NEW APPROACHES TO DIAGNOSTICS
The Impact of Biomarkers in Alzheimer’s Disease

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Scope of the talk

- 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
What is a biomarker?

- **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”

Ideal biomarkers of AD

- 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

- Primary prevention
- Disease modification
- Palliation
- Pathology

Symptoms:
- 3 years
- MCI
- AD
## Tests for Alzheimer’s

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<th>Use in Clinical Management</th>
<th>Utility in Clinical Trial</th>
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<td>risk of acquiring dementia</td>
<td>Enriching at risk populations c.f. APOE and MCI trials</td>
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<td>Early diagnosis</td>
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<td>Pharmacogenomics c.f. NICE and ACHEIs</td>
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AddNeuroMed

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

Current methods of diagnosing AD

- **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
Current methods of diagnosing AD

- **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
Current methods of diagnosing AD

- **Cerebrospinal fluid**
  - Monitoring of brain-derived protein levels
  - Well established markers related to disease pathology – Aβ, tau, phospho-tau
  - Moderately sensitive and specific
  - Some markers may correlate with stage and/or progression
  - Poor leakage into blood
  - Of limited diagnostic potential
Current methods of diagnosing AD

- Cerebrospinal fluid

Current methods of diagnosing AD

- 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)

Sample → 2DE-gel → Image analysis → MS/Bioinformatics

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

Sample → Labeling → HPLC/MS; HPLC/MS/MS → Bioinformatics
SELDI – Control vs AD
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
Towards multiplexed assays

8 Protein MRM Array

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

Instrumentation
1. Triple Quadrapole
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
MS tau phosphorylation assays

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
Conclusion

- 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
Conclusion

- 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
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