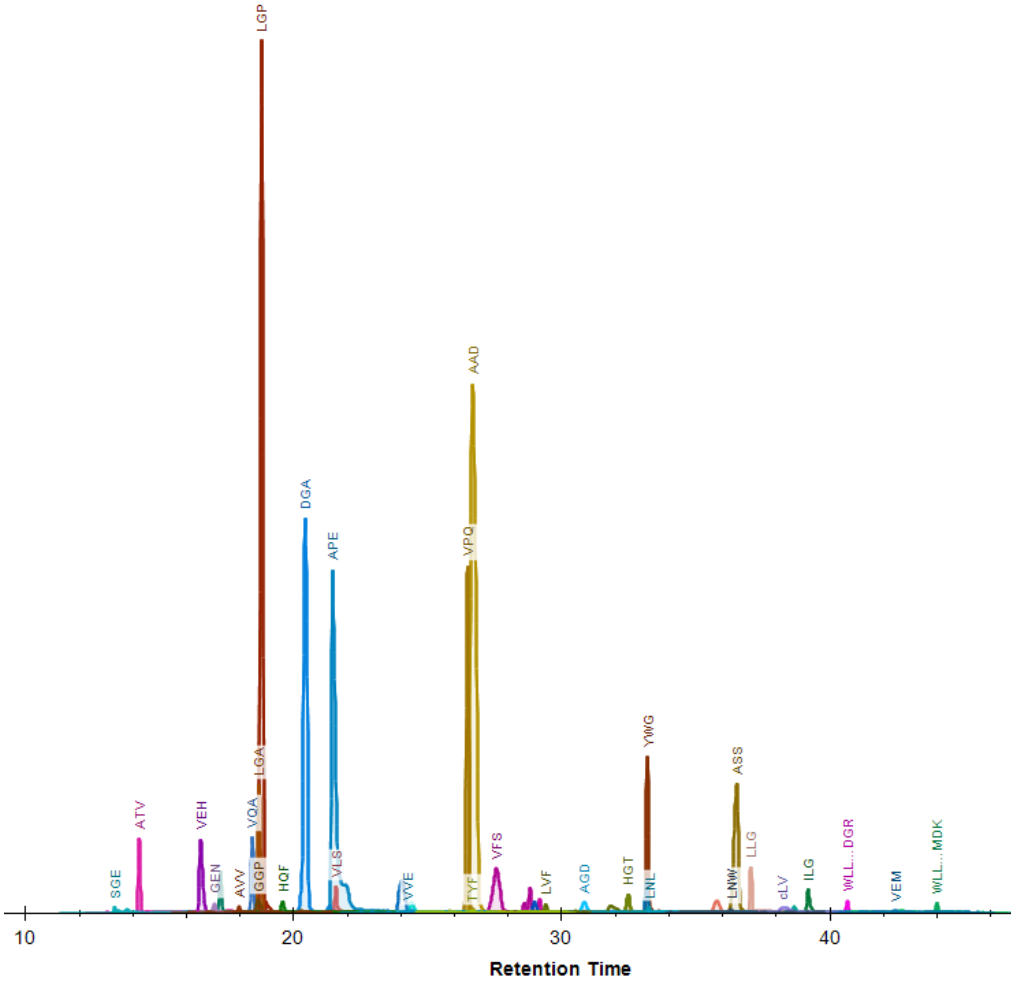
Signature peptides

+internal standards (heavy peptides)

LC-MS/MS

Quantitation

Multiplex



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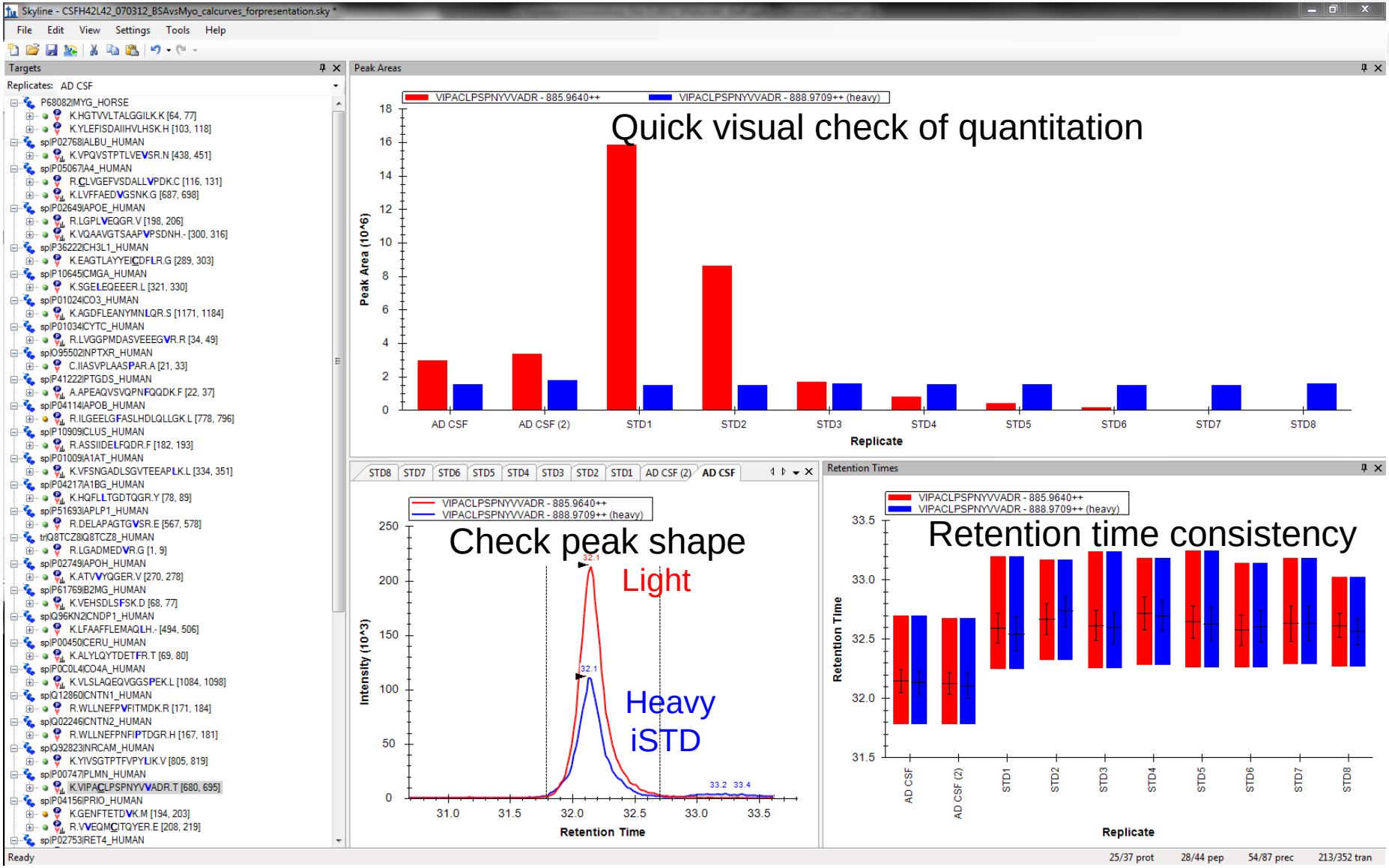
Multiplexing enabled by targeted-proteomics software



Open-source software available from MacCoss lab at University of Washington:
https://brendanx-uw1.gs.washington.edu/labkey/project/home/software/Skyline/begin.view

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Performance of targeted proteomics (39-peptide) quantitative assay evaluated in CSF

Characteristics of purchased CSF	Cognitively Normal	Mild Cognitive Impairment	Alzheimer's Disease
n	10	5	45
Sex, M/F	7/3	2/3	30/15
Age, mean (range)	68.8 (64-75)	74 (66-80)	76.9 (61-90)
MMSE score (range)	29.4 (25-30)	23.4 (21-26)	19.7 (6-27)

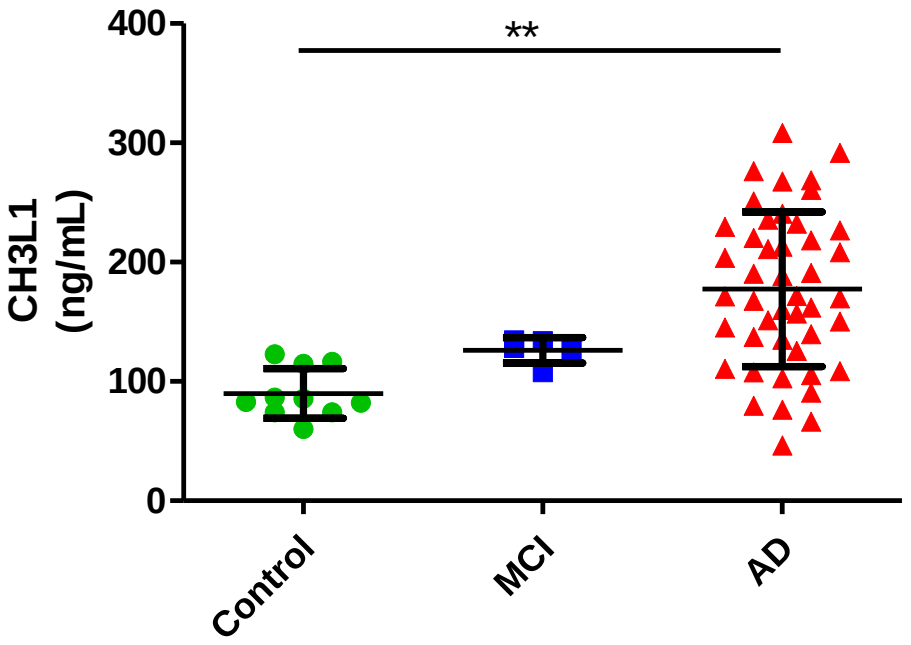
1. Compare levels between groups
   - Are results consistent with unbiased proteomic results?

2. Characterize change over-time in biomarkers (0, 3-6, 12 mo.)
   - Stable or variable?
   - Increasing or decreasing?

Statistically significant discrimination between controls and Alzheimer's confirmed for Chitinase 3 like protein 1

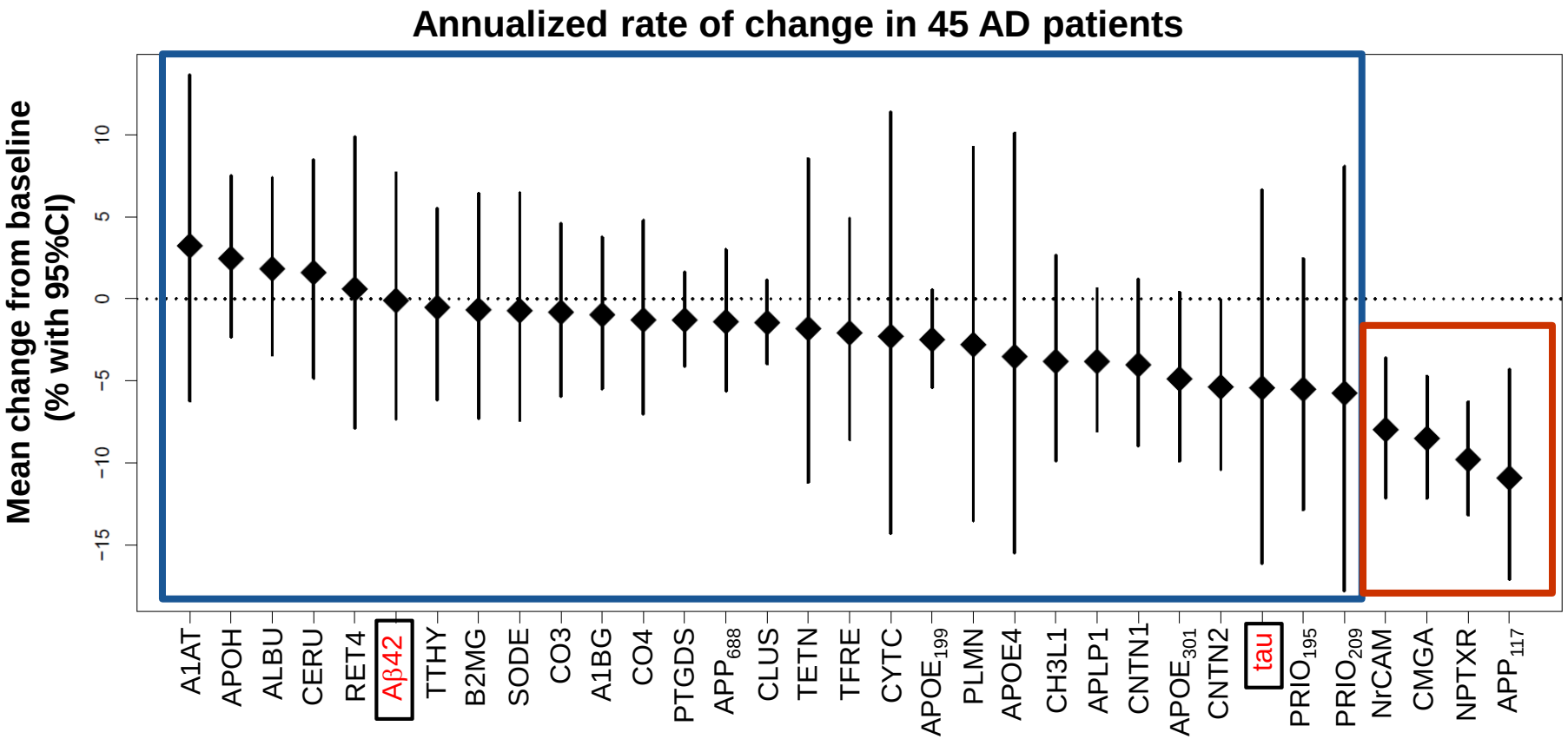
Control (n=10) vs. AD (n=45)		
Biomarker	**corrected p-value**	**Fold difference**
Aβ42	*<0.001*	*0.60*
CH3L1_290	0.003	1.6
Total Tau	*0.004*	*2.0*
TTHY_56	0.006	1.2
p-tau181	*0.0070*	*3.3*
A4_117	0.031	0.7
CO3_1172	0.031	1.4

P values represent linear regression comparison of log values (corrected by Benjamini & Hochberg method), adjusting for age and sex



Wildsmith et al. Mol. Neurodegener. 2014 9:22

The majority of CSF biomarkers are stable over 1 year
4 biomarkers decline in Alzheimer's patients



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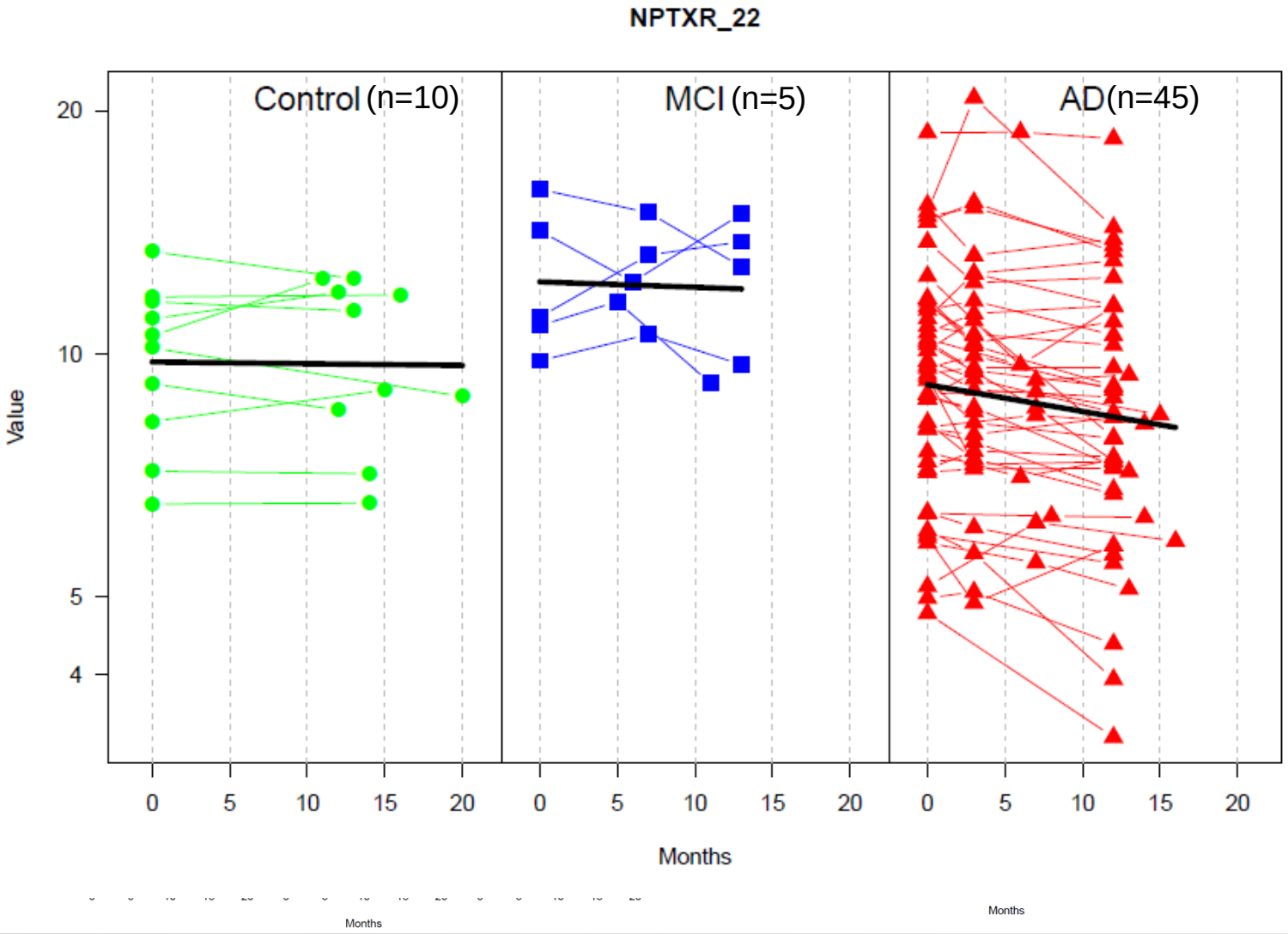
Multiplexed LC/MS assay identifies four potential markers of progression in CSF from AD patients



Wildsmith et al. Mol. Neurodegener. 2014 9:22

Current data QC and analysis workflow for multiplexed MRM

Generate Data

Peak integration and calibration curve generation

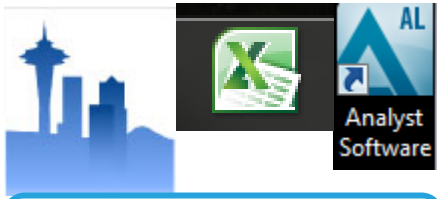
QC sample data

Statistical analysis and data visualizations

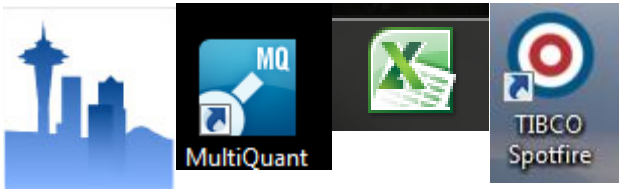
MQ MultiQuant 2.1

TIBCO Spotfire

Large data files from multiple platforms creates data management challenges



Method and data generation

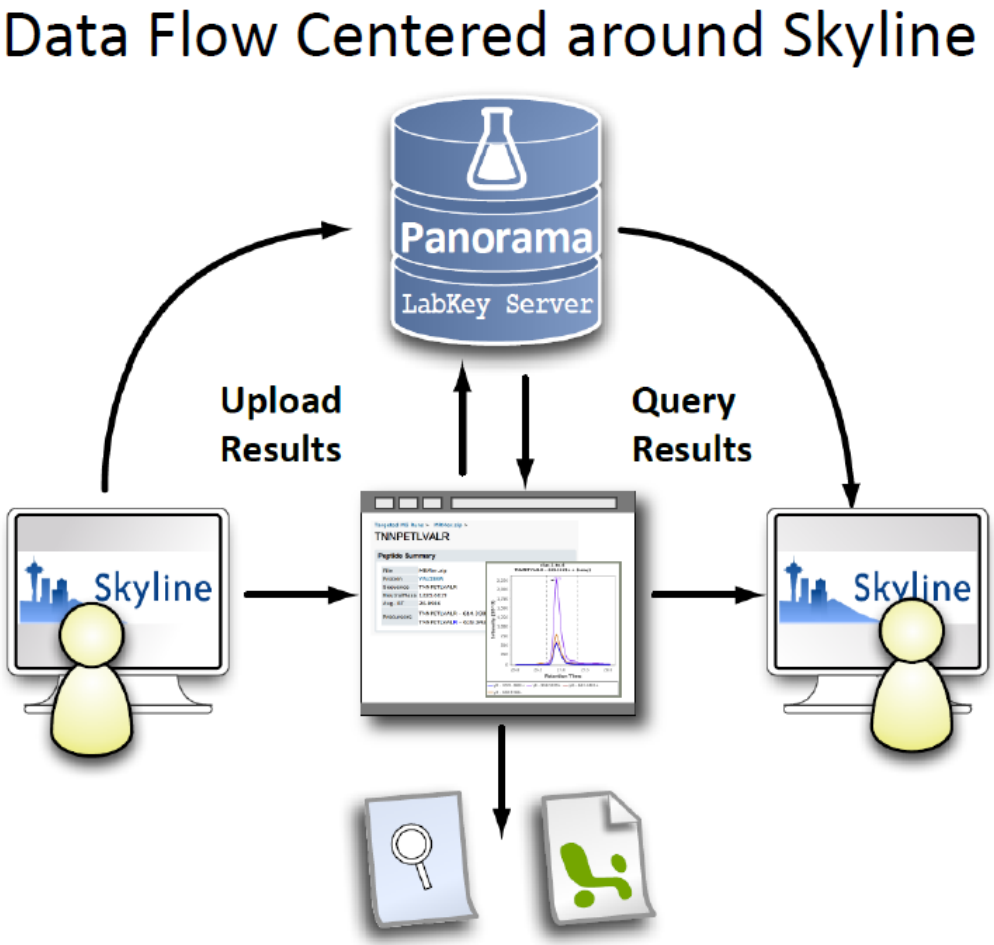


Data QC and analysis

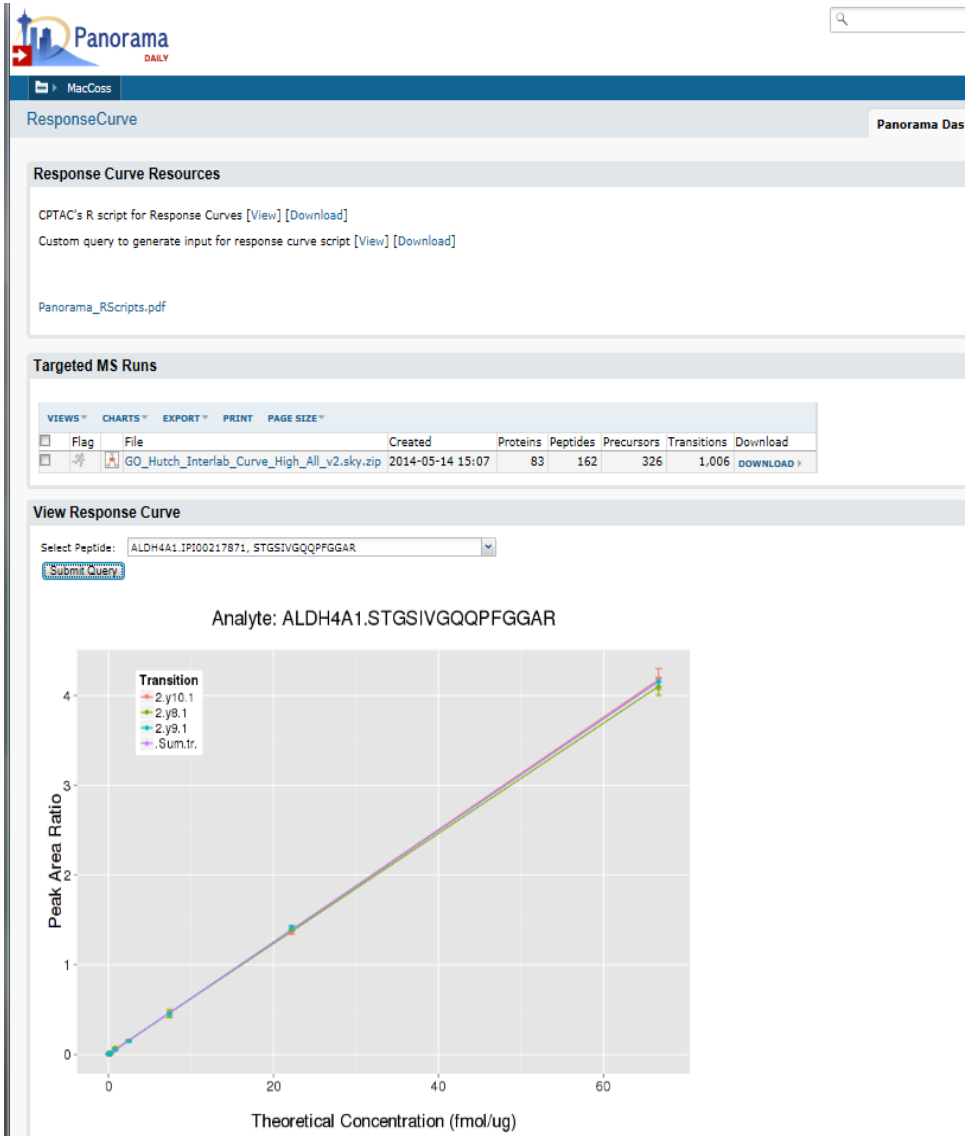
Types of files generated:

- Skyline method and data visualization files
- Instrument method file
- Raw MS data
- Quantitation file
- Excel spreadsheets for import to Spotfire
- Spotfire QC file
- Spotfire biomarker result file
- Additional (externally generated) biostats *.csv files and R plots

Data management simplification with Panorama



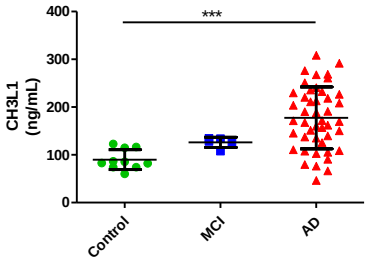
Analyzing data directly in Panorama

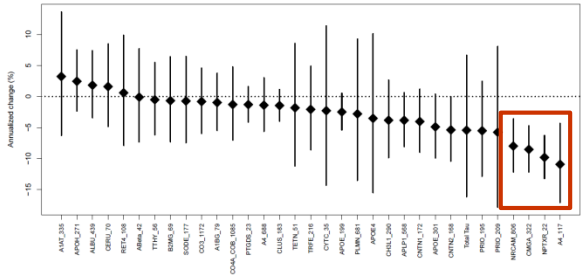


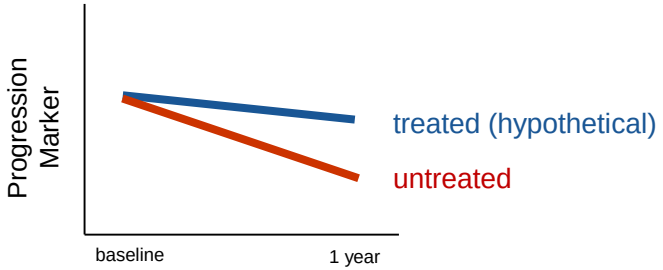
Panorama tutorial: Panorama_Rscripts.pdf (Vagisha Sharma)

https://daily.panoramaweb.org/labkey/project/MacCoss/vsharma/CPTAC/ResponseCurve/begin.view?

Targeted proteomics accelerates biomarker development







- Confirmed potential diagnostic markers
- 4 candidate disease progression markers
- Will any of these markers show pharmacodynamic potential? (stay tuned)

Next steps:

1. Expand panel and test on additional Alzheimer's cohorts
2. Transition to Panorama and simplify data management

Panorama

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Acknowledgements

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PD Biomarkers

Lee Honigberg

Rod Mathews

Paul Fielder

Stephen Schauer

Ashley Smith

DevSci MS

Surinder Kaur

Sami Mahrus

Research proteomics (MCP)

Jennie Lill

David Arnott

Wendy Sandoval

Spotfire (PK/PD)

Joshua Haznedar

Nonclinical Biostats

Yuda Zhu

Panorama Partners

Josh Eckels (Labkey)

Brendan MacLean (UW)

Vagisha Sharma (UW)

Tom Dunkley (Roche)

Michel Petrovic (Roche)

UW Targeted Proteomics Course 2014

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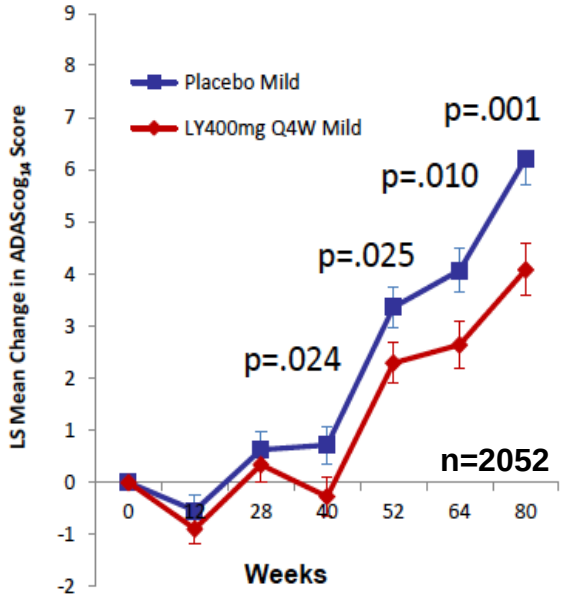
Appendix

Biomarker and efficacy data from recent Ph III anti-Aβ trials diverge

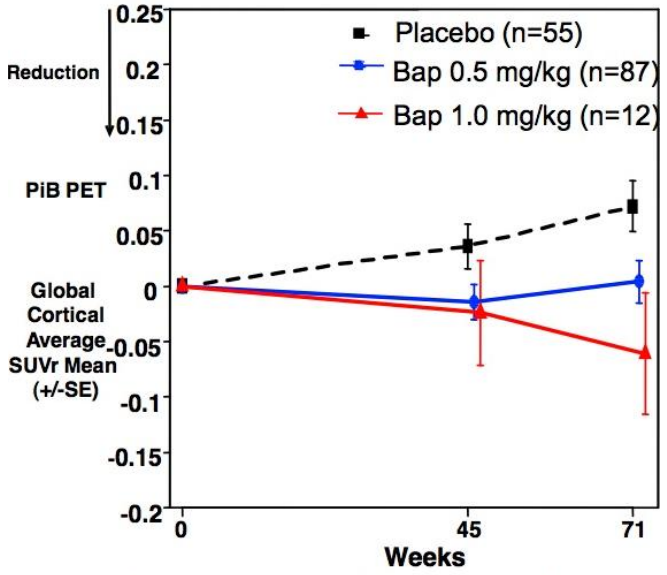
Solanezumab (Anti-monomeric Aβ)



Pooled Analysis
Mild Subjects Only - ADASCog

No change in biomarkers

Bapineuzumab (Anti-monomeric and aggregated Aβ)

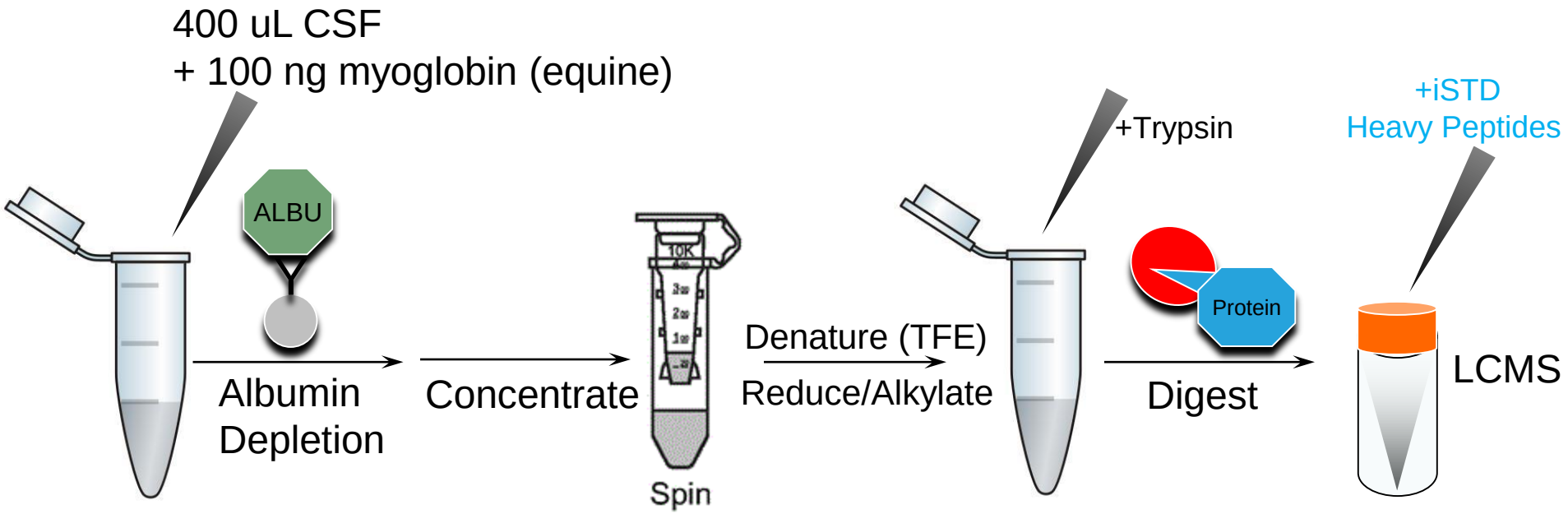


Placebo vs Bap 0.5 mg/kg p=**0.027**
Placebo vs Bap 1.0 mg/kg p=**0.028**

Pooled Analysis
Amyloid PET

No change in cognition

CSF sample preparation

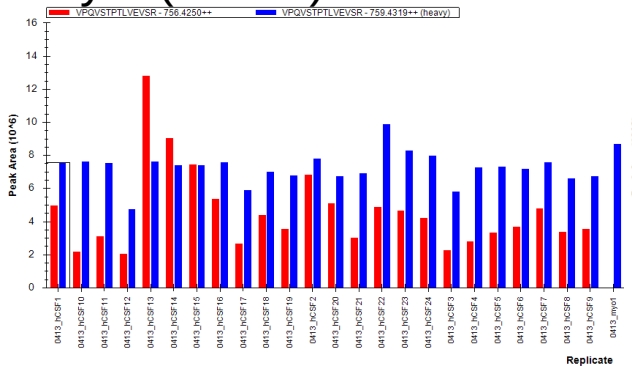


Variable albumin depletion effects some but not all proteins

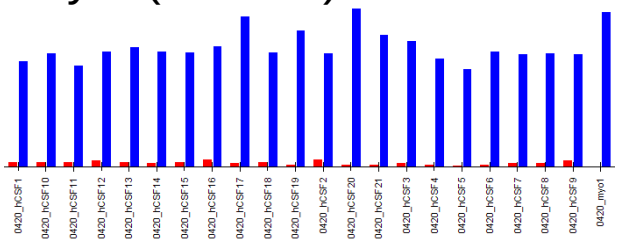
The majority of albumin was depleted

Albumin

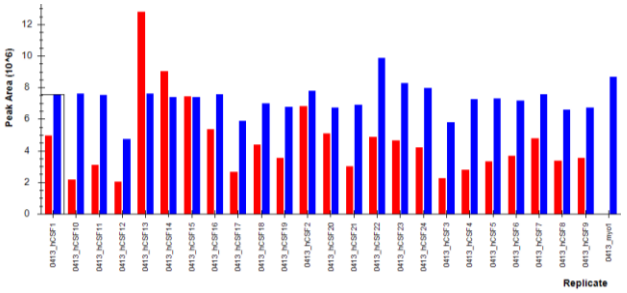
Day 1 (99.5%)

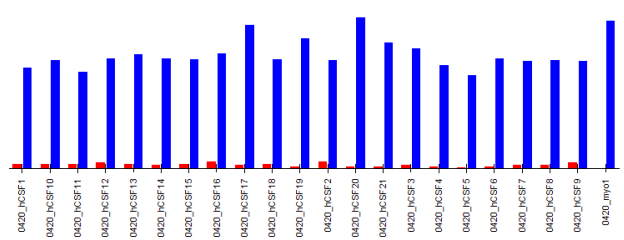


Day 2 (99.96%)

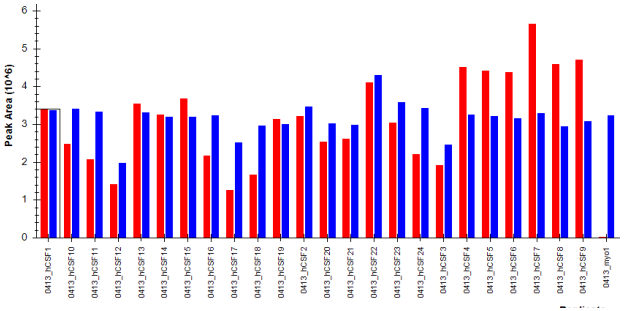


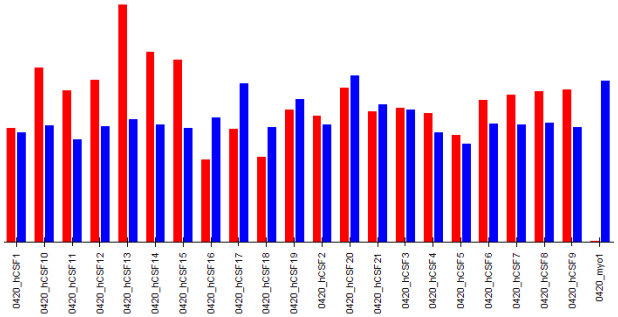
Clusterin
(affected protein)





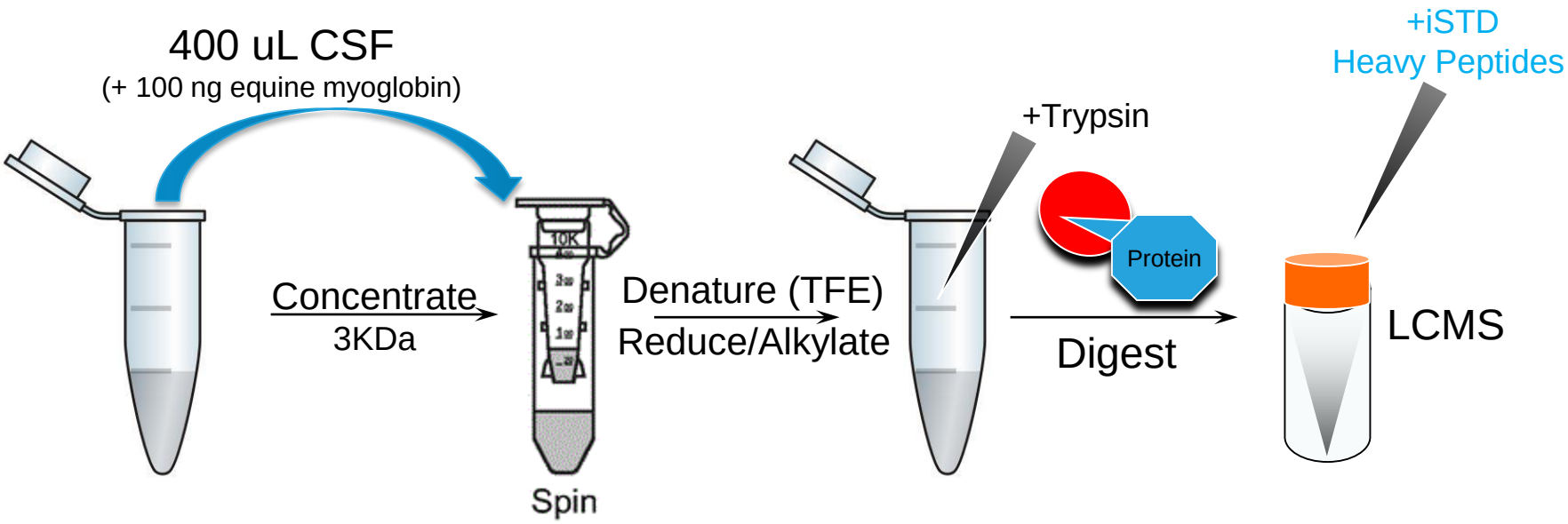
NPTXR
(non-affected protein)



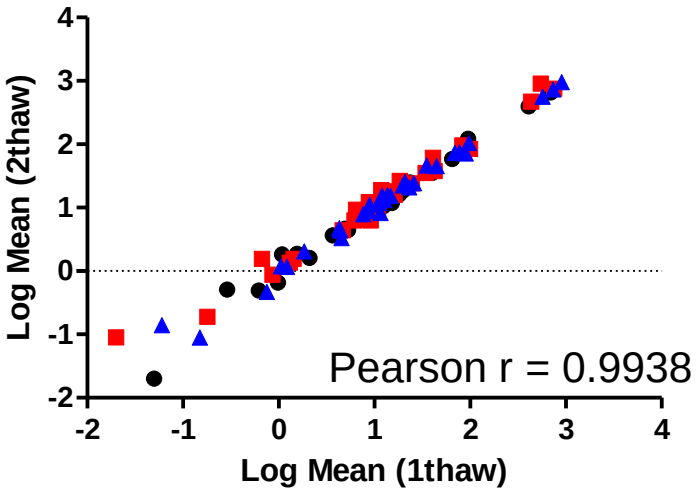


However, 9 candidate biomarkers are affected by depletion column variability
(APP, ApoE, ApoE4, CO3, CO4, CLUS, CMGA, SODE, VTDB)

Revised CSF sample preparation



Supplemental figure 1. The majority of peptides are stable after one or two freeze-thaw cycles. Log of the mean ratio (light to heavy peptide pair) observed for 42 peptides between 1 or two freeze thaw cycles in CSF from three AD patients. (shape and color by patient).



Targeted proteomic MRM assay development summary

- LOD and LOQ for 40 peptide panel established in artificial CSF matrix (10 ug/ml BSA digest)

- 32/40 peptides representing 28 CSF proteins are above LOQ in AD CSF
  - 5/8 peptides below LOQ are plasma contamination markers
  - 3/8 peptides below LOQ are VILIP-specific peptides

- Intra-assay CV is ≤10% for 32 peptides

- Inter-assay CV is <20% for 27 peptides

Sensitivity range (ng/mL → μg/mL) is peptide specific

Protein	Peptide Identifier	LOD (fmol)	LOQ (fmol)	Range normal CSF (fmol) (n=10)	Range AD CSF (fmol) (n=45)	Range normal CSF (ng/mL) (n=10)	Range AD CSF (ng/mL) (n=45)
Plasminogen	PLMN_681	0.004	0.04	2-11	2-19	36-250	48-436
Amyloid precursor protein	A4_117	0.4	4	22-67	21-114	479-1446	455-2474
Transthyretin	TTHY_56	1	2	1004-1714	766-1961	3989-6808	3043-7789

Representative low, medium and high abundance proteins

Comparison table of LOD and LOQ CSF for MRM vs. ELISA

CSF Biomarker	Assay	LOD (ng/mL)	LOQ (ng/mL)	LOD (nM)	LOQ (nM)
CH3L1/YKL-40	ELISA	5.4	20	0.1	0.5
CH3L1/YKL-40	MRM	18.5	92.7	0.4	2.2
NrCAM	ELISA	1	4	0.01	0.03
NrCAM	MRM	6.3	62.6	0.04	0.4
CMGA	ELISA	20	90	0.4	1.8
CMGA	MRM	0.4	44.1	0.01	0.9

Peptide levels by diagnostic group

Spotfire visualization of multiplex results