



NEW APPROACHES TO DIAGNOSTICS

The Impact of Biomarkers in Alzheimer's Disease

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TVLSN Meeting
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- Biomarkers for Alzheimer's disease
- Current methods of diagnosis
- Application of ProteoSHOP™ technologies to biomarker discovery
- Mass spectrometry of tau phosphorylation
- Future perspectives of multi-analyte diagnostics

- **Biomarker**

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

- **Clinical endpoint**

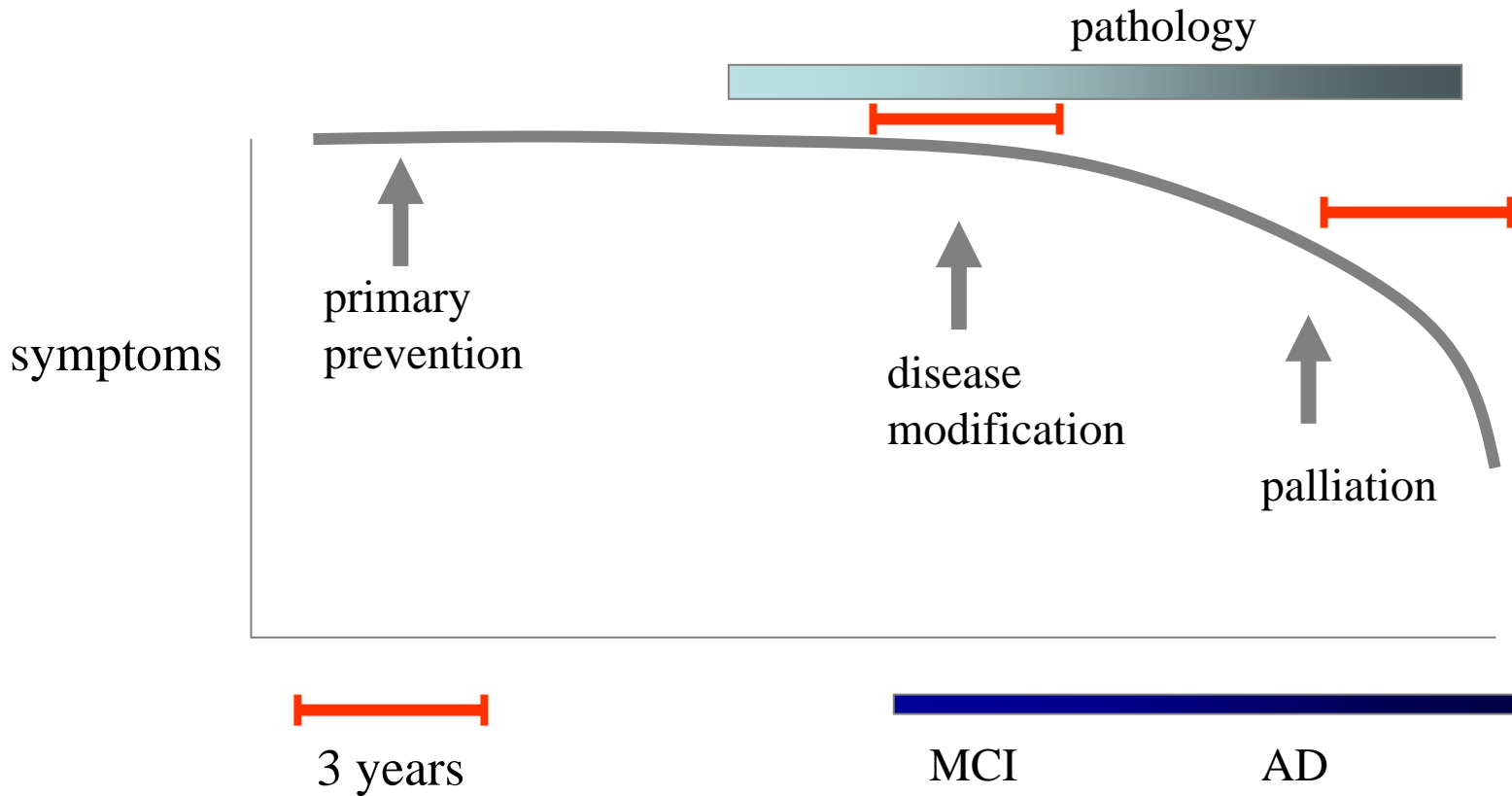
“a characteristic or variable that reflects how the patient feels, functions or survives.”

- **Surrogate endpoint**

“sub-set of biomarkers intended to substitute for clinical endpoints”

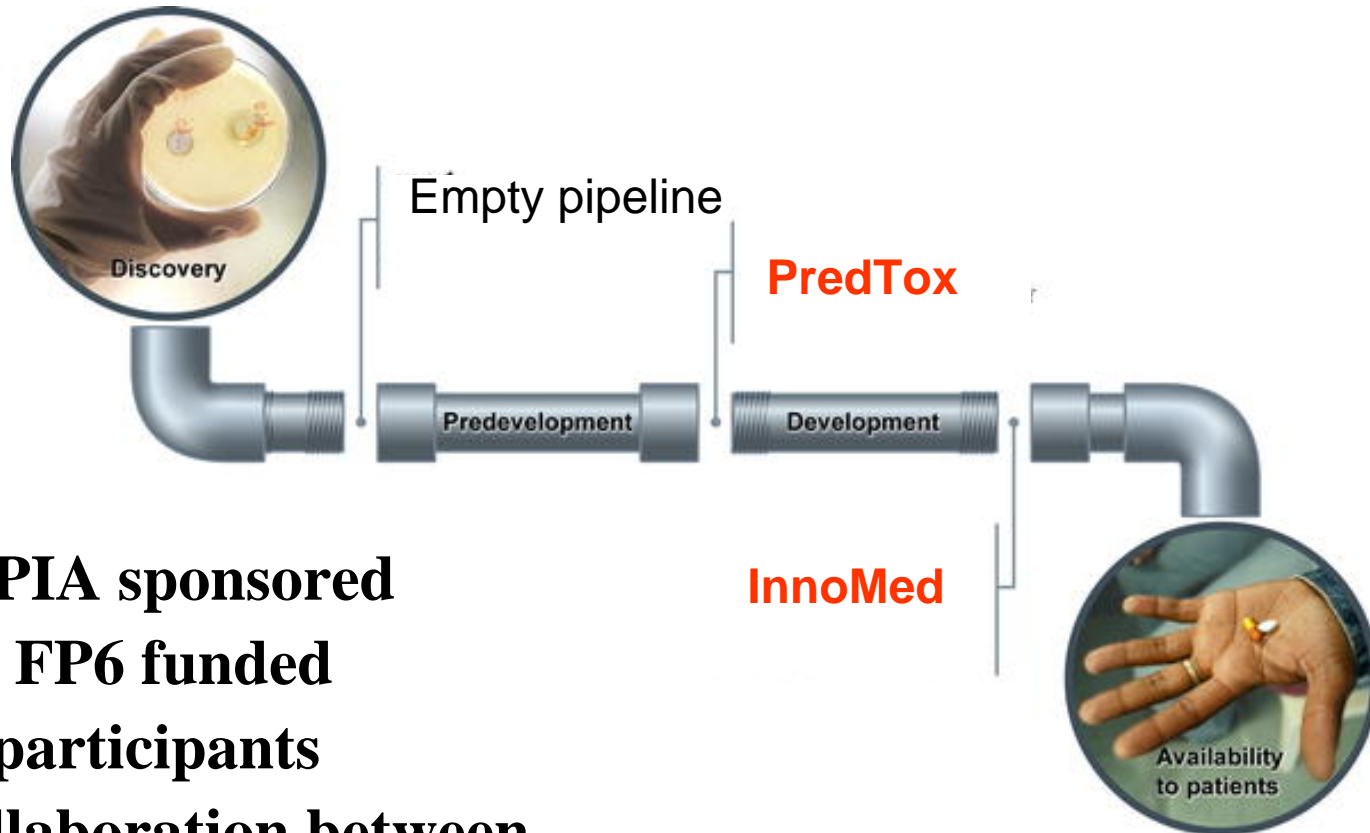
- Detectable before clinical symptoms
- Sensitivity & Specificity >80 % in neuropathologically confirmed cases
- Reliable, reproducible & inexpensive
- Non-invasive & simple to perform
- Confirmed by at least two independent studies
- Maximise effect of disease-modifying therapy

AD disease progression



Tests for Alzheimer's

Test purpose	Use in Clinical Management	Utility in Clinical Trial
Prediction	risk of acquiring dementia	Enriching at risk populations c.f. APOE and MCI trials
Early diagnosis	dementia vs no dementia	Early intervention
Differential diagnosis	AD vs other dementia	Increased specificity
Evaluating therapy	surrogate marker	Monitoring efficacy
Predicting outcome	responders vs non-responders	Pharmacogenomics c.f. NICE and ACHEIs



- **EFPIA sponsored**
- **EU FP6 funded**
- **44 participants**
- **Collaboration between pharma, SMEs, academia**

■ Clinical examination

- Based on detailed questioning of patient and carer(s)
- Can reliably differentiate MCI from AD
- Cannot readily predict MCI converters
- Inherently difficult to precisely stage AD
- Hard to score improvement from symptomatic treatment

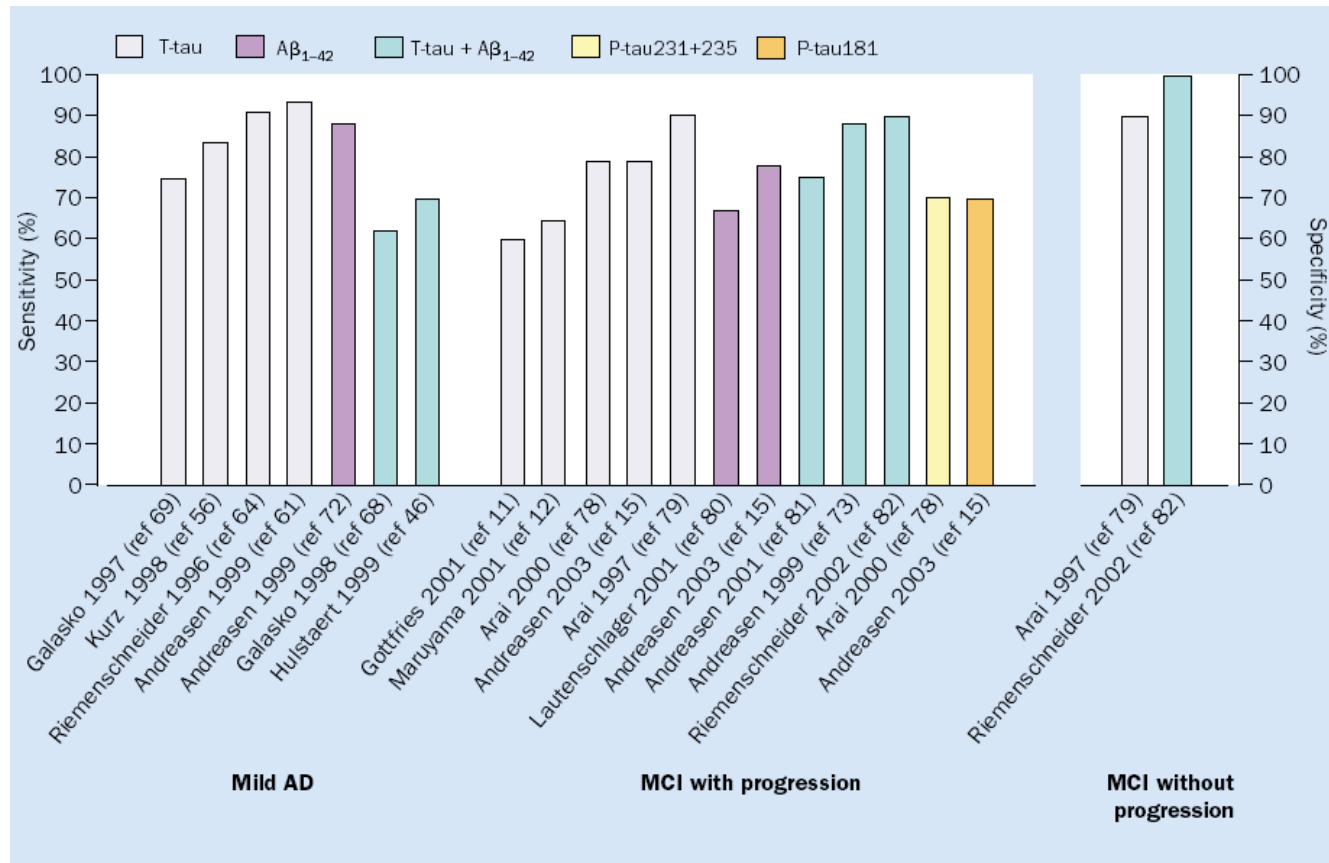
▪ Brain imaging

- Can show structural changes – but not truly diagnostic
- Newer functional methodologies in development e.g. beta-amyloid ligand function
- Regular imaging may assist with staging and progression
- Must be used in combination with clinical examination

■ Cerebrospinal fluid

- Monitoring of brain-derived protein levels
- Well established markers related to disease pathology – $A\beta$, tau, phospho-tau
- Moderately sensitive and specific
- Some markers may correlate with stage and/or progression
- Poor leakage into blood
- Of limited diagnostic potential

■ Cerebrospinal fluid



Blennow, K. and Hampel, H. (2003). CSF markers for incipient Alzheimer's disease. *Lancet Neurol.* 2, 605-613.

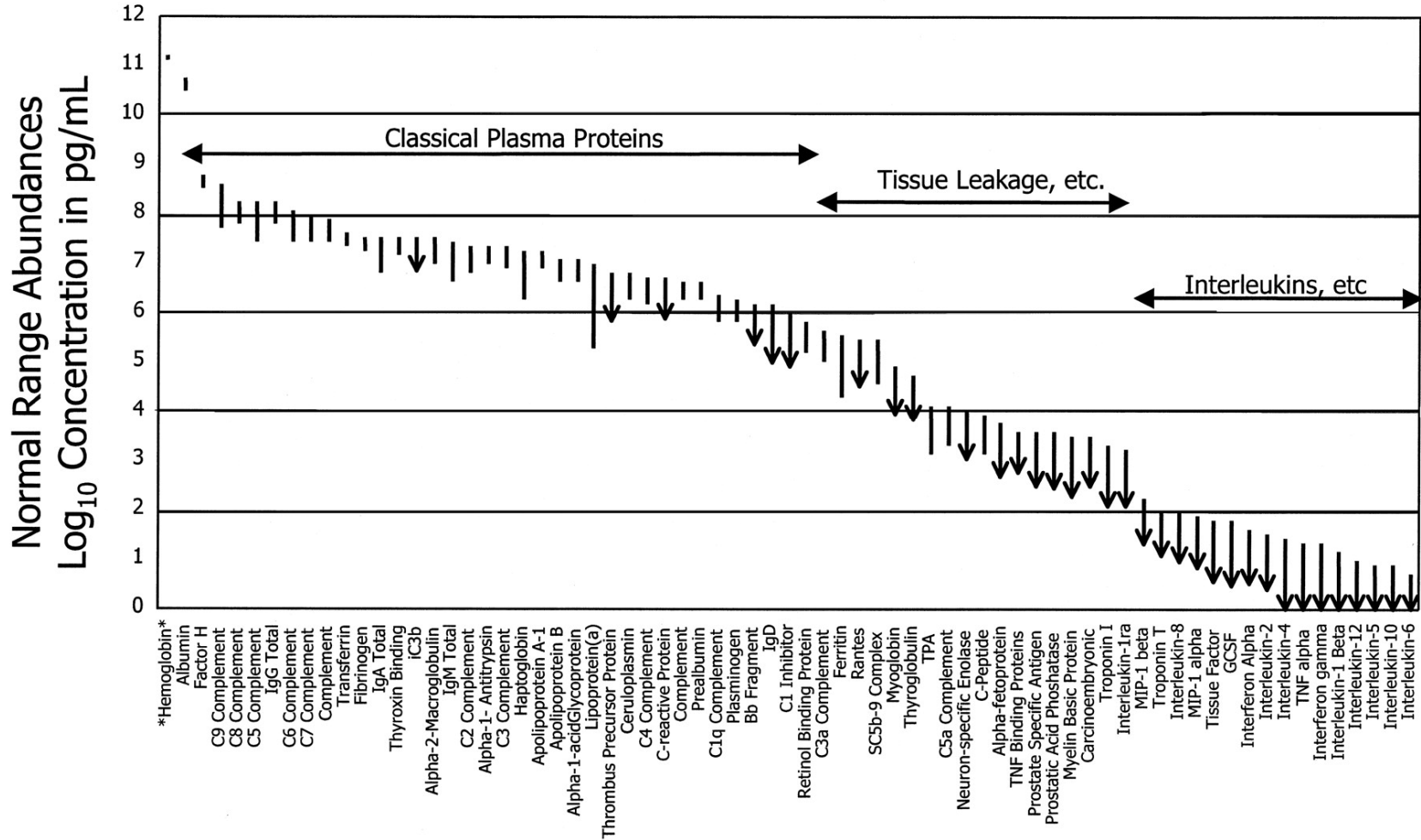
■ Where are we today?

- No routine non-invasive methodology with sufficient sensitivity or specificity for diagnosis
- No routine correlation to disease stage for accurate monitoring of progression
- No disease modifying therapeutics approved
- Benefit of symptomatic treatments difficult to measure accurately
- Lack of early diagnostics limit clinical management of AD
- Need to discover blood biomarkers of AD

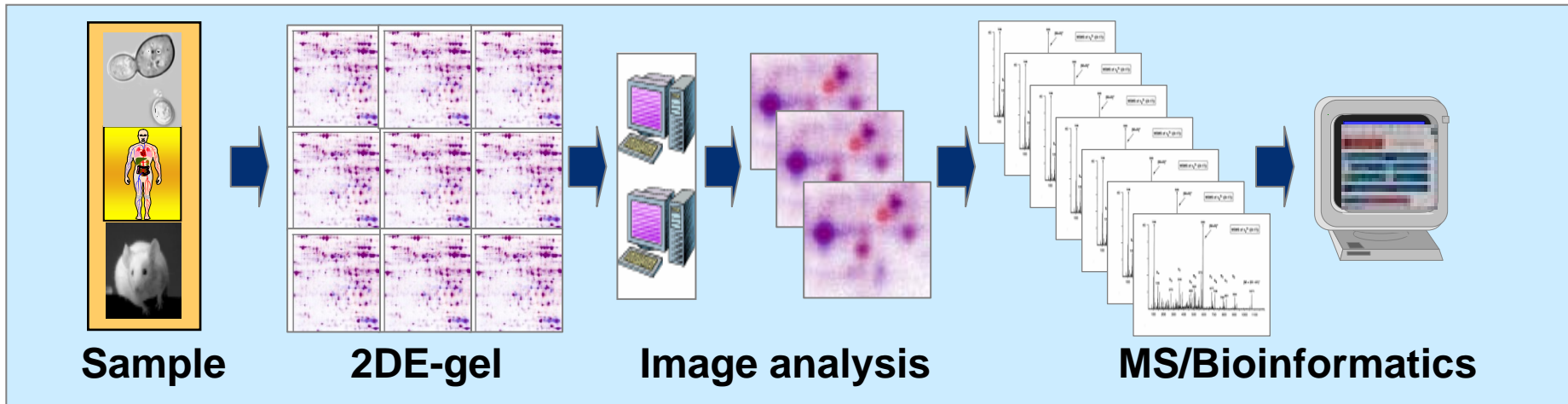
■ Proteomic analysis of blood

- Three different proteomics approaches: 2-D Gel, SELDI, Isotopic mass labels (qPST)
- Cohort of 50 patients and 50 age/sex matched controls
- Over 30 candidate biomarkers identified
- Ten proteins seen by 2 or more methods prioritised for evaluation
- ELISA & Western blot assays for 6 markers already developed

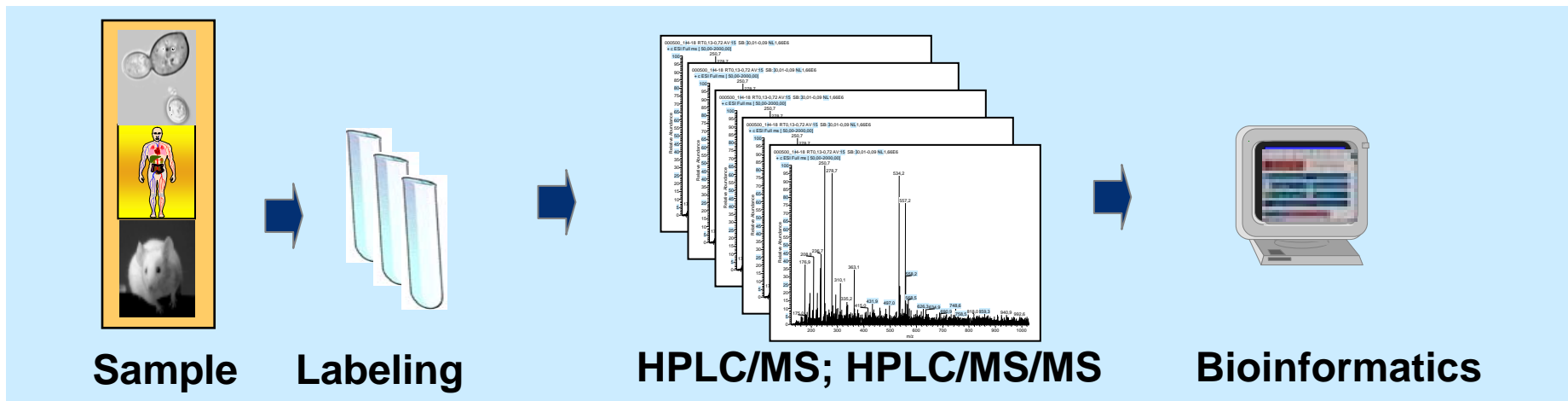
■ The Plasma Proteome Challenge



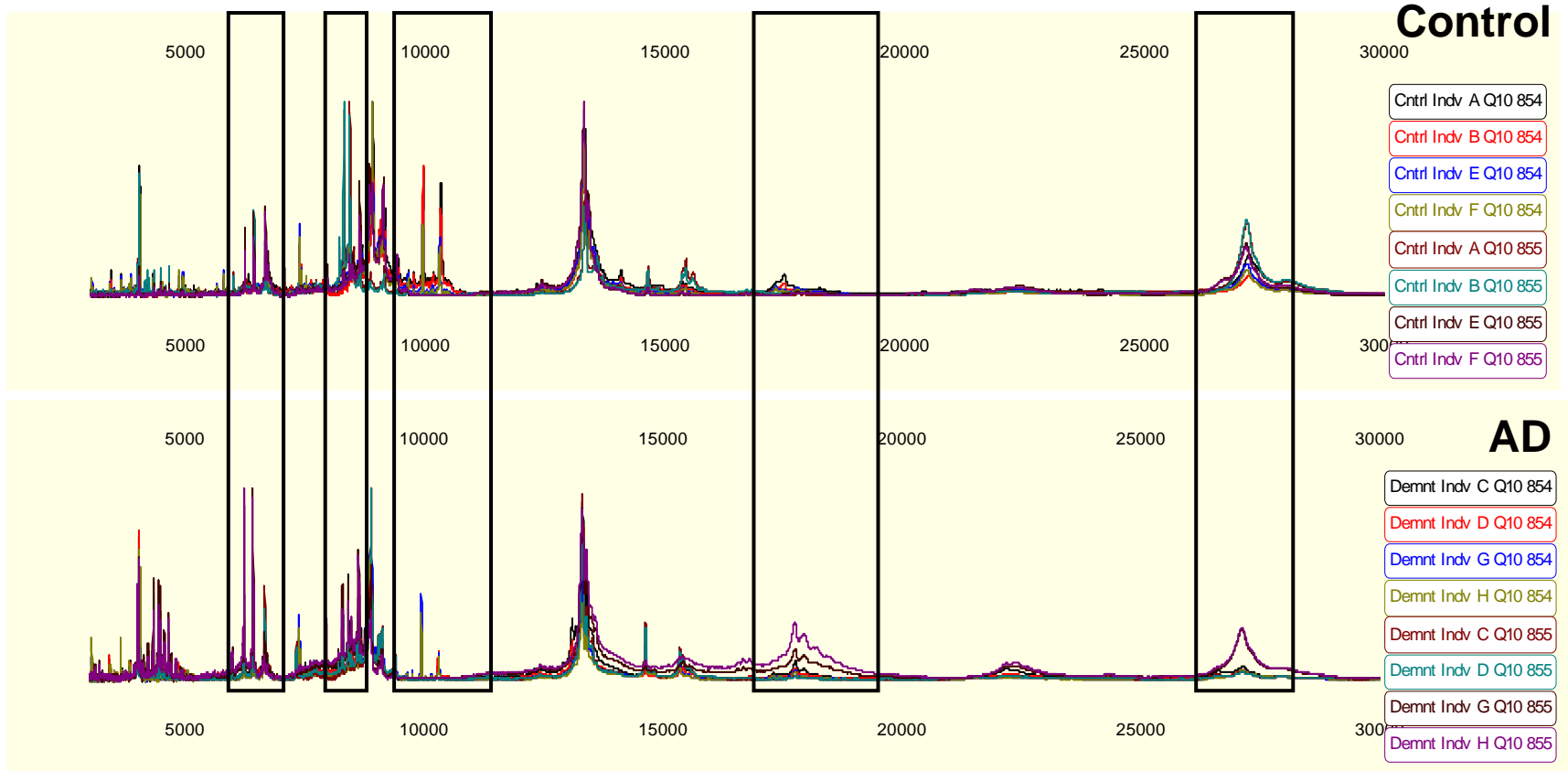
The 2-DE Process (Gel based)



Gel-free Technologies (PST[®]; qPST[™]; TMT[®])



SELDI – Control vs AD



doi:10.1093/brain/awf279

Brain (2006) 129, 3042–3050

Proteome-based plasma biomarkers for Alzheimer's disease

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Candidate blood biomarkers include:

Complement Factor H

Haptoglobin

Alpha-2-microglobulin

Clusterin

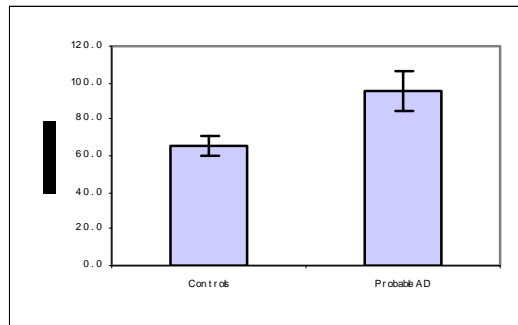
Complement C3a

Complement C4a

Evaluation of Candidate Biomarkers

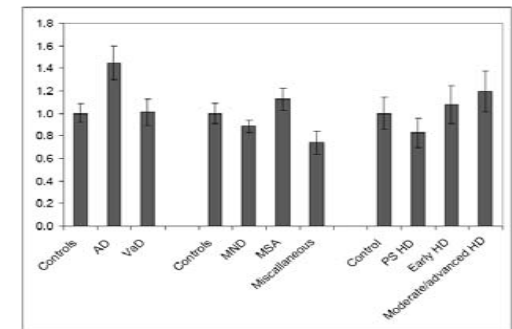
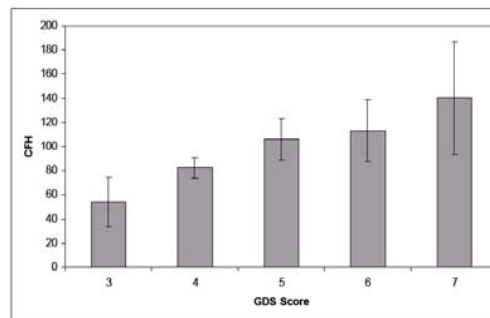
Western blot

- 121 AD patients
- 205 non-demented controls
- 100 Motor Neuron disease (MND) patients
- 18 Multiple System Atrophy (MSA) patients
- 14 non-demented neurological patients
- 55 Huntington's disease patients

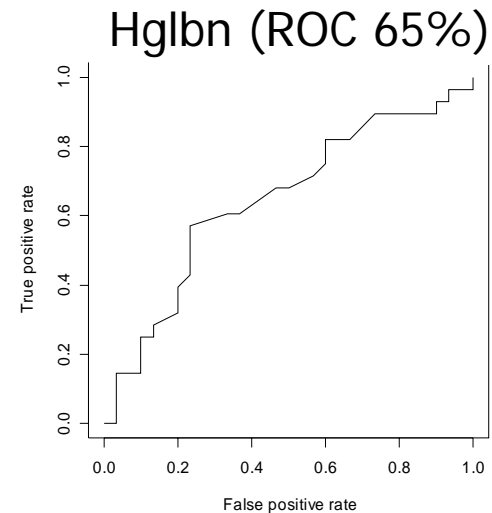
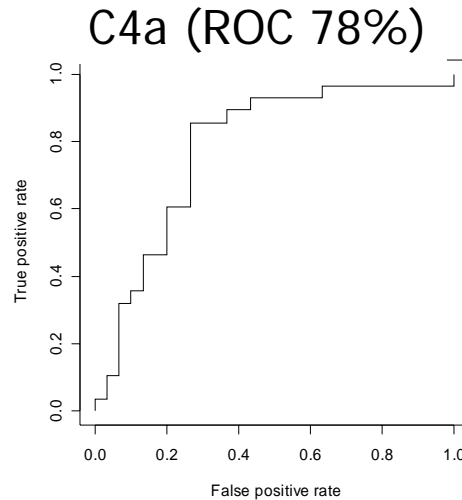
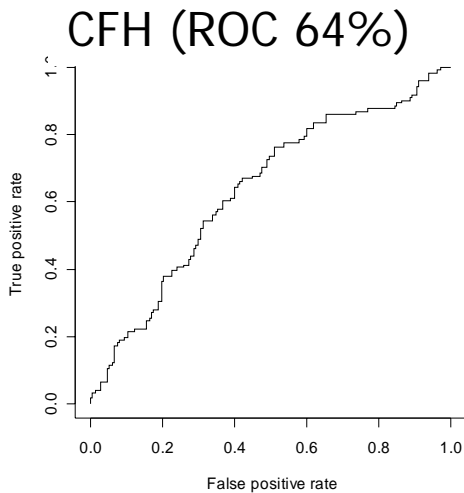


Complement Factor H (CFH)

- CFH up-regulated in AD
- Western blot validation 121 AD vs 148 controls
- Significant 45% increase in AD ($p < 0.01$)
- Significant correlation with MMSE score ($p < 0.05$)
- Spot intensity correlates with GDS
- No elevation in other dementias



- **Biomarker assay performance**



- Single marker assays do not have required specificity
- Better methods required
- Multiplex analysis will be necessary for complex diseases like AD
- Availability and quality of antibodies for ELISA-type validation is time limiting factor for development

8 Protein MRM Array

- 72 individual transitions
- At least 2 peptides per protein
- Use of standards for quantitation

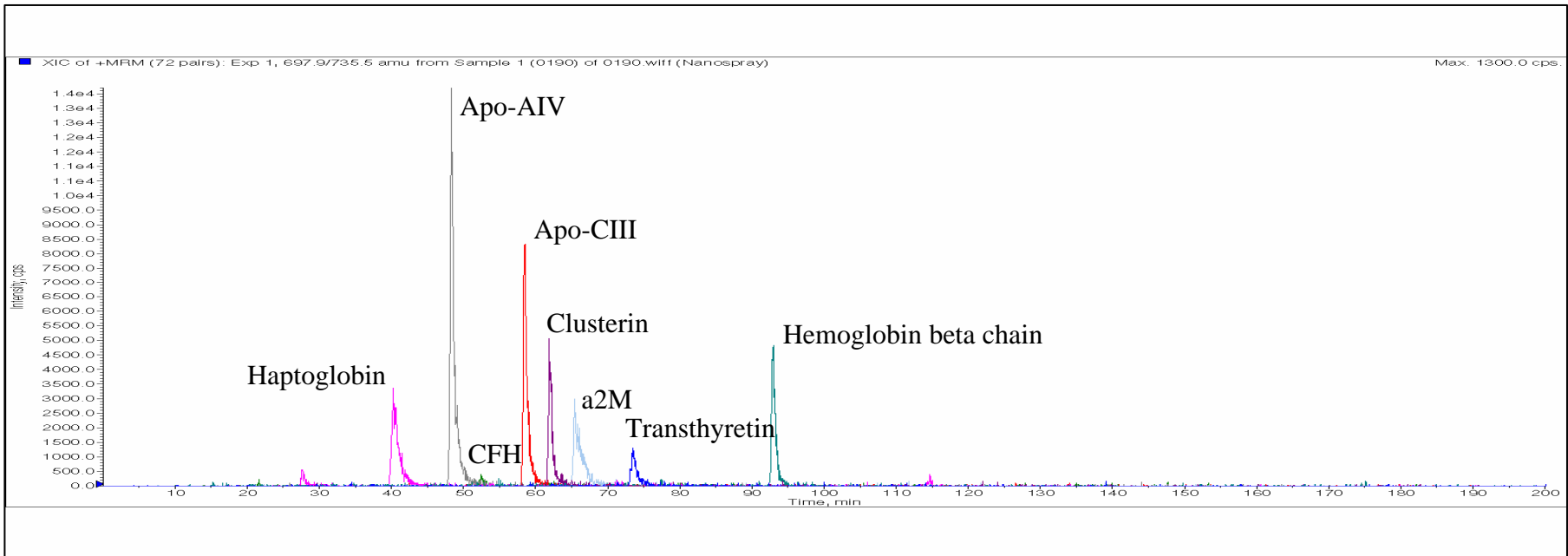


Instrumentation

1. Triple Quadrupole
2. Hybrid MS: QTRAP

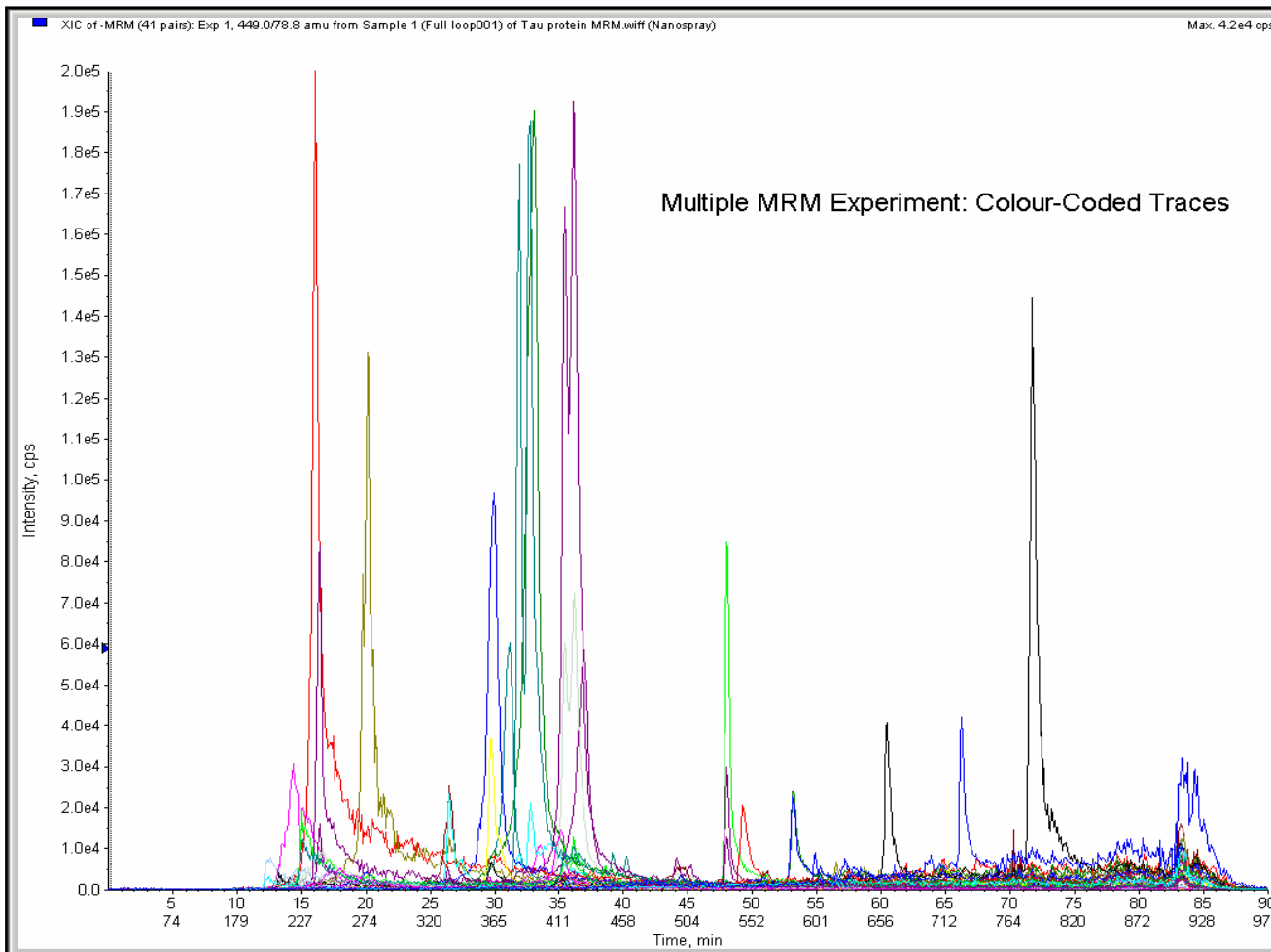
Application

- Wide range of analytes
- Qualitative or Quantitative



- Hyperphosphorylated PHF Tau is a classic AD pathology
- Phospho-tau peptides seen in CSF but not yet in blood
- Phosphosite specific MS analysis method developed and applied to PHF tau extracted from AD brains
- In a first analysis 19 phosphorylation sites were mapped to the tau molecule
- High sensitivity MS using MRM revealed 37 total sites - several previously unseen
- Provides potential new markers in blood – if MS methodologies are sensitive enough

Monitoring 41 unique p79 events via an MRM experiment



Relevant Sample:

Recombinant protein

Cell line

Mouse models

PHF Tau (Human)

Creation of panels:

Choice of protease

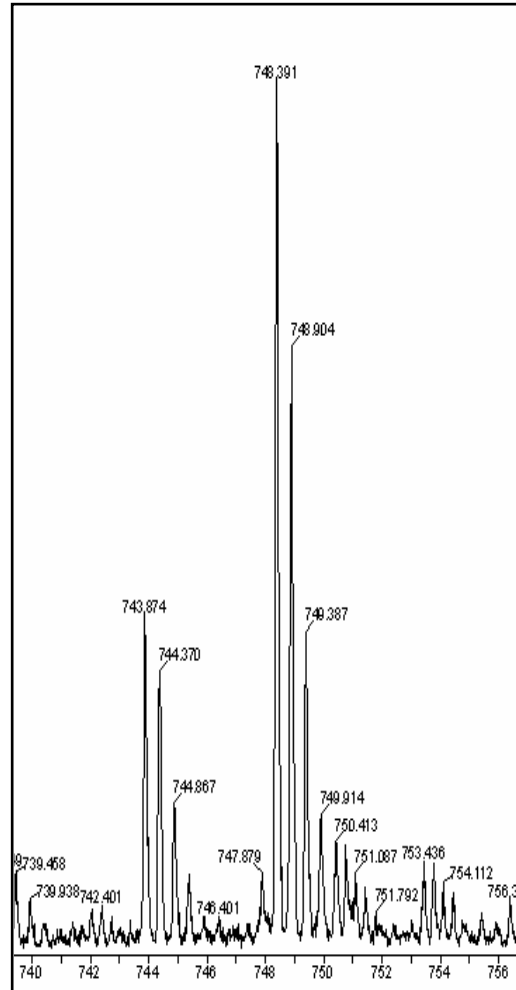
Site directed or
Kinase directed

- Current AD diagnostics lack accuracy or are too problematic for routine use
- Proteomics strategy has identified candidate biomarkers of AD in blood, but....
- Single immunological assays lack adequate sensitivity and specificity
- Mass spectrometry offers improvements in sensitivity and quantitation capabilities without need for antibodies
- Need to develop and validate AD-Biomarkers in medium throughput multiplex assays

- New diagnostic paradigms based on multiplex detection of blood biomarkers will have a significant impact on clinical management of AD
- Biomarker support for drug development will improve drug efficacy and safety whilst reducing size, length and cost of clinical trials
- Proteomics studies of AD are providing new insights into disease pathology providing new targets and biomarkers

Proteome Sciences plc

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