inoviv

OVERVIEW

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PROTEOMIC BIOMARKERS HAVE BECOME CRITICAL TO PHARMACEUTICAL R&D PIPELINES GLOBALLY[1]

[1]Lee, J.-M., Han, J.J., Altwerger, G. and Kohn, E.C., 2011. Proteomics and biomarkers in clinical trials for drug development. Journal of Proteomics, 74(12), pp.2632–2641

THE USE OF PATIENT SELECTION BIOMARKERS HAS INCREASED DRAMATICALLY SINCE THE SEQUENCING OF THE HUMAN GENOME*

*Clinical Development Success Rates 2006-2015 - BIO, Biomedtracker, Amplion 2016

R&D EXCELLENCE IS BEING RESHAPED BY BIOMARKERS

WE HAVE NEW EXPECTATIONS

THE SUPPORT IS WIDESPREAD

FDA

Approve companion diagnostics developed alongside new therapies*

DOCTORS

Demand precision medicine in the clinic

PATIENTS

Need the right treatments

*Marton, M.J. and Weiner, R., 2013. Practical Guidance for Implementing Predictive Biomarkers into Early Phase Clinical Studies. *BioMed Research International*, 2013, pp.1–9.

ALL THERAPEUTIC AREAS ARE TRANSITIONING

Cardiovascular

Oncology

CNS

Infectious diseases

LEADING PHARMA COMPANIES HAVE ADOPTED BIOMARKER-DRIVEN CLINICAL TRIAL MODELS

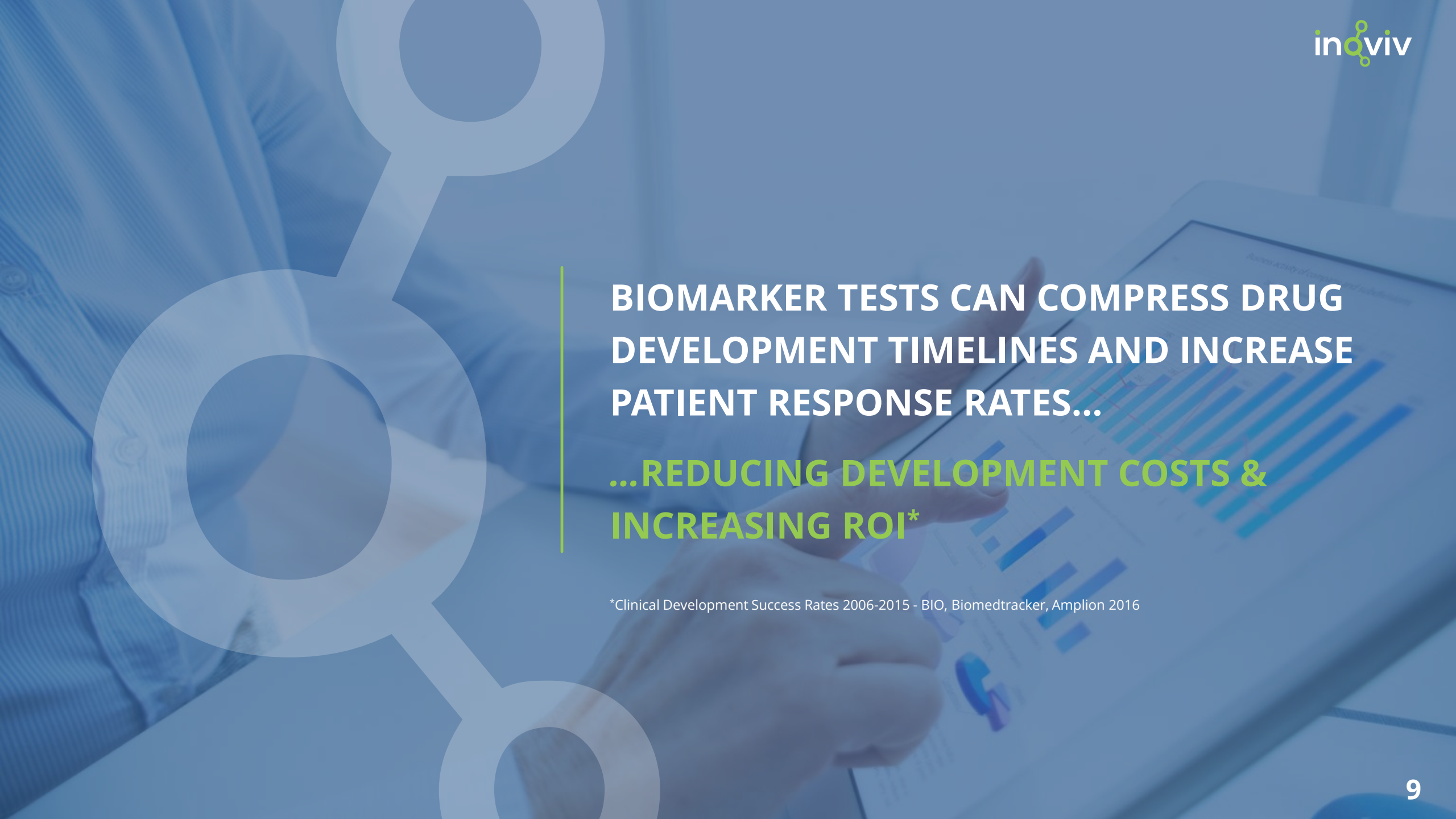
'We estimate 30 - 40 % of novel drugs in the pharma pipeline are developed in conjunction with a biomarker' - Mckinsey & Company

99.6% OF NEURODEGENERATION TRIALS HAVE BEEN UNSUCCESSFUL TO DATE*

CLINICAL TRIAL SUCCESS RATES ARE 3X HIGHER WHEN A BIOMARKER STRATEGY IS IMPLEMENTED**

*Cummings, J.L., Morstorf, T. and Zhong, K., 2014. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimer's Research & Therapy, 6(4), p.37.

**Clinical Development Success Rates 2006-2015 - BIO, Biomedtracker, Amplion 2016



BIOMARKER TESTS CAN COMPRESS DRUG DEVELOPMENT TIMELINES AND INCREASE PATIENT RESPONSE RATES...

...REDUCING DEVELOPMENT COSTS & INCREASING ROI*

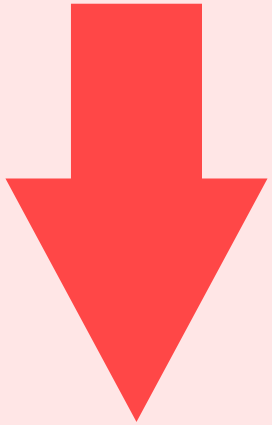
*Clinical Development Success Rates 2006-2015 - BIO, Biomedtracker, Amplion 2016

BIOMARKER PANELS CAN INCREASE CLINICAL TRIAL SUCCESS RATES

Bristol-Myers Squibb

Lack of biomarker strategy for **Opdivo** non-small cell lung cancer treatment resulted in **failed clinical trial**

-17%
Share price



-$22B
Market cap

MERCK
INVENTING FOR LIFE

Pro-biomarker strategy for Keytruda resulted in treatment **approval** for non-small cell lung cancer

+7%
Share Price

+$12B
Market cap

Source: LaMotta, LL, 2018. How biomarkers cost Bristol-Myers the lung cancer market. BiopharmaDive,13 February 2017.

THIS REDEFINES R&D EXCELLENCE

DRUG DEVELOPMENT

NO BIOMARKER STRATEGY	BIOMARKER-LED
Latest biomarker technologies not utilized	Biomarker assays developed to characterize disease models
Traditional patient selection methods compromise patient response rates	Stratified patient populations increase drug response rates
Increased risk of failing to meet clinical endpoints	Rich treatment efficacy data generated, delivering deeper insights on the mechanisms of action
8.4% clinical trial success rate*	25.9% clinical trial success rate*

*Anon, 2016. Biomarker Tests for Molecularly Targeted Therapies.

HOWEVER, CURRENT BIOMARKER TESTS ARE SEVERELY LIMITED

INOVIV OFFERS A UNIQUE TECHNOLOGY SOLUTION FOR BIOMARKER DEVELOPMENT

CUSTOM ASSAY DESIGN

- Enabling pharmaceutical companies to benefit from a test **tailored to the drug**

RELIABLE

- Data generated in **CLIA-accredited GLP/GCP labs** with FDA-compliant quality management systems

RICH DATA

- **Treatment efficacy** across multiple aspects of disease pathology
- 50+ quantified biomarkers per sample delivering **deep insights** on the mechanism of action

HIGH THROUGHPUT

- Assays with **50+ fully calibrated biomarkers** per 10 minute run and **3,000** samples per day

PROFICIENCY with protein, peptide, lipid, and metabolite analytes in a range of tissue types and biofluids

REPRODUCIBLE measurements across different laboratories and instruments

VALIDATION

- **Full technical** and **clinical validation** of biomarkers to support clinical trials

INOVIV DEVELOPS BIOMARKER ASSAYS FOR DIAGNOSTIC, PATIENT STRATIFICATION AND TREATMENT MONITORING APPLICATIONS TO SUPPORT THERAPIES

WE HAVE DEVELOPED A UNIQUE MASS SPECTROMETRY-BASED TARGETED PROTEOMICS PLATFORM, ENABLING 10X GREATER MULTIPLEXING THAN CONVENTIONAL METHODS

- WE HAVE TECHNICAL PROFICIENCY AND YEARS OF EXPERIENCE IN BIOMARKER PANEL DEVELOPMENT FOR BOTH PRECLINICAL STUDIES AND CLINICAL TRIALS
- OUR WORKFLOWS HAVE BEEN APPLIED ACROSS MULTIPLE THERAPEUTIC PROGRAMS AND OPTIMISED FOR DISTINCT CLINICAL TRIAL STRATEGIES

INOVIV IS AT THE FOREFRONT OF MASS SPECTROMETRY BIOMARKER TESTING IN NEURODEGENERATIVE DISEASES

- World's first comprehensive Alzheimer's and Parkinson's disease targeted biomarker panels
  - All aspects of disease pathology covered in one robust assay
- Biomarker panels used across multiple clinical trials
  - 3 Parkinson's disease (preclinical, Ph1 and Ph2)
  - 2 Alzheimer's disease (Ph1 and Ph2)
- 200+ neuroscience publications in peer-reviewed journals

- 4 assays brought into the clinic by our CSO, still in use at Great Ormond St Hospital (London, UK)
- Experience bench-to-bedside
  - Pre-clinical
  - Clinical trials
  - Healthcare settings
- GLP/ GCP and CLIA-accredited laboratories
- Can analyse up to 3000 samples per day

- Targeted proteomics mass spectrometry dataset across 279 Alzheimer's patients
- 60+ mass spectrometry publications in peer-reviewed journals
- 50+ fully calibrated biomarkers per 10 minute run
- Latest mass spectrometry systems with the highest available sensitivity and throughput

CASE STUDIES

Anonymised to protect confidentiality

1

Top 25 Global Pharma

- Developing **Alzheimer's disease** therapeutic
- Required a biomarker panel for **patient stratification**
- Inoviv designed a bespoke panel of 50 biomarkers

2

Phase 2 US Pharma

- Developing **Alzheimer's disease and aging frailty** therapeutics
- Required an exploratory **treatment efficacy** panel
- Inoviv designed a bespoke panel of 150 biomarkers

3

US-headquartered international small Pharma (ongoing project)*

- Pre-clinical **Parkinson's disease** therapeutics
- We worked with them to begin developing a panel **aimed at diagnosing PD**
- Inoviv designed a **bespoke panel** of 20 biomarkers

*Results to be available early 2020

LEADERSHIP & ADVISORY BOARD

Combined over 1,200 publications among all team members

Michael Dove

CEO

Ernestas Sirka

CSO

Dr Roman Fischer

Head of Discovery Proteomics at **University of Oxford**

Professor Adrian Harris

Cancer Research UK Professor of Medical Oncology at **University of Oxford**

Professor Michael Heneka

Director Dept. Neurodegenerative Diseases & Gerontopsychiatry at **University of Bonn**

Jason Foster

Former General Manager at **Indivior**

Former Europe Marketing Director at **Reckitt Benckiser**

OUR LABS

We contract specialist laboratories based in the US and in the UK. All laboratories are equipped to support clinical trials.

United Kingdom

UK laboratories are **GLP/ GCP** and are based near **London** and **Manchester**.

United States

US laboratories are **GLP** and **CLIA-accredited** for high complexity testing.

The labs are based in **California**, **Indianapolis** and **Idaho**.

OUR PROCESS

Our standard process for working with pharmaceutical companies

✓ Establish **biomarker needs** & begin to **tailor biomarker panel** ✓ Share proposed overview of the panel	**1 month**
✓ **MILESTONE 1:** Licence provided for biomarker panel & **full panel shared** ✓ **MILESTONE 2: Panel reviewed,** developed further and **finalized**	**1 month**
✓ Develop study plan & project plan ✓ Set up test in lab & complete analytical validation	**5 months**
✓ **OPTIONAL MILESTONE 3:** Proof of concept/ feasibility study	**1-3 months**
✓ Receive biofluid samples at our laboratory ✓ **MILESTONE 4: Testing and data analysis** ✓ Reports provided	**ongoing**

STANDARD DOCUMENTS

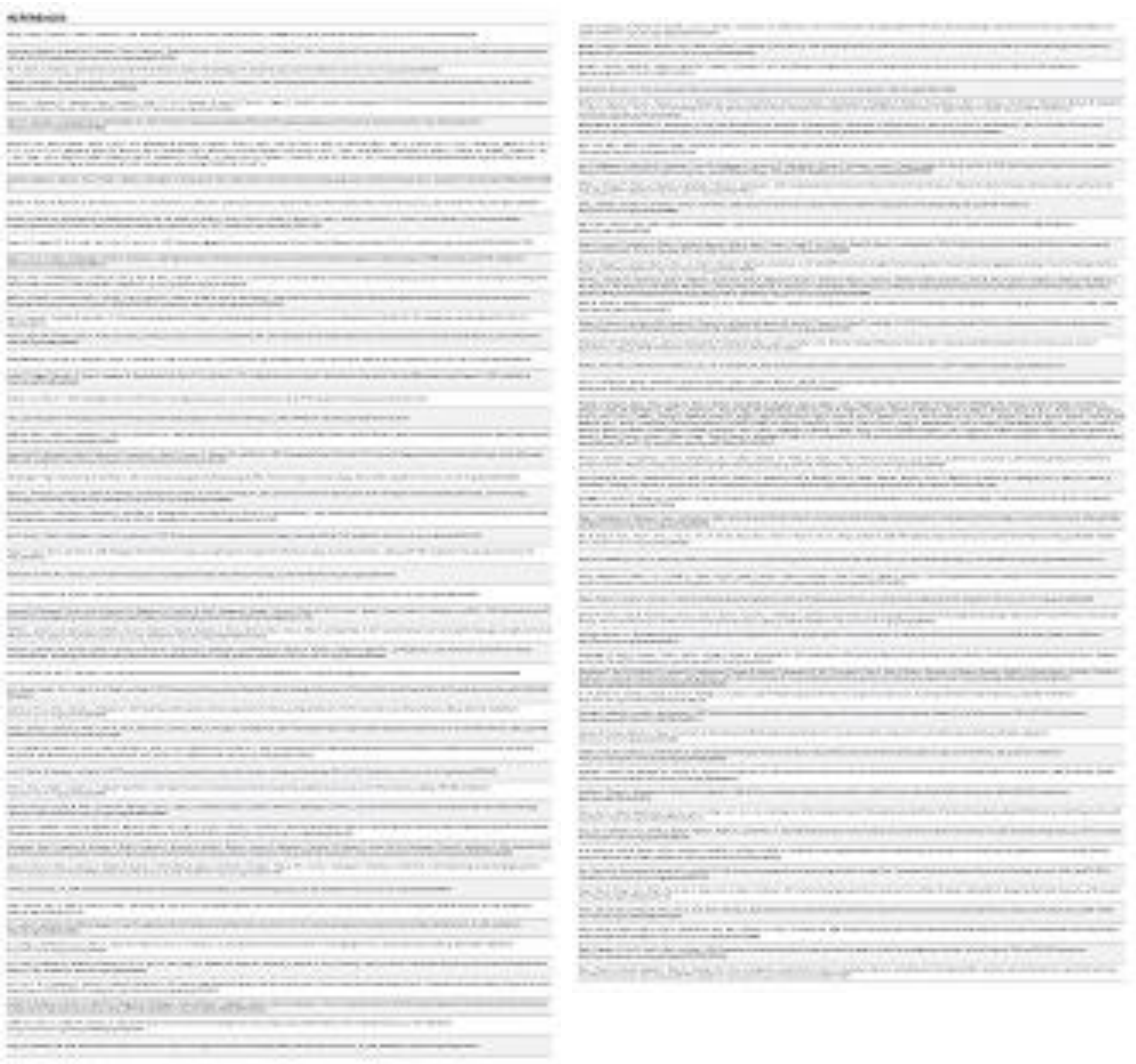
Biomarker Panel Template (can be amended as required)

Biomarker Panel Overview

INCLUDES

- List of biomarkers
- Relevant disease process
- Rationale for Inclusion in the Biomarker Panel
- References

Full Reference List



STANDARD DOCUMENTS

Our templates (can be amended as required)

Study Plan

Report PDF

Tabulated Data Excel

PARKINSON'S DISEASE BIOMARKER ASSAY PROOF OF CONCEPT PROJECT PLAN

[DATE]

TEMPLATE

Project Overview

Pharma PM & Lead Scientist:

Inoviv PM: Michael Dove

Inoviv Lead Scientist: Ernestas Sirka

Start/ End Dates:

Project Description:

Project Metrics	Status
Schedule	GREEN
Budget	GREEN
Stakeholder Management	GREEN
Logistics	GREEN
Technical	GREEN

Milestone	Due	Expected	Responsible
Finalise & sign NDA			
Select biofluid samples for PoC			
Finalise & sign Inoviv commercial contract			
Order chemicals for mass spectrometer			
Test samples & generate data			
Analyse data & provide report			

Actions planned next 2 weeks

- Finalise & sign NDA
- Discuss structure of PoC/ samples to use

Key discussion areas

Risks/ Issues

DISCOVERY PROTEOMICS

- **Discovery Proteomics** allows for **unbiased identification of patient signatures** within a broad and otherwise heterogenous patient population, which could be used to **discriminate between responders vs non-responders.**
- Using the latest high resolution mass spectrometry systems with the **highest sensitivity, throughput and proteome coverage available to date** enables identification and quantification of **~800-1200 proteins in CSF** and **~500-700 proteins in plasma and urine.**
- **Leveraging this broad and deep coverage of the proteome** could not only help in **identifying patient signatures**, but also to uncover the different processes affected by treatment with a given therapeutic to **identify biomarkers that could potentially be used to demonstrate treatment efficacy.**
- These rich data can **support decision-making as part of a clinical development program** and provide markers which could then be **transferred onto a robust and reproducible targeted proteomics platform** suitable for ongoing testing.

INOVIV DISCOVERY LC-MS/MS PLATFORM

FEATURES

Detection approach	Unbiased, high resolution detection of multiple peptides per protein
Sensitivity	Femtomole on column
Specificity	High mass accuracy (< 5 ppm mass error) for both MS and MS/MS data, very low false discovery rate (< 1% peptide & protein)
Reproducibility	Intraday CV < 10 %
Linear dynamic range	Up to 5 orders of magnitude
Multiplexing	1000s of proteins quantified in a 60 min run. Unlimited sample numbers and conditions can be analysed in a single batch
Cross-reactivity across multiplexed biomarker panels	No cross-reactivity, antibody free detection
Speed of assay development	No method development required
Flexibility to amend assays	Sample preparation workflow can be optimised for PTMs (e.g phosphorylation) Results can be directly translated into targeted assay design
Quantification	Label-free, relative quantification for identification of regulated markers. Absolute amounts (ng/ml) can be estimated
Sample run time	30 – 60 minutes
Sample types	Plasma, CSF, urine, tissues, others
Sample volume required	Plasma: 100 µl CSF: 500 µl Urine: 5 ml Tissue: 5-10 mg

TARGETED PROTEOMICS

Targeted Proteomics enables multiplexed high throughput protein measurements on a reproducible platform, which in turn **enables full technical and clinical validation to support ongoing testing in clinical trials.**

Inoviv's Neurodegenerative Biomarker Panels for Targeted Proteomics combine **50+ fully calibrated biomarkers** to cover **multiple aspects of disease pathology**, including: inflammation, oxidative stress, mitochondrial damage, amyloid processing, endothelial dysfunction, axonal degeneration, synaptic degeneration, lysosomal dysfunction and others.

The panels can be tailored to the specific needs of a program incorporating biomarkers uncovered with discovery proteomics.

These data can demonstrate the effects of a given therapeutic on the relevant disease processes to **provide robust treatment efficacy data.**

All data are generated in **GLP/GCP laboratories with FDA-compliant quality management systems** so that the data can **support regulatory submissions** and to enable subsequent **translation of the assay into healthcare settings run from a CLIA-accredited lab.**

INOVIV TARGETED LC-MS/MS PLATFORM

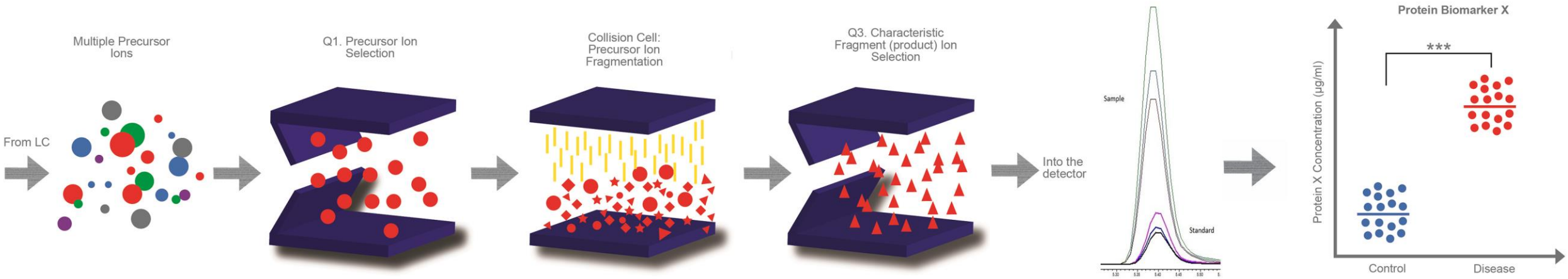
FEATURES

Detection approach	Detection of multiple peptides per protein biomarker
Sensitivity	0.5-5 ppm
Specificity	Multiple points of analyte identification and confirmation. Capable of distinguishing sequence variants, truncated isoforms or target proteins with different post-translational modifications.
Reproducibility	The highest possible reproducibility and robustness in multiple reaction monitoring (MRM) mode
Linear dynamic range	6 orders of magnitude
Multiplexing	50+ biomarkers per sample in a 10 min run; can be extended to hundreds
Cross-reactivity across multiplexed biomarker panels	No cross-reactivity, unlike antibodies/ ELISA
Speed of assay development	Short assay development timelines
Flexibility to amend assays	Capacity to rapidly adapt and update biomarker panel upon new scientific developments in the field
Absolute quantification	Fully quantitative and calibrated measurements
Sample run time	10 minutes
Sample types	Plasma, CSF, urine, tissues, others
Sample volume required	Plasma: 10 µl CSF: 100 µl Urine: 1ml Tissue: 5-10 mg

TARGETED MASS SPECTROMETRY WORKFLOW

Mass spectrometry (MS) is an analytical technique which quantifies biomarkers by utilising the mass-to-charge ratio (m/z) of a wide range of molecules

Schematic representation of the principles behind triple quadruple mass spectrometry (MRM-mode)



1. Mass spectrometry (MS) is coupled to liquid chromatography (LC) to separate the molecules prior to their introduction into the MS. After LC separation, the molecules are ionised in an electrospray ionisation source and introduced into the MS.

2. The pre-selected molecules of interest are isolated in the first quadrupole from the rest of the mixture, based on their characteristic mass-to-charge ratio (m/z).

3. Selected molecules are fragmented into characteristic fragments in a collision cell (a modified second quadrupole).

4. Characteristic fragments of selected molecules are isolated from the rest of the mixture in the third quadrupole based on their m/z.

5. The signal is recorded in the detector to achieve full quantification of molecules.

6. Fully quantitative measurements are ready for interpretation.

ANALYTICAL VALIDATION

Inoviv completes a standard validation as a minimum for every assay and can perform an extended validation at increased cost

STANDARD VALIDATION

Sensitivity (Lower limits of quantification (LLOQ))	X ng/ml for each biomarker at ±20% CV from ≥ 5 replicates in at least 3 runs
Linear range tested	X-Y ng/ml (assessed from: A blank (no analyte, no IS), a zero calibrator (blank plus IS), and at least six, non-zero calibrator levels)
Intra-day precision (%CV)	Aimed at below ±15% from at least 5 replicates
Inter-day precision (%CV)	Aimed at below ±15% from ≥ 5 replicates in at least 3 runs
Selectivity	Assessed from internal tracking of internal standard response stability and stability of product ion ratios; analysis of blank and zero calibrators
Matrix effects	Assessed from internal tracking of internal standard response stability and stability of product ion ratios

EXTENDED VALIDATION

Stability	For auto-sampler, bench-top, extract, freeze-thaw, stock solution and long-term stability at least three replicates at low and high QC concentrations are performed
Carryover	Aimed to not exceed 20% of LLOQ
Quality control (QC) samples	4 QC samples are established for ongoing testing at LLOQ, low, mid, and high concentration levels
Trueness (accuracy)	Aimed at ± 15% of nominal concentrations; except ± 20% at LLOQ
Recovery	Extracted samples at low, mid, and high QC concentrations versus extracts of blanks spiked with the analyte post extraction (at low, mid, and high concentration levels)

BIOMARKER ASSAY DEVELOPMENT AND REGULATORY PATHWAY

DISCLAIMER: information below on the regulatory pathway is for guidance only. Inoviv cannot guarantee approval by any regulatory body (FDA, EMA, MHRA and others). Regulations are subject to change and each biomarker project may be assessed differently.

1 Assay Design

1-2 months

- ✓ Establish biomarker needs for therapy in development
- ✓ Adapt Inoviv neurodegeneration biomarker panel according to needs
- ✓ Develop study plan, project plan, finalise report template

2 Determine pathway according to purpose of Assay

Exploratory Biomarker Assay

- ✓ Treatment efficacy data
- ✓ Deeper insights on the therapeutic agent mechanism of action

Integrated Biomarker Assay

- ✓ Patient stratification (without assigning treatment)
- ✓ Validate the Assay's clinical utility for use as an Integral Biomarker Assay for future clinical trials

Integral Biomarker Assay

- ✓ Clinical trial endpoint
- ✓ Patient stratification (including treatment selection)
- ✓ Diagnostic

3 Studies prior to Clinical Trial

Exploratory Biomarker Assay

Bioanalytical Method Validation — **6 months (if required)**

- ✓ Bioanalytical method development defines the design, operating conditions, limitations, and suitability of the method for its intended purpose.
- ✓ Establishment of the following bioanalytical parameters: sensitivity (LLOQ), linear range tested, Intra-day precision (%CV), Inter-day precision (%CV), selectivity and matrix effects.

Integrated Biomarker Assay

Bioanalytical Method Validation

- ✓ Bioanalytical method development defines the design, operating conditions, limitations, and suitability of the method for its intended purpose.
- ✓ Establishment of the following bioanalytical parameters: sensitivity (LLOQ), linear range tested, Intra-day precision (%CV), Inter-day precision (%CV), selectivity and matrix effects

6 months (if required)

Regulatory Requirements

- ✓ Biomarker protocol included in study plan submitted to regulatory body prior to clinical trial
- ✓ Biomarker supported by strong mechanistic and/or epidemiologic rationale

1-2 months

Integral Biomarker Assay

Regulatory Requirements: Bioanalytical Validation — **6-12 months**

- ✓ Biomarker supported by clear mechanistic rationale & clinical data; can be generated through integrated biomarker studies
- ✓ Clinical data providing strong evidence the biomarker has a specific clinical benefit
- ✓ Validated analytical methods provided for the quantitative evaluation of analytes with optimized extended validation parameters: Stability, Carryover, Quality control (QC) samples, Trueness, Recovery in addition to sensitivity (LLOQ), linear range tested, Intra-day precision (%CV), Inter-day precision (%CV), selectivity and matrix effects
- ✓ Sponsor provides clinical data through IND, NDA and BLA submissions to FDA for acceptance of biomarker for its intended purpose

4 Clinical Trial Biomarker Analysis

Ongoing

- ✓ Receive biofluid samples to our CLIA-certified/ GLP laboratories
- ✓ Testing and data analysis
- ✓ Biomarker Reports provided

DISCOVERY PROTEOMICS PUBLICATION

By Roman Fischer, Inoviv Scientific Advisor, and others

Annals of NEUROLOGY
An Official Journal of the American Neurological Association and the Child Neurology Society
AMERICAN NEUROLOGICAL ASSOCIATION

Cerebrospinal Fluid Macrophage Biomarkers in Amyotrophic Lateral Sclerosis

Alexander G. Thompson, BA, BMBCh,[1]* Elizabeth Gray, BSc, PhD,[1]*
Marie-Laëtitia Thézénas, MSc,[2] Philip D. Charles, MA, MSc,[2]
Samuel Evetts, BSc, MSc,[1] Michele T. Hu, MBBS, PhD,[1]
Kevin Talbot, MBBS, DPhil,[1] Roman Fischer, PhD,[2]
Benedikt M. Kessler, BM, PhD,[2] and Martin R. Turner, MA, MBBS, PhD[1]

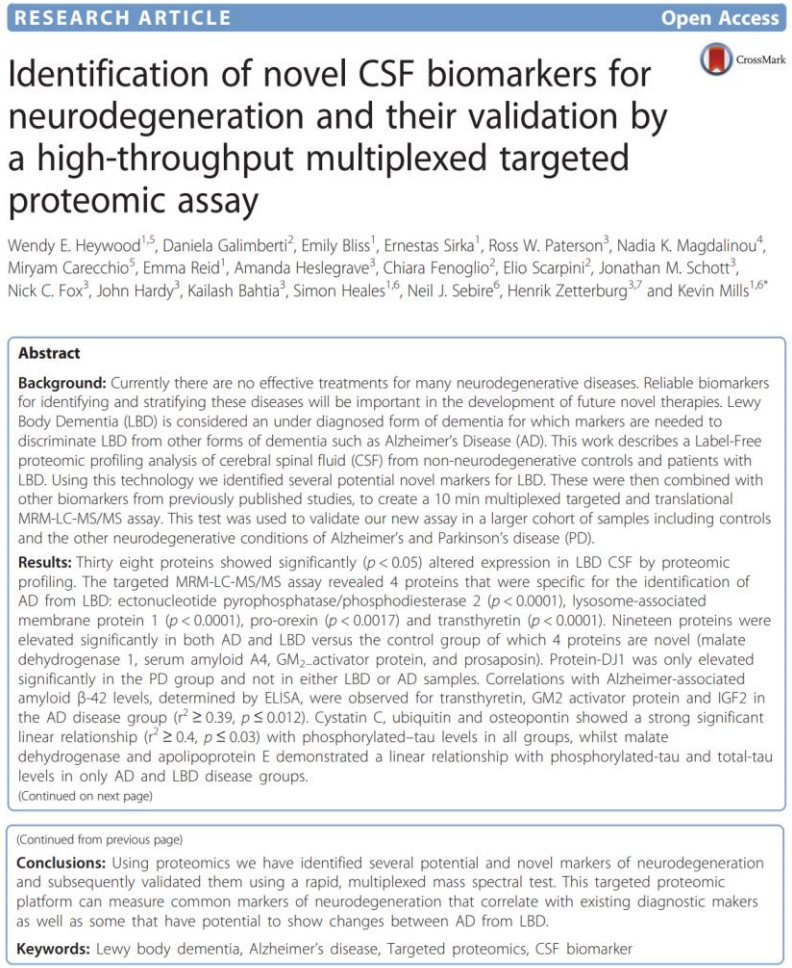
Objective: The neurodegenerative disease, amyotrophic lateral sclerosis (ALS), is a heterogeneous clinical syndrome involving multiple molecular pathways. The development of biomarkers for use in therapeutic trials is a priority. We sought to use a high-throughput proteomic method to identify novel biomarkers in individual cerebrospinal fluid (CSF) samples.
Methods: Liquid chromatography/tandem mass spectrometry with label-free quantification was used to identify CSF proteins using samples from a well-characterized longitudinal cohort comprising patients with ALS (n = 43), the upper motor neuron variant, primary lateral sclerosis (PLS; n = 6), and cross-sectional healthy (n = 20) and disease controls (Parkinsons' disease, n = 20; ALS mimic disorders, n = 12).
Results: Three macrophage-derived chitinases showed increased abundance in ALS: chitotriosidase (CHIT1), chitinase-3-like protein 1 (CHI3L1), and chitinase-3-like protein 2 (CHI3L2). Elevated CHI3L1 was common to ALS and PLS, whereas CHIT1 and CHI3L2 levels differed. Chitinase levels correlated with disease progression rate (CHIT1, $r = 0.56$, $p < 0.001$; CHI3L1, $r = 0.31$; $p = 0.028$; CHI3L2, $r = 0.29$, $p = 0.044$). CHIT1, CHI3L1, and CHI3L2 levels correlated with phosphorylated neurofilament heavy chain (pNFH; $r = 0.62$, $p < 0.001$; $r = 0.49$, $p < 0.001$; $r = 0.41$, $p < 0.001$). CHI3L1 levels, but not CHIT1 or CHI3L2, increased over time in those with low initial levels (gradient = 0.005 log abundance units/month, $p = 0.001$). High CHIT1 was associated with shortened survival (hazard ratio [HR] 2.84; $p = 0.009$). Inclusion of pNFH in survival models left only an association of pNFH and survival (HR 1.26; $p = 0.019$).
Interpretation: Neuroinflammatory mechanisms have been consistently implicated through various experimental paradigms. These results support a key role for macrophage activity in ALS pathogenesis, offering novel target engagement and pharmacodynamic biomarkers for neuroinflammation-focused ALS therapy.

ANN NEUROL 2018;83:258–268

- LC-MS discovery proteomics study to identify CSF biomarkers in ALS patients
- 773 protein groups were identified and quantified across conditions
- 19 protein groups were differentially abundant across different groups analysed
- 3 proteins were elevated in ALS compared to other groups: CHIT1, CHI3L1 and CHI3L2
- Levels of CHIT1, CHI3L1 and CHI3L2 correlate with disease progression rate and with pNFH (a marker of axonal damage)
- Baseline CHIT1 level is associated with survival

NEURODEGENERATION BIOMARKER PUBLICATION (1)

By Ernestas Sirka, Inoviv CSO and others



RESEARCH ARTICLE Open Access

Identification of novel CSF biomarkers for neurodegeneration and their validation by a high-throughput multiplexed targeted proteomic assay

Wendy E. Heywood[1,5], Daniela Galimberti[2], Emily Bliss[1], Ernestas Sirka[1], Ross W. Paterson[3], Nadia K. Magdalinou[4], Miryam Carecchio[5], Emma Reid[1], Amanda Heslegrave[3], Chiara Fenoglio[2], Elio Scarpini[2], Jonathan M. Schott[3], Nick C. Fox[3], John Hardy[3], Kailash Bahtia[3], Simon Heales[1,6], Neil J. Sebire[6], Henrik Zetterburg[3,7] and Kevin Mills[1,6*]

Abstract

Background: Currently there are no effective treatments for many neurodegenerative diseases. Reliable biomarkers for identifying and stratifying these diseases will be important in the development of future novel therapies. Lewy Body Dementia (LBD) is considered an under diagnosed form of dementia for which markers are needed to discriminate LBD from other forms of dementia such as Alzheimer's Disease (AD). This work describes a Label-Free proteomic profiling analysis of cerebral spinal fluid (CSF) from non-neurodegenerative controls and patients with LBD. Using this technology we identified several potential novel markers for LBD. These were then combined with other biomarkers from previously published studies, to create a 10 min multiplexed targeted and translational MRM-LC-MS/MS assay. This test was used to validate our new assay in a larger cohort of samples including controls and the other neurodegenerative conditions of Alzheimer's and Parkinson's disease (PD).

Results: Thirty eight proteins showed significantly ($p < 0.05$) altered expression in LBD CSF by proteomic profiling. The targeted MRM-LC-MS/MS assay revealed 4 proteins that were specific for the identification of AD from LBD: ectonucleotide pyrophosphatase/phosphodiesterase 2 ($p < 0.0001$), lysosome-associated membrane protein 1 ($p < 0.0001$), pro-orexin ($p < 0.0017$) and transthyretin ($p < 0.0001$). Nineteen proteins were elevated significantly in both AD and LBD versus the control group of which 4 proteins are novel (malate dehydrogenase 1, serum amyloid A4, GM_2-activator protein, and prosaposin). Protein-DJ1 was only elevated significantly in the PD group and not in either LBD or AD samples. Correlations with Alzheimer-associated amyloid β-42 levels, determined by ELISA, were observed for transthyretin, GM2 activator protein and IGF2 in the AD disease group ($r^2 \geq 0.39$, $p \leq 0.012$). Cystatin C, ubiquitin and osteopontin showed a strong significant linear relationship ($r^2 \geq 0.4$, $p \leq 0.03$) with phosphorylated–tau levels in all groups, whilst malate dehydrogenase and apolipoprotein E demonstrated a linear relationship with phosphorylated-tau and total-tau levels in only AD and LBD disease groups.

(Continued on next page)

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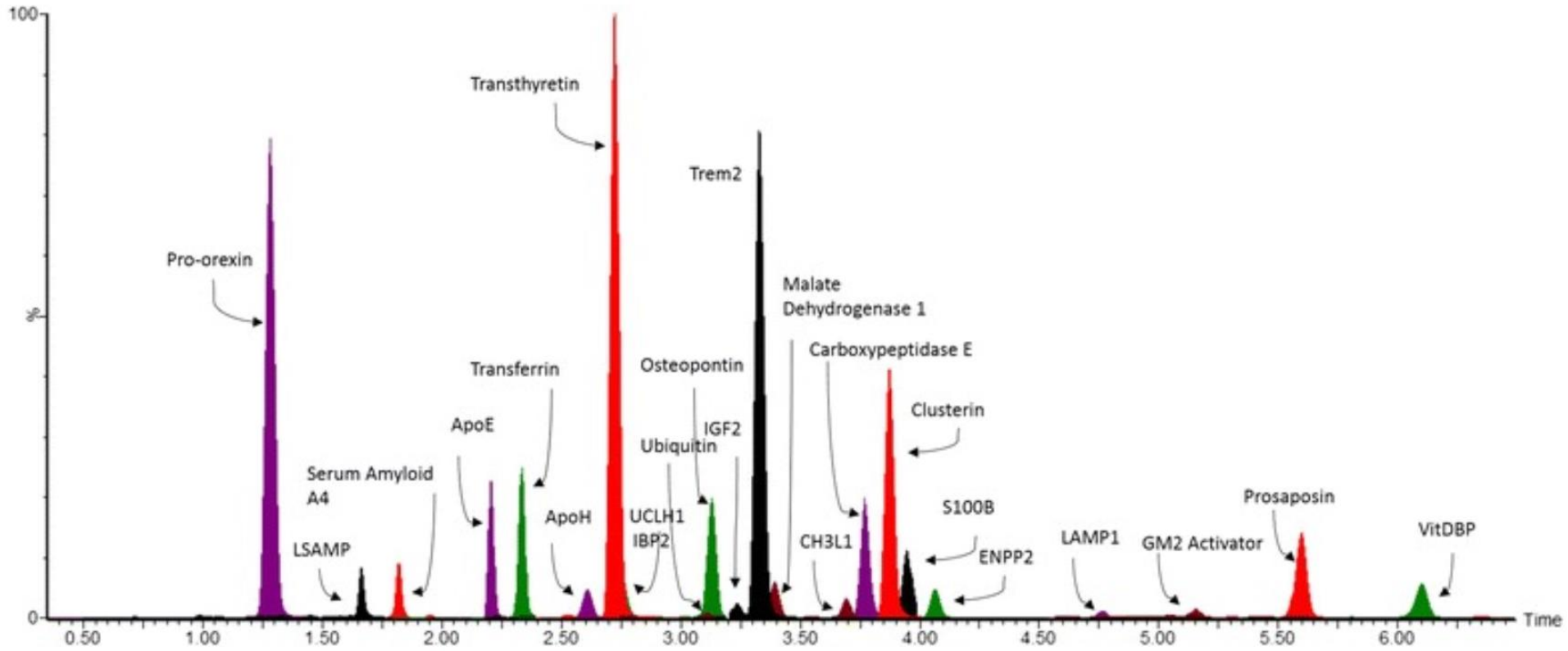
Conclusions: Using proteomics we have identified several potential and novel markers of neurodegeneration and subsequently validated them using a rapid, multiplexed mass spectral test. This targeted proteomic platform can measure common markers of neurodegeneration that correlate with existing diagnostic makers as well as some that have potential to show changes between AD from LBD.

Keywords: Lewy body dementia, Alzheimer's disease, Targeted proteomics, CSF biomarker

- Developed a targeted, multiplexed LC-MS/MS CSF biomarker assay
- 50+ biomarkers quantified simultaneously
- The assay contains previously reported as well as novel biomarkers for neurodegeneration
- 19 proteins specific to Alzheimer's disease and Lewy Body Dementia
- 4 proteins specific to Alzheimer's disease
- 7 novel proteins, previously not associated with neurodegeneration

NEURODEGENERATION BIOMARKER PUBLICATION (1)

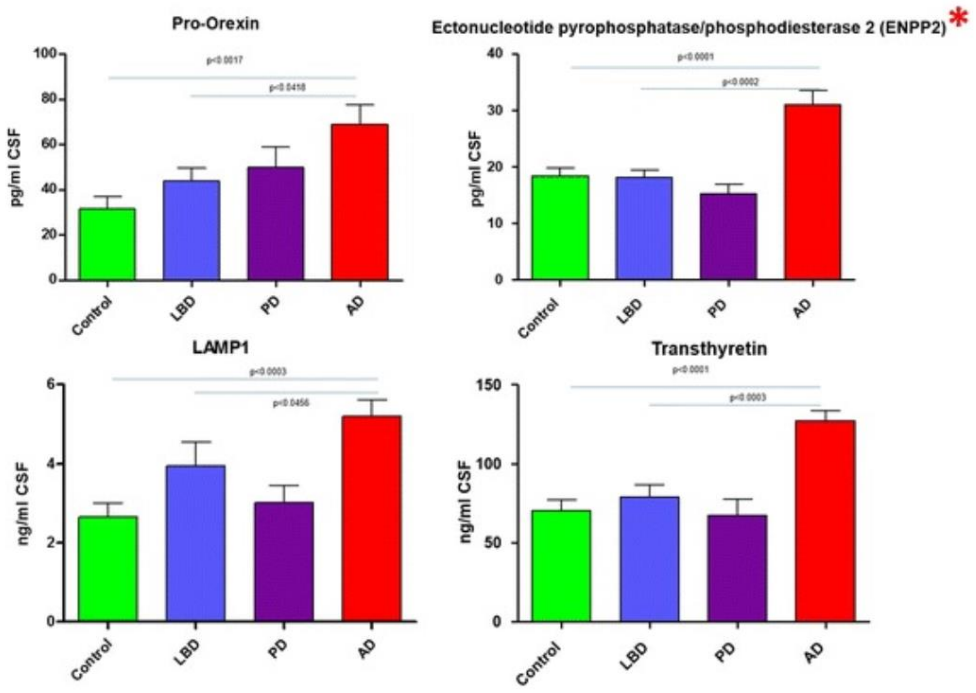
By Ernestas Sirka, Inoviv CSO and others



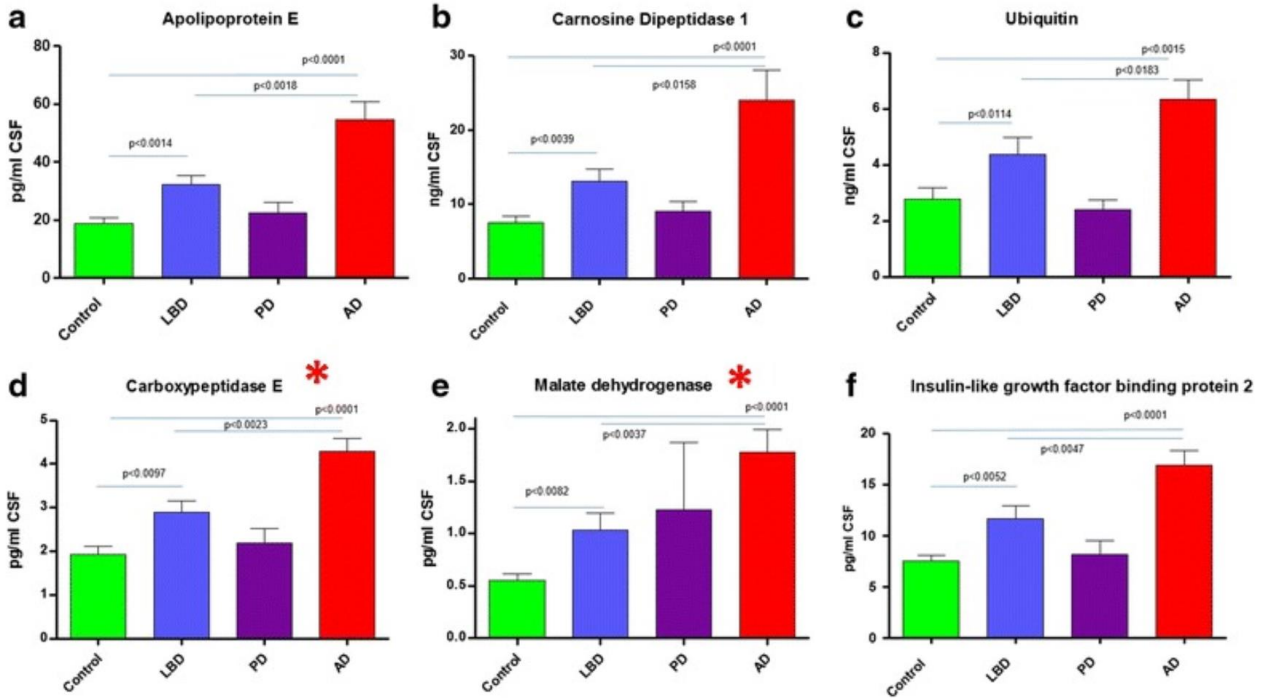
Overlaid chromatogram of the biomarker peptides included in the multiplexed targeted proteomic assay. The assay was developed to quantitate 50+ peptides in a 10 min LC run.

NEURODEGENERATION BIOMARKER PUBLICATION (1)

By Ernestas Sirka, Inoviv CSO and others



Alzheimer's disease specific markers. The results of the multiplexed MRM-based LC-MS/MS assay of protein biomarkers quantitated in the CSF of control, Lewy body dementia (LBD), Parkinson's disease (PD) and Alzheimer's disease (AD) patient samples. * Denotes a new marker not described previously.



Common dementia markers that are significantly elevated in AD compared to LBD. Graphs **a**-**f** show the results of the targeted multiplexed assay of protein biomarkers quantitated in the CSF samples of control, Lewy body dementia, Parkinson's and Alzheimer's disease patients.* Denotes new biomarkers not described previously. Modified from Mol Neurodegener. 2015; 10: 64.

NEURODEGENERATION BIOMARKER PUBLICATION (2)

By Ernestas Sirka, Inoviv CSO and others

ORIGINAL ARTICLE

A targeted proteomic multiplex CSF assay identifies increased malate dehydrogenase and other neurodegenerative biomarkers in individuals with Alzheimer's disease pathology

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Alzheimer's disease (AD) is the most common cause of dementia. Biomarkers are required to identify individuals in the preclinical phase, explain phenotypic diversity, measure progression and estimate prognosis. The development of assays to validate candidate biomarkers is costly and time-consuming. Targeted proteomics is an attractive means of quantifying novel proteins in cerebrospinal and other fluids, and has potential to help overcome this bottleneck in biomarker development. We used a previously validated multiplexed 10-min, targeted proteomic assay to assess 54 candidate cerebrospinal fluid (CSF) biomarkers in two independent cohorts comprising individuals with neurodegenerative dementias and healthy controls. Individuals were classified as 'AD' or 'non-AD' on the basis of their CSF T-tau and amyloid Aβ1–42 profile measured using enzyme-linked immunosorbent assay; biomarkers of interest were compared using univariate and multivariate analyses. In all, 35/31 individuals in Cohort 1 and 46/36 in Cohort 2 fulfilled criteria for AD/non-AD profile CSF, respectively. After adjustment for multiple comparisons, five proteins were elevated significantly in AD CSF compared with non-AD CSF in both cohorts: malate dehydrogenase; total APOE; chitinase-3-like protein 1 (YKL-40); osteopontin and cystatin C. In an independent multivariate orthogonal projection to latent structures discriminant analysis (OPLS-DA), these proteins were also identified as major contributors to the separation between AD and non-AD in both cohorts. Independent of CSF Aβ1–42 and tau, a combination of these biomarkers differentiated AD and non-AD with an area under curve (AUC) = 0.88. This targeted proteomic multiple reaction monitoring (MRM)-based assay can simultaneously and rapidly measure multiple candidate CSF biomarkers. Applying this technique to AD we demonstrate differences in proteins involved in glucose metabolism and neuroinflammation that collectively have potential clinical diagnostic utility.

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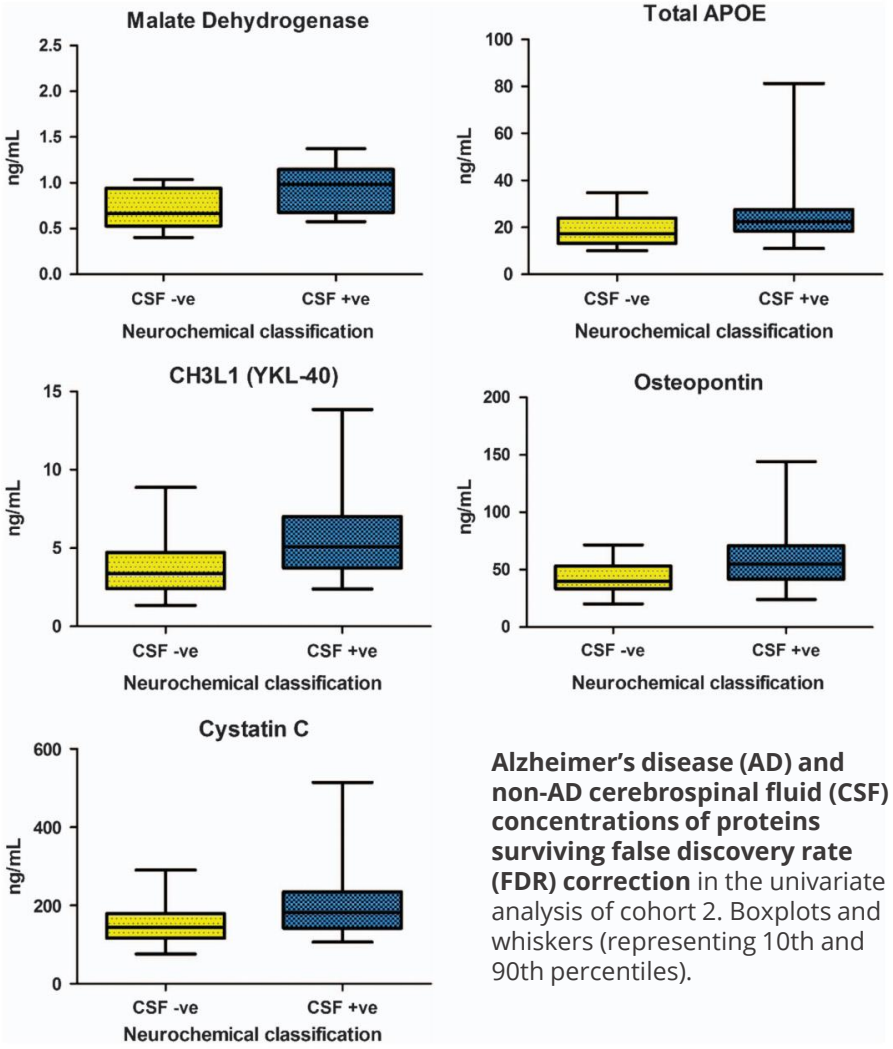
- Previously validated 10min targeted LC-MS/MS assay; further cohort of Alzheimer's disease samples
- 50+ biomarkers assessed in two independent cohorts
- Univariate and multivariate statistical analysis
- 5 significantly elevated proteins in both cohorts, differentiating AD from non-AD CSF with the AUC=0.88
- Demonstrated differences in proteins involved in glucose metabolism and neuroinflammation

NEURODEGENERATION BIOMARKER PUBLICATION (2)

By Ernestas Sirka, Inoviv CSO and others



Alzheimer's disease (AD) and non-AD cerebrospinal fluid (CSF) concentrations of proteins surviving false discovery rate (FDR) correction in the univariate analysis of cohort 2. Boxplots and whiskers (representing 10th and 90th percentiles).

Table 2A. Univariate analysis comparing biomarkers in AD and non-AD CSF from Cohort 2

	P-value (cohort 1)	*P-value (cohort 2)*	*Fold change in cohort 2*
Malate dehydrogenase[a]	**0.005***	**<0.001***	**2.12**
Total APOE[a]	**<0.001***	**0.005***	**1.55**
Chitinase-3-like protein 1(YKL-40)[a]	**<0.001***	**<0.001***	**1.52**
Osteopontin[a]	**<0.001***	**<0.001***	**1.50**
NCAM1	0.03	0.38	1.40
UCLH1	0.003*	0.88	1.30
Cystatin C[a]	**0.008***	**0.003***	**1.28**
Beta-amyloid 40	*<0.001**	*0.01*	*1.28*
CNDP1	*0.01**	*0.03*	*1.26*
V-Set and transmembrane domain containing protein 2A	0.03	0.06	1.25
Fibrinogen A	0.03*	0.83	1.24
IBP-2	*0.007**	*0.04*	*1.20*
S100B	<0.001*	0.06	1.20
TREM2	0.001*	0.05	1.18
Serum amyloid p-component	0.007*	0.33	1.14
CD166	0.03	0.25	1.12
Pro-orexin	<0.001	0.22	1.11
TIMP metallopeptidase inhibitor 1	0.03	0.5	1.05
IGF2	0.005*	0.72	0.97
Glutathione-S-transferase omega-1	0.006*	0.75	0.91
ENPP2	0.05	0.11	0.89

Abbreviations: AD, Alzheimer's disease; CNDP1, carnosine dipeptidase 1; CSF, cerebrospinal fluid; FDR, false discovery rate; IBP-2, insulin-like growth factor-binding protein 2; IGF2, insulin-like growth factor 2; NCAM1, neural cell adhesion molecule 1; OPLS-DA, orthogonal projection to latent structures discriminant analysis; TREM2, triggering receptor expressed on myeloid cells 2; UCLH1, ubiquitin carboxyl-terminal esterase 1. *Denotes a *P*-value that survived FDR correction. Bold indicates a biomarker that differentiated neurochemical AD from non-AD—significant after FDR correction in test and validation cohorts. Italics indicate a biomarker that differentiated neurochemical AD from non-AD—significant after FDR correction in test cohort only. [a]Denotes biomarkers also identified using OPLS-DA analysis where subjects were classified neurochemically.

NEURODEGENERATION BIOMARKER PUBLICATION (3)

By Ernestas Sirka, Inoviv CSO and others

nature medicine

LETTERS

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Fetal gene therapy for neurodegenerative disease of infants

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For inherited genetic diseases, fetal gene therapy offers the potential of prophylaxis against early, irreversible and lethal pathological change. To explore this, we studied neuronopathic Gaucher disease (nGD), caused by mutations in *GBA*. In adult patients, the milder form presents with hepatomegaly, splenomegaly and occasional lung and bone disease; this is managed, symptomatically, by enzyme replacement therapy. The acute childhood lethal form of nGD is untreatable since enzyme cannot cross the blood-brain barrier. Patients with nGD exhibit signs consistent with hindbrain neurodegeneration, including neck hyperextension, strabismus and, often, fatal apnea[1]. We selected a mouse model of nGD carrying a *loxP*-flanked neomycin disruption of *Gba* plus Cre recombinase regulated by the keratinocyte-specific K14 promoter. Exclusive skin expression of *Gba* prevents fatal neonatal dehydration. Instead, mice develop fatal neurodegeneration within 15 days[2]. Using this model, fetal intracranial injection of adeno-associated virus (AAV) vector reconstituted neuronal glucocerebrosidase expression. Mice lived for up to at least 18 weeks, were fertile and fully mobile. Neurodegeneration was abolished and neuroinflammation ameliorated. Neonatal intervention also rescued mice but less effectively. As the next step to clinical translation, we also demonstrated the feasibility of ultrasound-guided global AAV gene transfer to fetal macaque brains.

NEURODEGENERATION BIOMARKER PUBLICATION (3)

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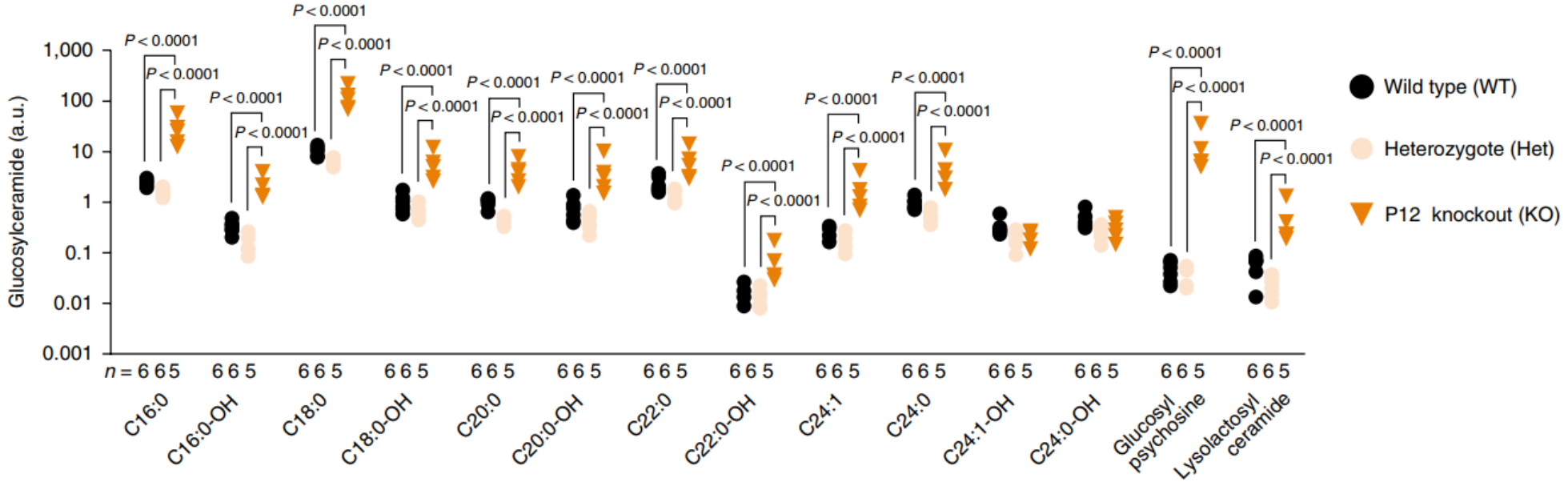

- Developed a 10min targeted LC-MS/MS assay
- Multiplexed lipid measurements
- Clear-cut differences in biomarker levels between control and disease as well as pre- and post-treatment