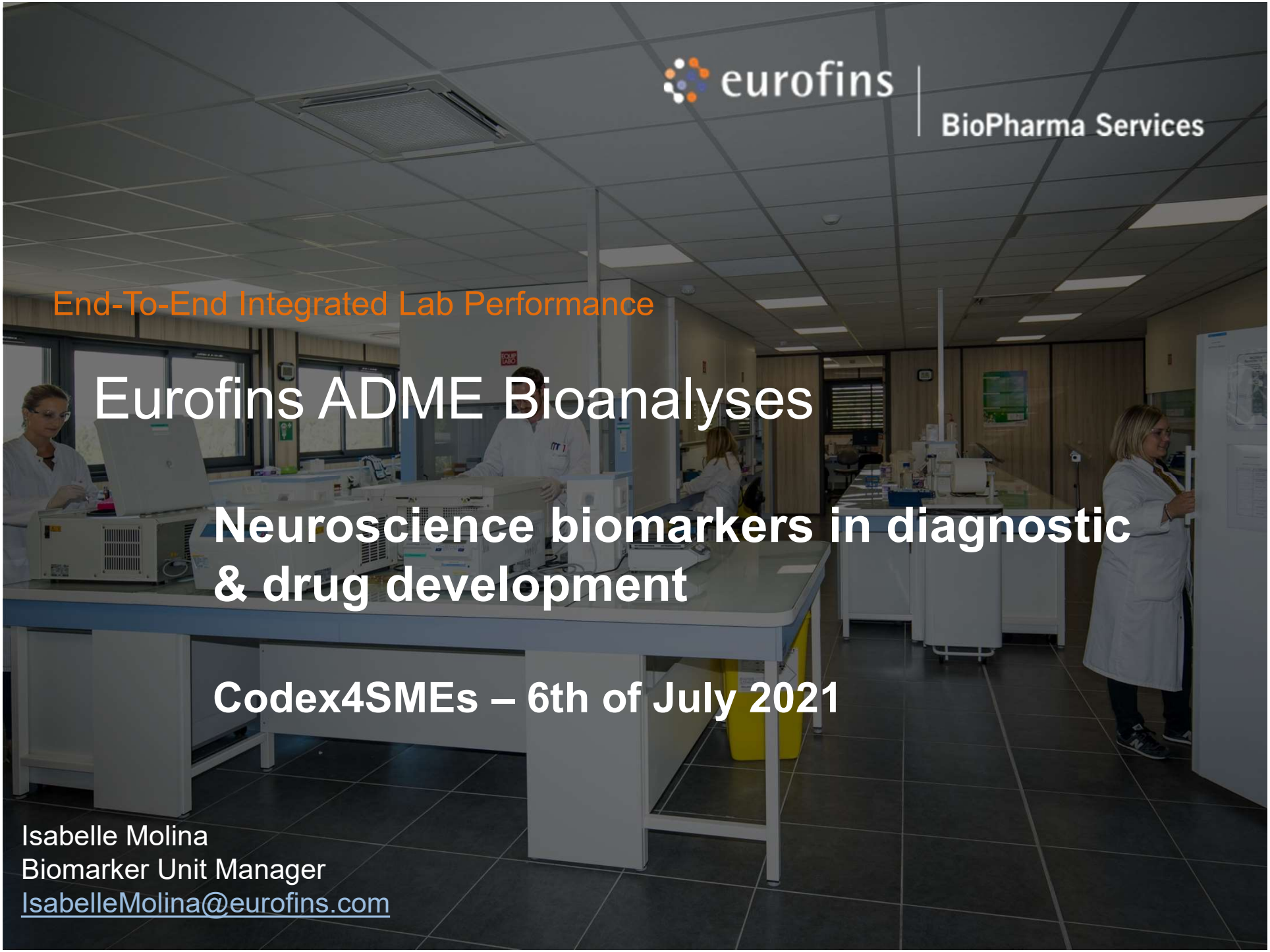

End-To-End Integrated Lab Performance

Eurofins ADME Bioanalyses

Neuroscience biomarkers in diagnostic & drug development

Codex4SMEs – 6th of July 2021

Isabelle Molina
Biomarker Unit Manager
IsabelleMolina@eurofins.com

Biomarkers – Definition (NIH)

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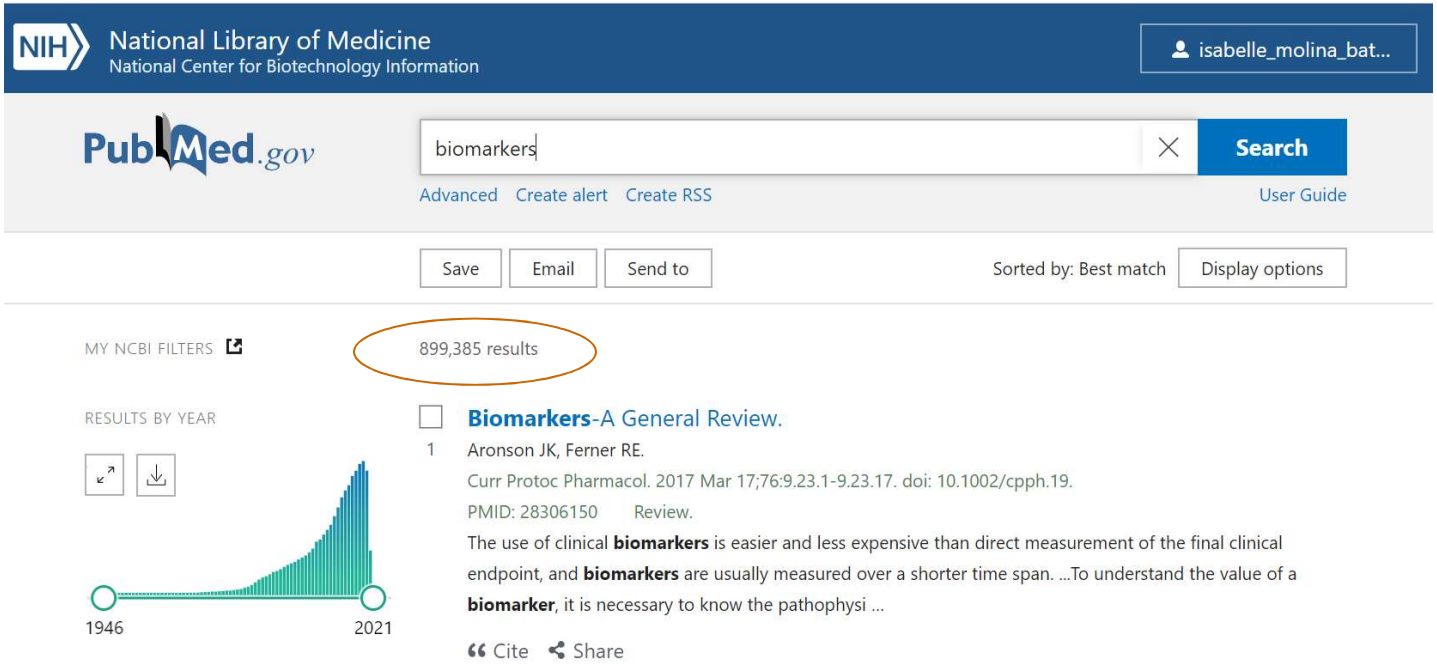
Supporting DMPK from screening to registration

A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

They inform about natural history of a disease or drug activity. They can be used as metrics.



Biomarkers - History

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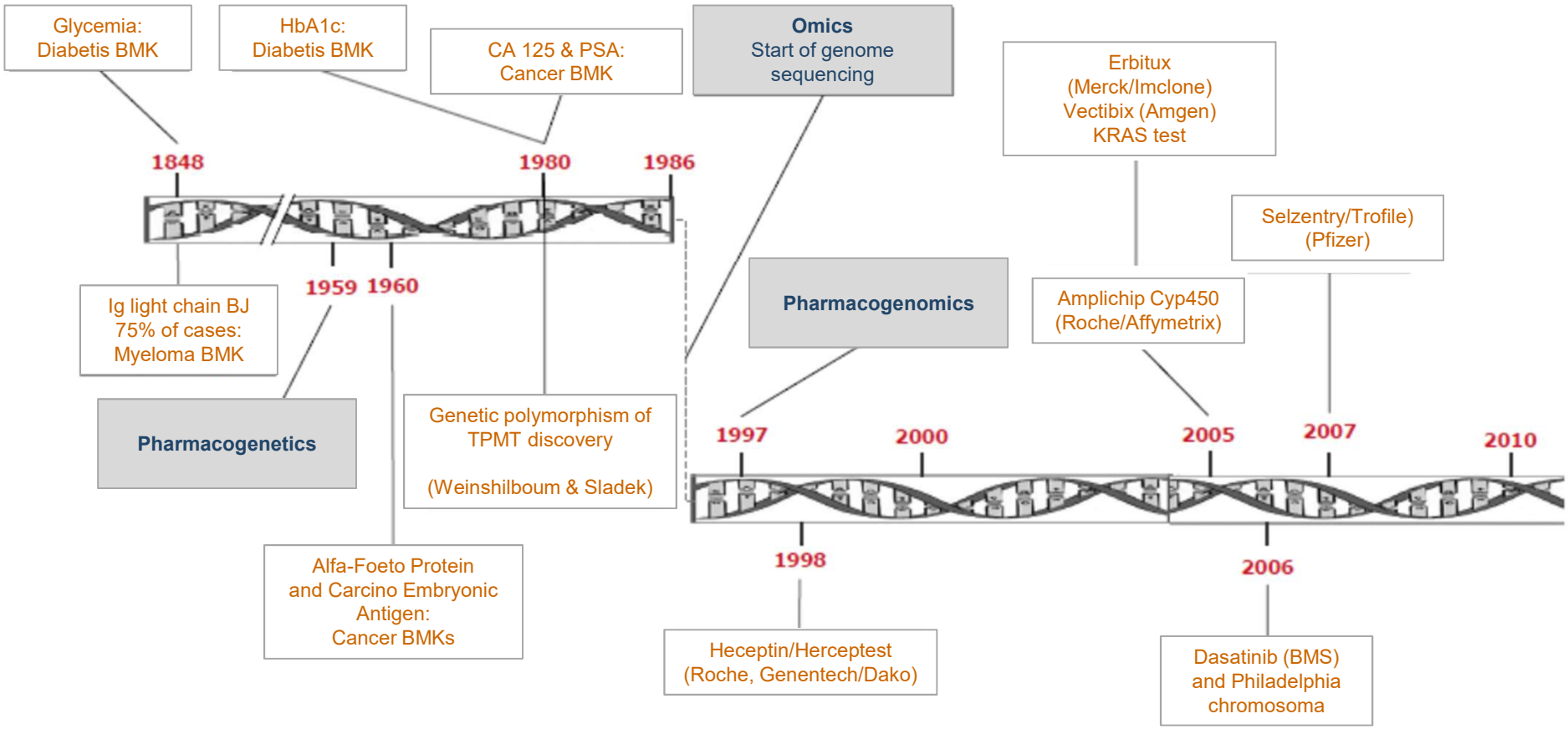
Supporting DMPK from screening to registration



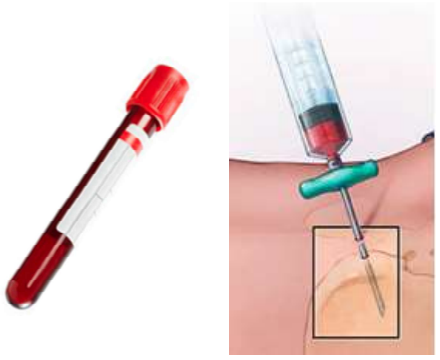
Ig: Immunoglobulin
BJ: Bence-Jones proteins
PSA: prostate specific antigen
TPMT: thiopurine-methyltransferase

Adapted from: BMS : Bristol-Myers Squibb

Biomarkers - Types

- Invasive biomarkers

proteins, genes (DNA, RNA), carbohydrates, metabolites, enzymatic activity: *tissue biopsies & biofluids*
e.g. creatinine for kidney function, LDL cholesterol for cardio diseases, PSA for prostate cancer…



Credit: https://www.tymora-analytical.com/

- Non-Invasive Biomarkers

ultrasound, elastometry: *physical technologies*
MRI, PET, echography: *imagery*

Credit: https://www.echosens.com/products/fibroscan-expert//

An imaging biomarker is a biological characteristic that is detectable on an image (analytical or qualitative).
e.g. blood pressure to check on heart diseases and cardiovascular risk, FibroScan® (kPa) for liver fibrosis, liver steatosis and portal hypertension assessment.

Biomarkers diversity & Biofluids

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Supporting DMPK from screening to registration

Human genome

≈ 35 000 genes

↓

300 000 mRNA
(alternative splicing)

↓

over 1 million functional proteins
(post translational modifications)

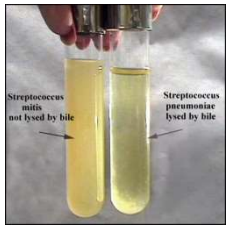
Human proteomes

urine
≈2500 proteins
sysdiag.cnrs.fr/DUP/

plasma
serum
≈3000 proteins
HUPO PPP Omenn, 2007

CSF
≈2600 proteins
Schutzer, Plos One 2010

tears
≈1500 proteins
Zhou.L, J of Proteomics, 2012



Saliva
≈1100 proteins
Denny, JPR, 2008
30% of plasma/tears proteins

Biomarkers – Assay types

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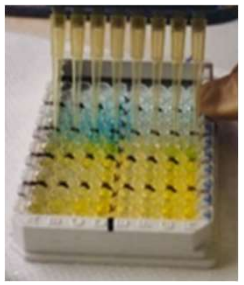
Supporting DMPK from screening to registration



- Quantitative or semi-quantitative assays

Method of analysis whose response is the amount of analyte measured either directly (counting in a mass or volume), or indirectly (color absorbance, impedance, etc.) in a certain amount of sample.

Quantitative: biochemistry, hormonology , blood counts…

Semi-quantitative : OD with a threshold effect (EIA or RIA) - Serology

- Qualitative assays

Method of analysis whose response is qualitative (presence/absence of the analyte) and measured either directly or indirectly (color absorbance, impedance, etc.) in a certain amount of sample

Immuno-chromatography (Western-Blot, lateral-flow, etc.), latex Agglutination, Immuno-electrophoresis,

Immunofluorescence (auto-antibody, serology, bacteria screening…)

Biomarkers
Dx & Drug development

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Supporting DMPK from screening to registration

Two major types of BMK with different rules of validation and registration – IVD

- Medical Device: BMK used independently to a specific drug, such as diagnostic or monitoring assays

Diagnostic / Early detection:

Disease vs. Healthy, Gene test for monogenic diseases (like cystic fibrosis), PSA (prostate cancer), cardiac markers…

Monitoring:

HbA1c (diabetes), Immune markers (after transplantation), titer (after vaccination)

Registration : Europe « Directive 98/79/CE DMDIV » – US « FDA 21CFR 820 »

- Companion BM

BMK used once before treatment to predict patients response to treatment

BMK used with drug development: safety BMK, efficacy BMK, dose selection

Registration : recommendations from EMA « Draft guideline on key aspects for the use of pharmacogenomic methodologies in the pharmacovigilance evaluation of medicinal products», January 2014 and FDA « FDA guidance In vitro companion diagnostic devices » August, 2014.

Biomarkers – Context Of Use

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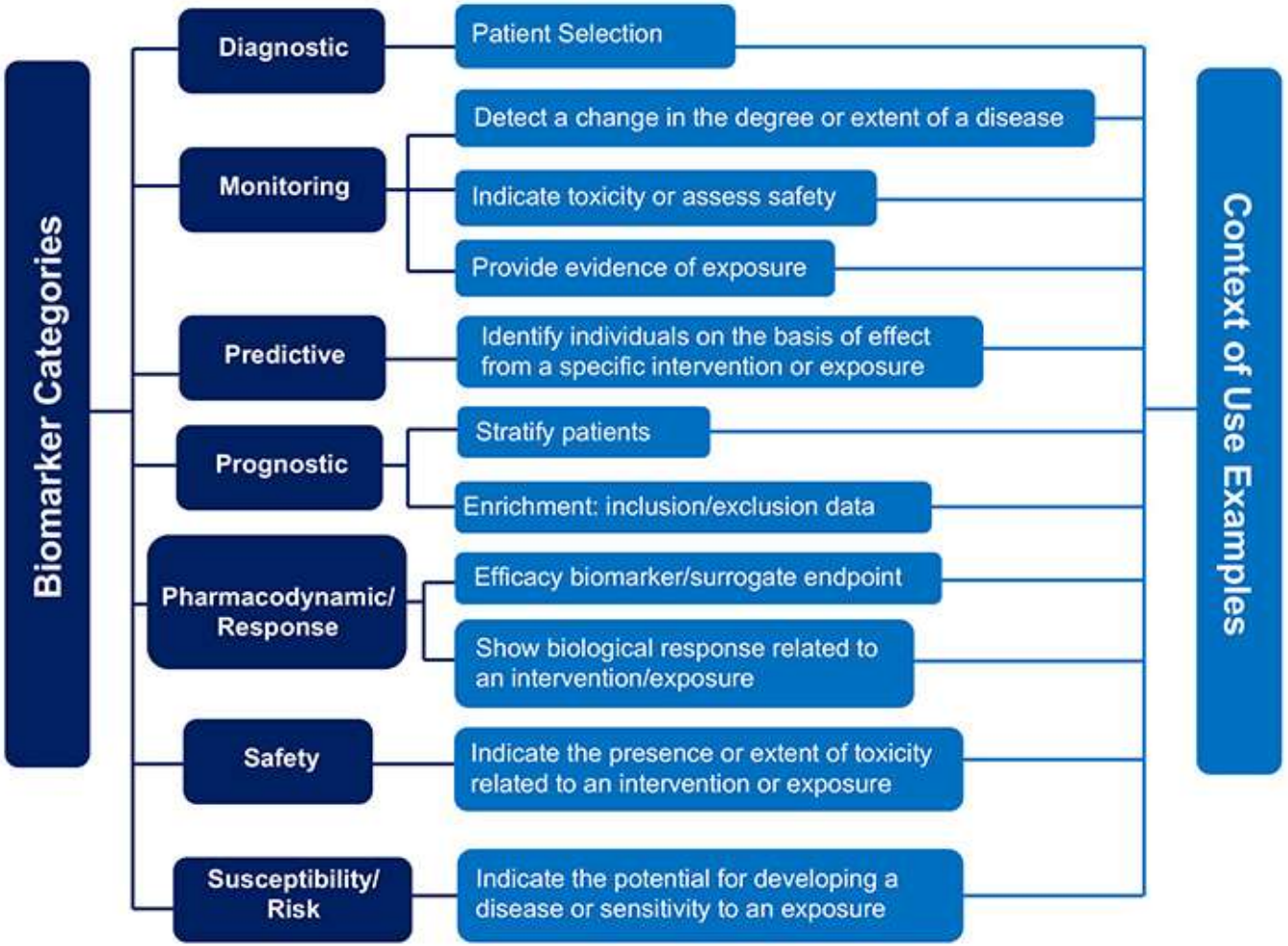
Supporting DMPK from screening to registration



COU drives everything !

https://www.fda.gov/drugs/biomarker-qualification-program/context-use

Diagnostic

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Supporting DMPK from screening to registration

- Five major phases and decision gates (or Milestone) in the Biomarker development process: 3 – 4y (optimal timelines)

*Customer needs definition
*Mkg Product design
*Time line
*Resources
*Business Plan
6-9 months

*Product Design specification
*Formulation
*Design freeze
*Analytical & clinical validations
9-12 months

*Product optimization vs industrial and economic constraints
*R&D batches validation
6-9 months

*Transfer to manufacturing
*Production of Pilot batches
*Performance validation
6-9 months

*Customer needs definition
*Time line
*Resources-
*Business Plan

$$Se = \frac{TP}{FN + TP}$$

- Diagnostic sensitivity

Ability of an IVD examination procedure to identify the presence of a target marker associated with a particular disease or condition

A test with 100% sensitivity correctly identifies all patients with the disease. A test with 80% sensitivity detects 80% of patients with the disease (true positives) but 20% with the disease go undetected (false negatives).

Drug development

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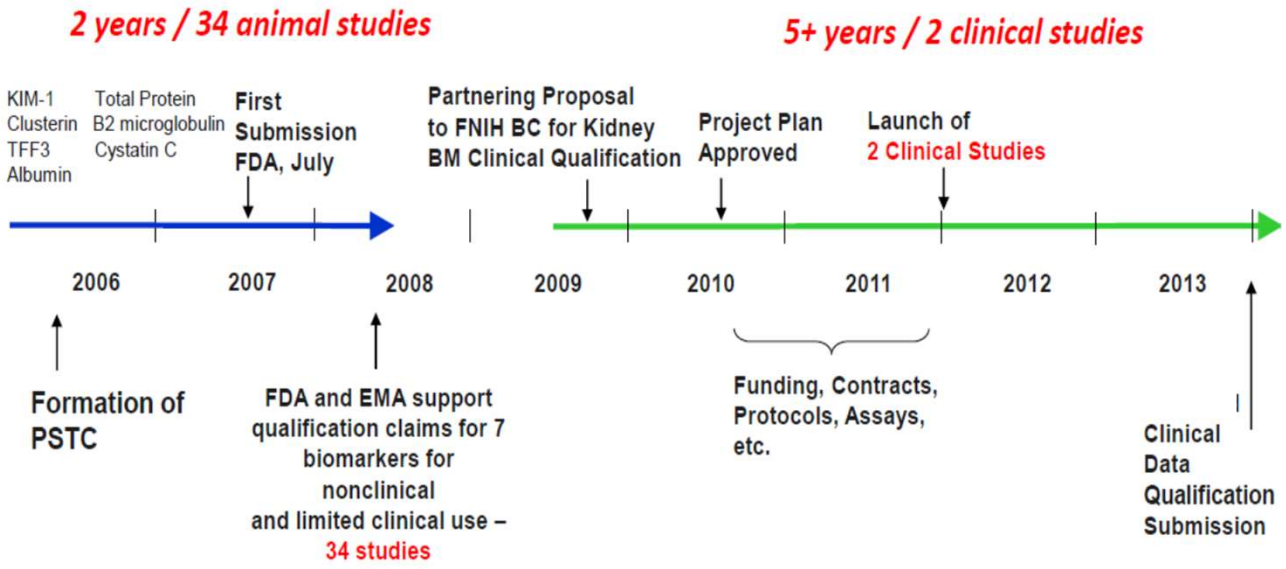
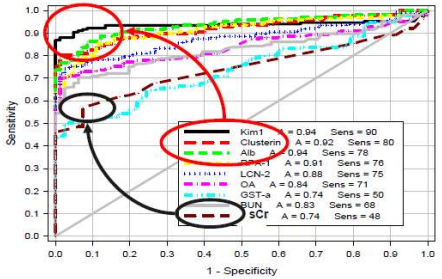
Supporting DMPK from screening to registration

Predictive safety testing consortium: Kidney Safety Biomarkers Qualification 7+ y timelines



- Example: New kidney safety biomarkers can outperform serum creatinine and BUN
  - FDA, EMA, PMDA Qualification



- Enable continued clinical development of drugs suspected of human irrelevant animal toxicities
- Ensuring patient safety
- Reducing drug failures and loss of time

BMK process

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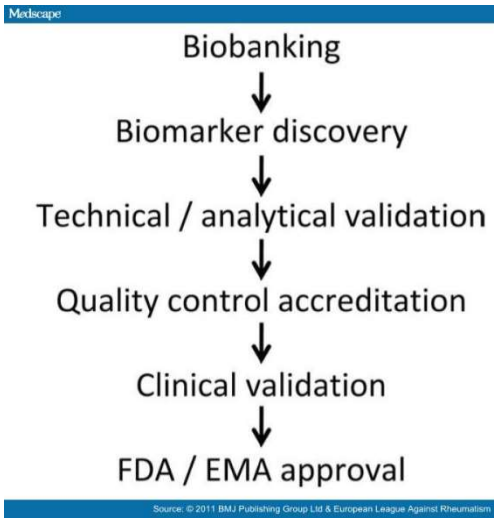
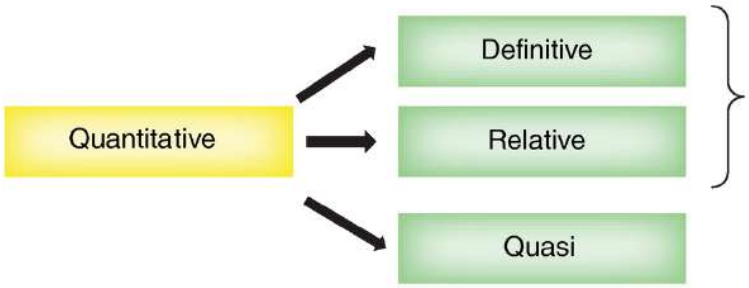
Supporting DMPK from screening to registration



- **Biobanking:**
  - Define and establish validation cohorts with biosamples (well characterized, proper collection & storage temperature…) => can be decisive for success or failure
  - Retrospective cohort (adapt number sample/statistic analysis)
- **BM discovery : Design input**
  - Technologies used (proteomic, genomic, transcriptomic…)
  - Robustness of the data (nb of sample : diseased vs healthy group, sample preparation, statistic analysis…)
  - Utility vs existing BM (market analysis, profitability, business plan…)
  - Intended use => Product Design Requirement
- **Validations :**
  - **Writing a Product Design specification**
  - **Analytical validation: Define the reliability and robustness of the test**
    - ✓ Precision, Stability, robustness studies
  - **Clinical validation: Define performance and relevance of the test in the given application**
    - ✓ Clinical sensitivity, specificity
- **Clinical utility / Clinical evidence:**
  - Demonstration of the potential usefulness
  - Added value to patient management decision-making

EBF

Biomarker assays are not PK assays



Biomarker levels determined using a reference standard

Reference material is representative of test samples (parallelism)

Reference material not available or not representative of test samples (non-parallelism)

Biomarker levels expressed in terms of a characteristic of the test sample (e.g. assay signal, titre, % bound)

Qualitative

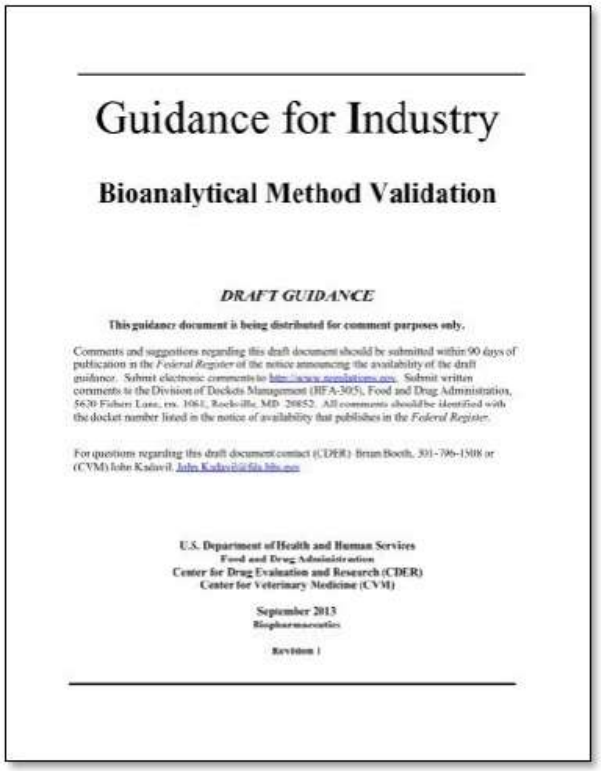
Biomarker levels expressed in nominal (positive or negative) or ordinal terms (e.g. 1 to 5 scoring scale)

Bioanalysis (2020) 12(20), 1427–1437

COU for biomarkers is not the same as for PK assays
May change depending on data and decisions being made

EBF

Things started to look different …..



Guidance for Industry

Bioanalytical Method Validation

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Brian Booth, 301-796-1308 or (CVM) John Kadavil, John.Kadavil@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

September 2013
Biopharmaceutics

Revision 1

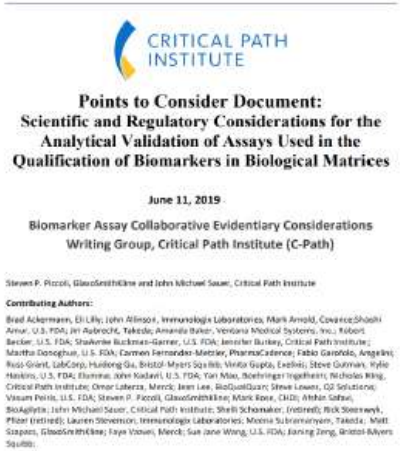
Selective, sensitive, and validated analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) and biomarkers are critical for the successful conduct of nonclinical and/or biopharmaceutics and clinical pharmacology studies. Validating bioanalytical methods includes performing all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix (e.g., blood, plasma, serum, or urine) is reliable and reproducible for the intended use. Fundamental parameters for this validation include the following:

- Accuracy
- Precision
- Selectivity
- Sensitivity
- Reproducibility
- Stability

EBF

So do we need guidance?

- Regulatory guidance cannot cover all eventualities for biomarkers within pharmaceutical development
- Existence of white papers for things to consider
- Regulation will only serve to remove the act of thinking and create “tick box” mentality
- What is needed is scientific and “**biomarker thinking**”
- Following guidance doesn’t automatically make an assay the right assay for the intended purpose to get the right data



CRITICAL PATH INSTITUTE

Points to Consider Document: Scientific and Regulatory Considerations for the Analytical Validation of Assays Used in the Qualification of Biomarkers in Biological Matrices

June 11, 2019

Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path)

White Paper

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

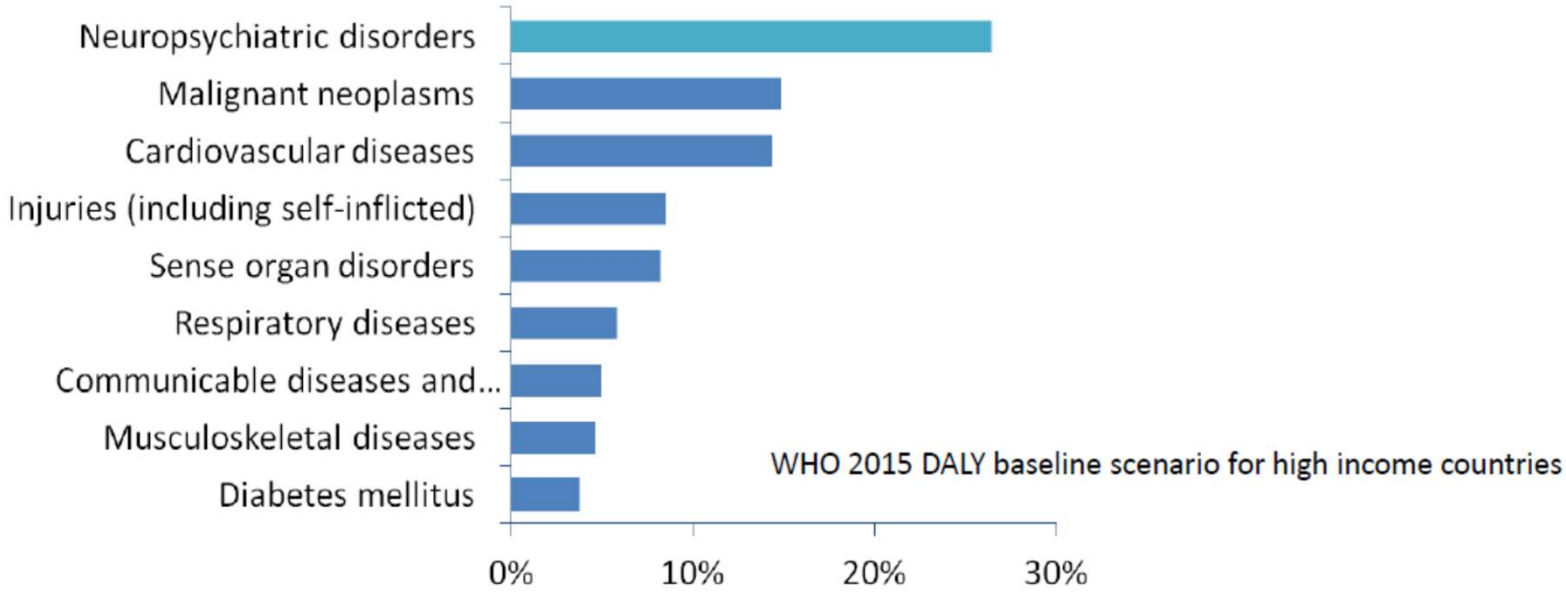
Update to the European Bioanalysis Forum recommendation on biomarkers assays; bringing context of use into practice

Neurodegenerative diseases

eurofins | ADME BIOANALYSES

Supporting DMPK from screening to registration

Share of 2015 Burden of disease (% of Total Disability Adjusted Life Years)



WHO Statement: *« ….unless immediate action is taken globally, the neurological burden is expected to become an even more serious & **unmanageable threat to public health** »*

Neurodegenerative diseases

eurofins | ADME BIOANALYSES

Supporting DMPK from screening to registration

- **NDD affect millions people worldwide in 2021**
- Alzheimer's disease and Parkinson's disease are the most common
- 1/3 of the European population – 179 million persons – is affected by at least one brain disorder
- in 2020, an estimated 5.4 million Americans were living with Alzheimer's disease. An estimated 930,000 people in the United States could be living with Parkinson's disease by 2020.
- NDD occur when nerve cells in the brain or peripheral nervous system lose function over time and ultimately die.
- Although treatments may help relieve some of the physical or mental symptoms associated with neurodegenerative diseases, there is currently no way to slow disease progression and no known cures.

Neurodegenerative diseases

eurofins | ADME BIOANALYSES

Supporting DMPK from screening to registration

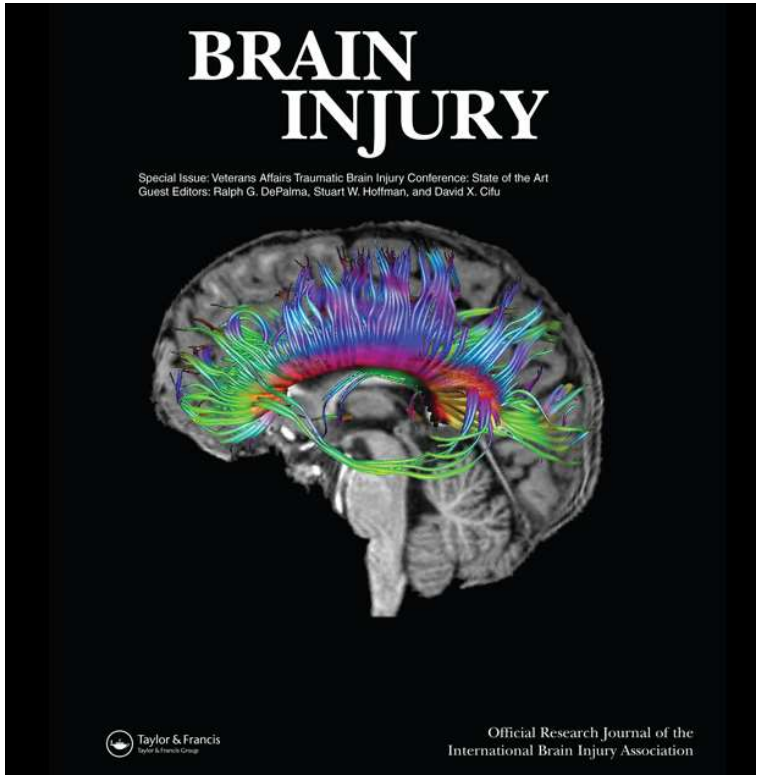
The Human brain is the most complex object of study in the history of science and one of the most inaccessible

- 2% of body weight, but consumes 20% of the oxygen that is used
- composed of about 100 billion nerve cells

- Expertise is scattered, approach to research is fragmented, and resulted datasets are siloed

- Incentives for research and collaboration are lacking and/or misaligned

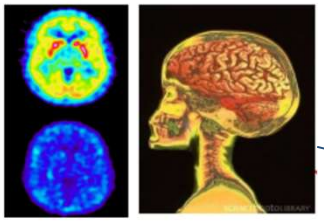
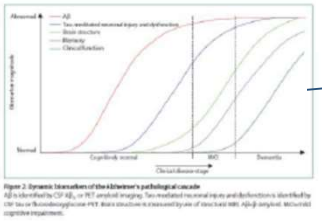
- Declining federal and industrial support is leading to diminished pipelines



Neurodegenerative diseases

eurofins | ADME BIOANALYSES

Supporting DMPK from screening to registration

Challenges		Pitfalls
Uncertain target engagement Difficult to detect PD or AD effects in CNS compartment without using reliable analytical methods		Errors in dose selection
‘Noisy readouts’ Cognitive function, mood, psychosis, pain		Need large N to detect small signals
Population heterogeneity Synfromic classification in neurology and psychiatry		Diagnostic uncertainty, Low responder rates
Insidious onset and slow progression Larger and longer trials		Larger and longer trials

Use of Biomarkers in NDD

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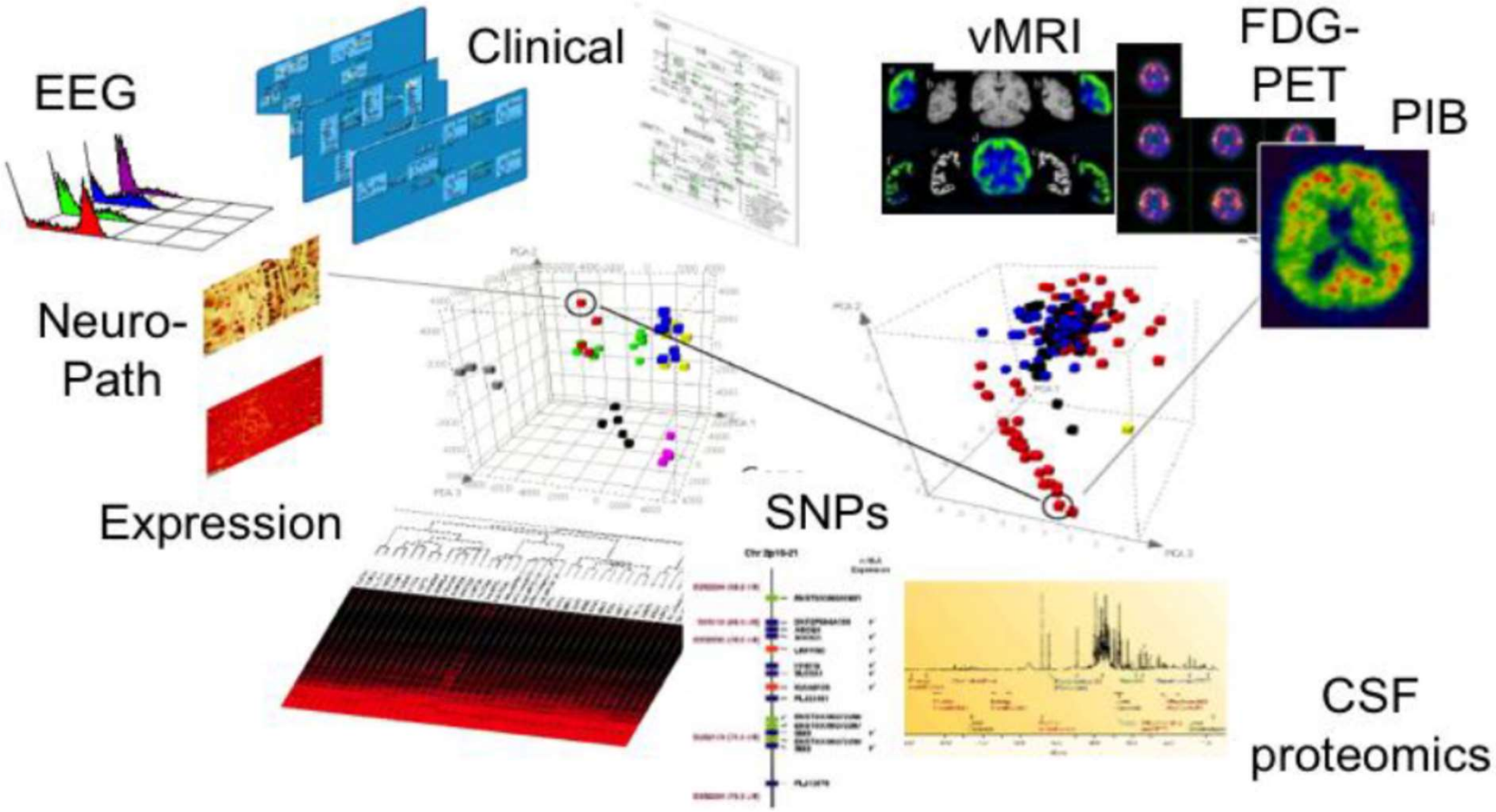
Supporting DMPK from screening to registration

Biomarkers in neurodegenerative diseases are heterogenous, composite and complex



NDD Biomarkers

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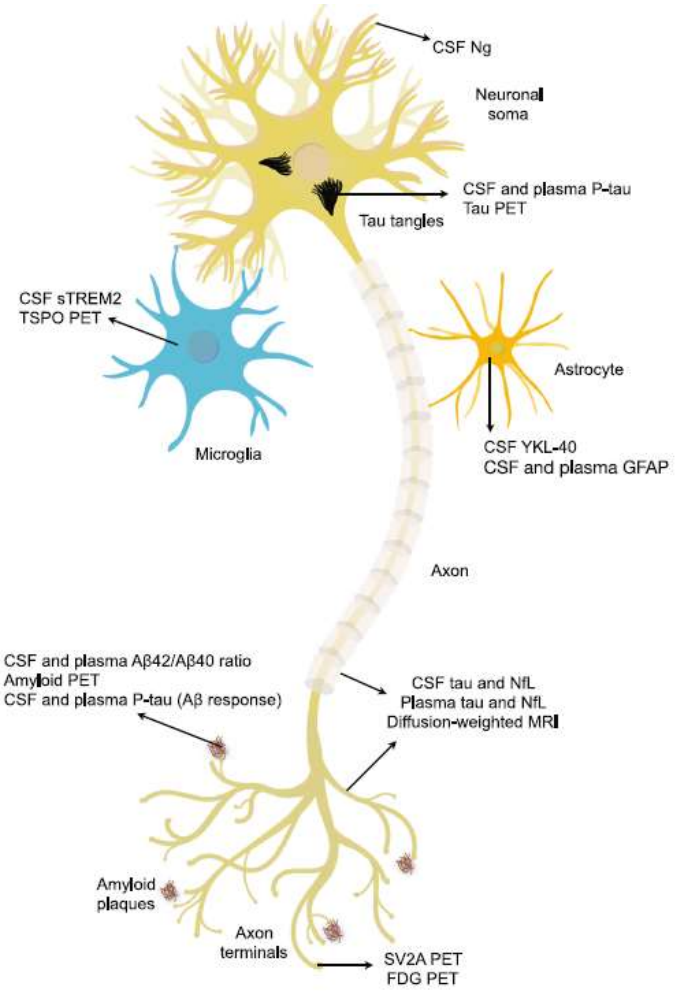
Supporting DMPK from screening to registration

Neurodegenerescence	Biomarker	Matrix
Amyloidopathy	Aß42, Aß40, ratio Aß42/Aß40	CSF
Tau pathophysiology	P-tau181, P-Tau231, P-Tau217 non P-Tau, Tau aggregates	CSF
Neurodegeneration	Total-tau	CSF
Axonal damage	Neurofilament –Light NfL	CSF, serum
Synucleopathy	α-synuclein, β-sheet α-synuclein	CSF
Synaptic dysfunction	Neurogranin	CSF
Microglial responses	sTREM2	CSF
Astrogial-related responses	GFAP, YKL40, S100	CSF, serum
Neuroinflammation	Interleukins multiplex panels	serum
Fronto temporal labor degeneration	TDP43	CSF, serum
Prion	PrPc	CSF



Henrik Zetterberg et al., Molec Psychiatry, 2020

Alzheimer's disease

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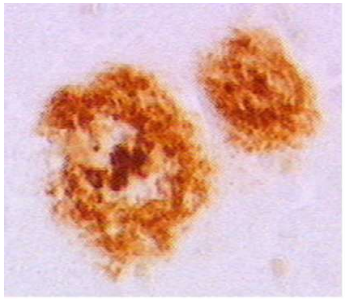
Supporting DMPK from screening to registration

Post-mortem confirmed diagnostic

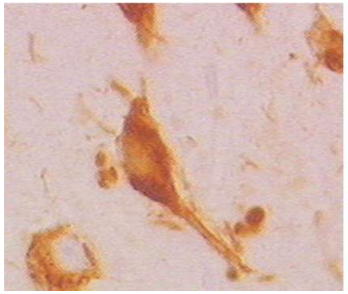
There are 3 consistent neuropathological hallmarks:

– Amyloid-rich senile plaques
– Neurofibrillary tangles
– Neuronal degeneration

These changes eventually lead to clinical symptoms, but they begin years before the onset of symptoms

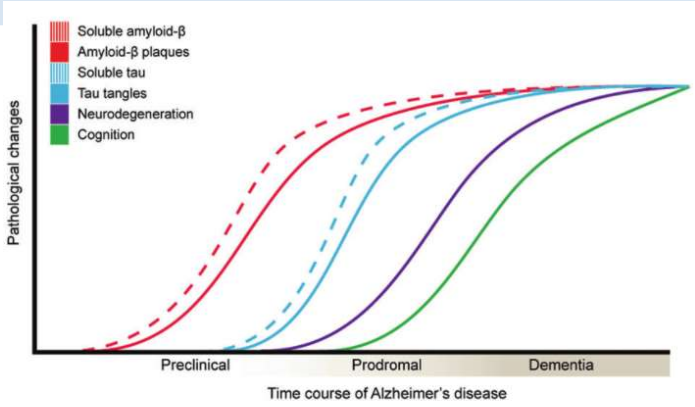


Immunocytochemical staining of senile plaques in the isocortex of a brain of a human with AD (anti-amyloid antibody)



Immunocytochemical staining of neurofibrillary tangles in the isocortex of the brain of a human with AD (anti-tau antibody)

The deposition of Ab plaques occurs long before any clinical symptoms and as many as 20 years before the onset of dementia.



Hypothetical time course of pathological changes in Alzheimer's disease (AD), in which biomarkers for amyloid-β become abnormal [cerebrospinal fluid (CSF) amyloid-β 1-42 preceding PET], followed by abnormal tau (CSF p-tau preceding PET), neurodegeneration, and cognitive decline. Adapted with permission from Jack et al. [182] and 2013 [172], Nordberg [238], and McDade and Bateman [235]

NIA-AA Research A-T-(N) Framework

NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease

A: Aβ biomarkers determine whether or not an individual is in the Alzheimer's continuum
Aggregated Aβ or associated pathologic state
CSF Aβ42 or Aβ42/ Aβ40 ratio
Amyloid PET

T: Pathologic tau biomarkers determine if someone who is in the Alzheimer's continuum has Alzheimer's disease.
Aggregated Tau or associated pthologic state
CSF phosphorylated tau
Tau PET

Staging disease severity

(N): Neurodegenerative/neuronal injury biomarkers
Neurodegeneration or neuronal injury
CSF total Tau
Anatomic MRI and/or FDG PET

(C): Cognitive symptoms

AT(N) profiles	Biomarker category	
A-T-(N)-	Normal AD biomarkers	
A+T-(N)-	Alzheimer's pathologic change	Alzheimer's continuum
A+T+(N)-	Alzheimer's disease	
A+T+(N)+	Alzheimer's disease	
A+T-(N)+	Alzheimer's and concomitant suspected non Alzheimer's pathologic change	
A-T+(N)-	Non-AD pathologic change	
A-T-(N)+	Non-AD pathologic change	
A-T+(N)+	Non-AD pathologic change	

C.R. Jack, Jr et al., Alzheimers Dement. 2018

AD CSF Biomarkers

eurofins | ADME BIOANALYSES

Park et al. *Experimental & Molecular Medicine* (2020) 52:556–568
https://doi.org/10.1038/s12276-020-0418-9

Experimental & Molecular Medicine

REVIEW ARTICLE Open Access

New fluid biomarkers tracking non-amyloid-β and non-tau pathology in Alzheimer's disease

Sun Ah Park[1,2,3], Song Mi Han[1,3] and Chae Eun Kim[1,3]

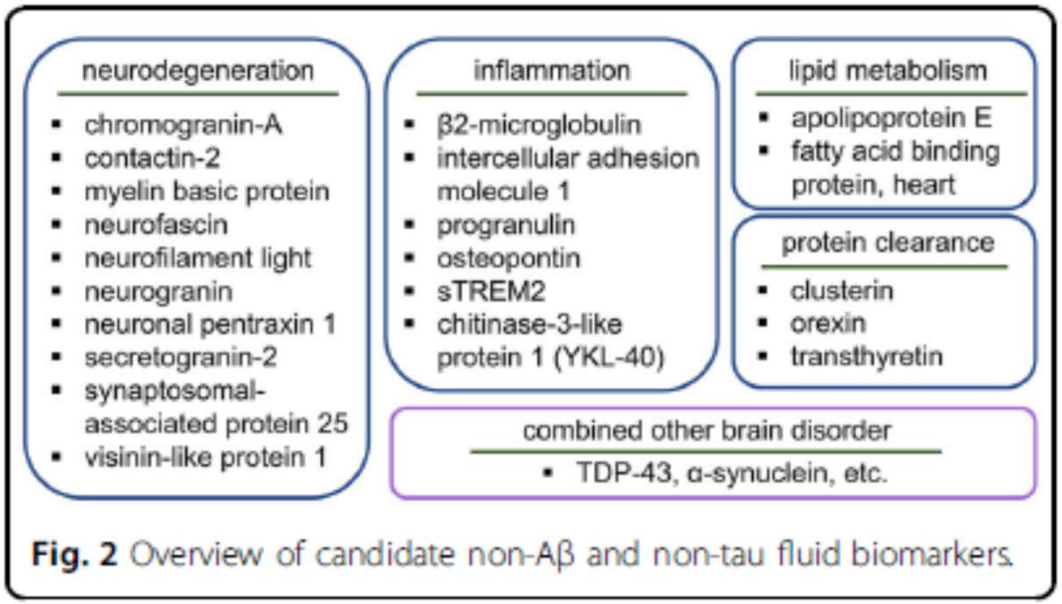


Fig. 2 Overview of candidate non-Aβ and non-tau fluid biomarkers.

Table 2 Sets of new biomarker combinations.

Combination
CSF neurogranin, YKL-40[93]
CSF chromogranin-A, FABP-3, matrix metalloproteinase-2, pancreatic polypeptide levels + regional brain volume on MRI + CSF $A\beta_{42}$, $pTau_{181}$, tTau levels[53]
CSF neurogranin, NFL + CSF tTau levels[20]
CSF FABP-3, IL-10, NFL[80]
CSF neurogranin, NFL[94]
CSF neurogranin, SNAP-25, VILIP-1, YKL-40[9,98]
CSF neurogranin, NFL + CSF tTau levels[95]
CSF clusterin, fractalkine, MCP-1, sTREM2, YKL-40[71]
CSF neurogranin, NFL, YKL-40 + CSF tTau[22]

AD blood biomarkers

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Supporting DMPK from screening to registration

doi:10.1093/brain/awaa286 BRAIN 2020: Page 1 of 8 | 1

BRAIN
A JOURNAL OF NEUROLOGY

REPORT

Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease

Niklas Mattsson-Carlgren,[1,2,3] Shorena Janelidze,[1] Sebastian Palmqvist,[1,4]

Plasma P-tau 181, P-tau 217 and Other Blood-Based Alzheimer's Disease Biomarkers in a Multi-Ethnic, Community Study

Adam M. Brickman[1-3], Jennifer J. Manly[1-3], Lawrence S. Honig[1,3], Danurys Sanchez[1,2], Dolly Reyes-Dumeyer[1,2], Rafael A. Lantigua[1,4], Patrick J. Lao[1-3], Yaakov Stern[1-3], Jean Paul Vonsattel[1,5], Andrew F. Teich[1,3,5], David Charles Airey[6], Nicholas Kyle Proctor[6], Jeffrey L. Dage[6], and Richard Mayeux[1-3]

JAMA | Original Investigation

Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders

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Time course of phosphorylated tau181 in blood across the Alzheimer's disease spectrum

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Neurodegeneration

ORIGINAL RESEARCH

Diagnostic and prognostic value of serum NfL and p-Tau$_{181}$ in frontotemporal lobar degeneration

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Molecular Psychiatry
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ARTICLE

Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study

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Serum GFAP associated with cognitive decline

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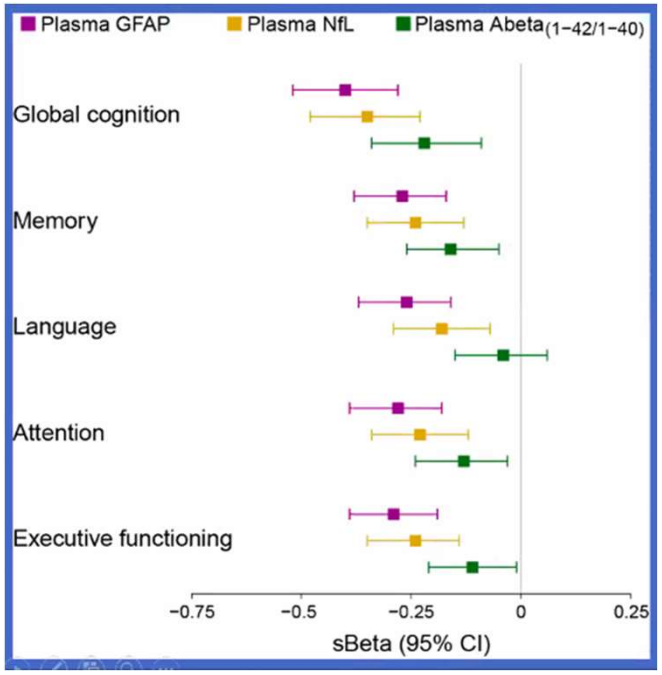
Supporting DMPK from screening to registration

Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study



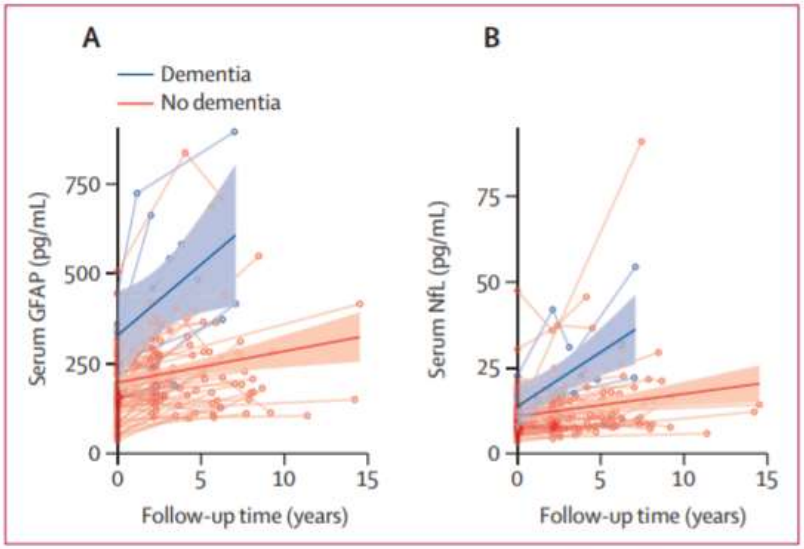


Figure 2: **Spaghetti plots of longitudinal serum GFAP (A) or NfL (B) levels for individuals with and without progression to dementia over time**
Serum GFAP (A) and NfL (A) levels were plotted against time, colour coded for progression to dementia over time or no progression to dementia over time. We superimposed average slopes per group, with 95% CIs. GFAP=glial fibrillary acidic protein. NfL=neurofilament light.

Inge M W Verberk et al., The Lancet, 2021

Alzheimer's disease

06/07/2021: **Aduhelm (aducanumab)** is the first novel therapy approved for Alzheimer's disease since 2003.

The screening of libraries of human memory B cells for reactivity against aggregated Aβ led to the molecular cloning, sequencing, and recombinant expression of aducanumab (BIIB037), a **human anti-Aβ monoclonal antibody** that selectively targets **aggregated forms of Aβ**, including **soluble oligomers** and **insoluble fibrils**.

Alzheimer's disease

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Supporting DMPK from screening to registration

First-in-human, double-blind, placebo-controlled, single-dose escalation study of aducanumab (BIIB037) in mild-to-moderate Alzheimer's disease, Biogen Cambridge USA

PK/PD approach

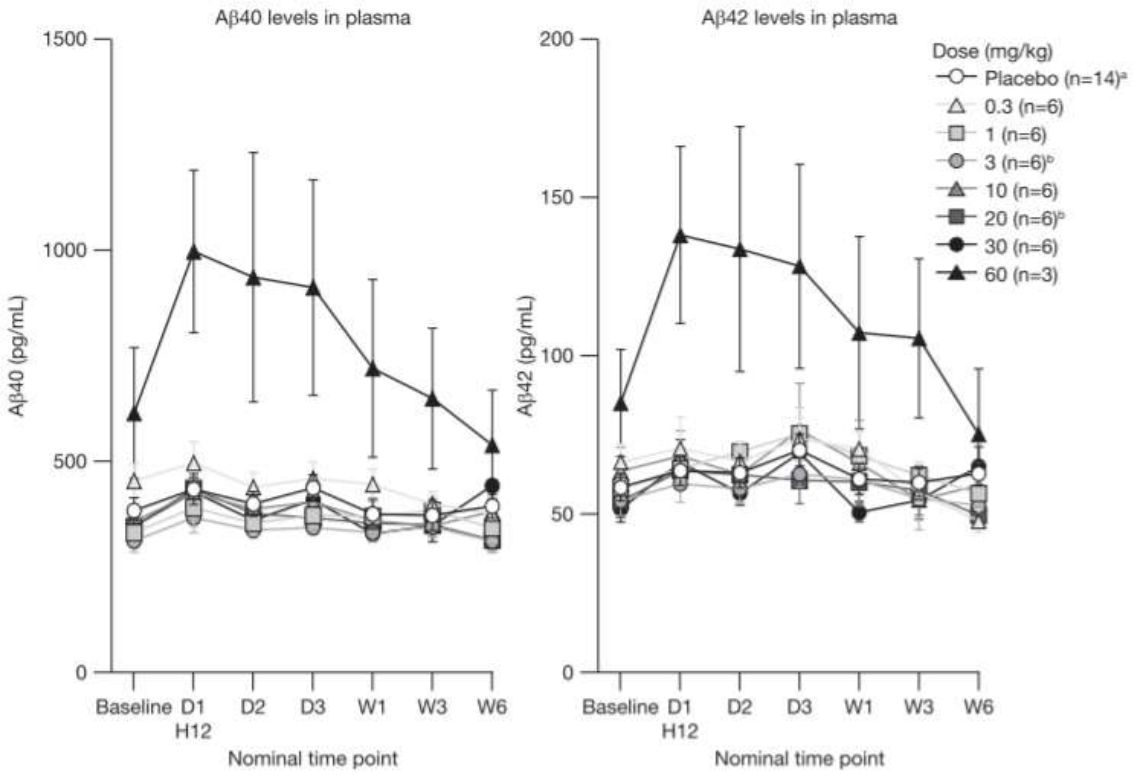

Fig. 2. Mean (SE) Aβ40 and Aβ42 plasma levels (extracted) after a single dose of aducanumab or placebo. [a]n = 13 at W3 and W6; [b]n = 5 at W3. Abbreviations: D, day; H, hour; SE, standard error; W, week.

James Ferrero et al., Alz&Dementia, 2016

Biomarkers

Immunoassays

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Supporting DMPK from screening to registration

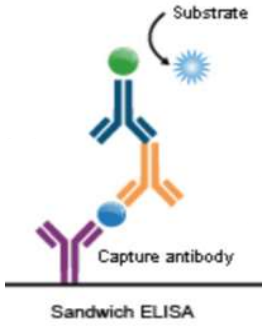
ELISA

chemiluminescence
colorimetry
fluorescence

plates/beads

sandwich
competition
direct/indirect
inhibition

ELISA



Sandwich ELISA

Method validation/qualification

commercial or in-house:
Fit-for-purpose approach
RUO / IUO / GCP GLP env

Reference interval for [BMK],
Optimize use of samples (pre-analytical)
Linearity, Parallelism
Calibration curve,
Selectivity
Specificity
Accuracy (deviation)
Precision
Sensitivity
Stability (short & long term)

100 to 200 samples per day

Spectramax M3 and i3x (Molecular Devices)

Meso QuickPlex SQ120 (Meso Scale Discovery)

ECL Electro chemiluminescence

automate

high sensitivity,
accuracy,
broad dynamic range
low background
low volume of sample

MSD-ECL

Biomarkers

LC-MSMS

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Supporting DMPK from screening to registration

Mass spectrometry LC-MSMS MRM/Single Plex

>1200 analytical methods developped in the lab (peptides & small molecules)

GHB (bioequivalence) FDA

Cortisol and hydrocortisol (clinical study BMK) ; FDA

Nicotine (bioequivalence) FDA

- 10 LC-MS/MS systems: API 4000 (3), API5500 (2), Shimadzu 8060 (3), API6500 (2)
- HPLC systems with UV, fluorescence and diode array detection
- Liquid scintillation counters (beta radiation)
- On line HPLC with radiometric detector

Method validation/qualification

Fit-for-purpose approach
RUO / IUO / GCP GLP env

Linearity
Selectivity & Specificity
Carry-over
Matrix effect
Recovery (exctraction)
Accuracy & Precision
Timeof run impact
Stability
freeze and thaw cycles
Stability - bench top
Stability long term

60 to 80 runs per day

Biomarkers

Biobanking

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Supporting DMPK from screening to registration

Discovery

Pre-clinical studies

Clinical studies

- Sample collection and storage procedures according to KOLs & literature guidelines (sample volume, DBS, -80°C)
- Samples tracking with Watson LIMS system
- Short turn around time for sample analysis & results sending

➢ Clinical Data Interchange Standards data (CDISC)

➢ FDA compliant study reports and/or customers' template

Take-Home message

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Supporting DMPK from screening to registration

- Biomarkers improve assesment of disease and drug effects: target engagement, pharmacodynamics (PK/PD), efficacy, disease progression;
- The effective use of biomarkers can increase the probability of succes
- Biomarker qualification (FDA) takes years including clinical and analytical BMK validations
- In drug development, validation of PK assays are not validation of BMK assays ‘Fit for purpose approaches’
- The second and third waves of AD BMKs used in combination in blood and or/CSF will help to sucess Pharma and Diag studies

Thank you