The cost effective development of a new blood based AD test

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Biotech in a Norwegian perspective...
Blood based assay for early diagnosis of Alzheimer’s Disease

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- Professor II, Institute of Clinical Medicine, University of Oslo

Research Discovery:
- Macrophage dysfunction causes amyloid plaque formation.
- Mid-domain amyloid beta peptides well suited for diagnosis and monitoring of Alzheimer’s disease
  - Macrophages in blood reflects their CNS counterparts
  - Prototype blood based immunoassay (ELISA) developed

Preliminary results demonstrates accuracy in line with current gold standards (beta-Amyloid spinal fluid tests)

The R&D history-
From macrophages in CSF to peptides in blood

1997-98: Macrophages isolated in spinal fluid from AD patients and patients with other conditions
2007-08: Peptide mining, identifying smaller fragments of beta-amyloid
2010: IP-MS-analysis of macrophages from AD-patients and patients with other conditions showed different patterns
2010: Peptides demonstrated in blood monocytes, in the lysosomal fraction
2013: Peptides after Cathepsin D digestion, demonstrating enzymatic catabolism of beta-amyloid in cultivated cells
The R&D history-
From identified peptides in blood to a diagnostic test

Applying the immuno-PCR test on patient cohorts demonstrating the diagnostic potential in pre-dementia stages of Alzheimer’s disease

The novel antibodies are specific for mid-domain peptide while the general Aβ-antibody is only specific for the longer fragments and not mid-domain

AD-mouse (TgArcSwe) stained with the antibodies showing a glial cell containing the new peptide, supporting the notion that the peptide is a post-phagocytic degradation product of Aβ42.

Development of the immuno-PCR method for detection of the new Aβ degradation-based peptide

New antibodies for beta-amyloid degradation developed. Several mouse monoclonal IgG antibodies resulting from immunization with beta-amyloid 21-31

New study confirms the initial proof of concept study

Proof of Concept # 1

- Levels of monocyte mid-domain Aβ differentiate controls from pre-dementia and Alzheimer dementia cases

Proof of Concept # 2

- A new, independent and larger cohort confirms the initial finding.

The test detects pathology in the pre-dementia stage (SCI & MCI)
Aβ 21-31 (mid domain) …Current procedure & immunoassay

Macrophage isolation

1. 4 ml heparin blood
2. Prenatrol E 10 ml (Elpal)
3. Crebrate HEPES
4. Gentamicin (20 min)
5. Phenteramine
6. Incubation PBS
7. Sterilize (10 min)
8. Lysis
dna
9. Phosphate
10. Gentamicin (20 min)
11. Phenteramine incubation
12. Phosphate
13. Toluene
14. Sterilize (10 min)
15. Phosphate
16. Blood Ruiz
17. Gentamicin (10 min)
18. Phosphate
19. Magneto separation
20. Cell culture
21. Lysis
22. Pipette

PCR immunoassay

Level cell counts
Aβ-31 fragment
Solid elution coated with Aβ3
capture antibody coated Aβ1-41
Aβhep-(Aβ31) sample

Top Level project plan
EU market launch in 2016

Blood sampling and macrophage isolation

<table>
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<tr>
<th>Partners</th>
<th>ELISA analytical development</th>
<th>Clinical documentation</th>
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Cost efficient partnerships ensures timeliness and product quality, ELISA-test using conventional technologies and instrumentation
Aβ 21-31 (mid domain)
Macrophage isolation...

1. 4 µM fluorescein
2. Polybrene 8 µg/ml (+/− 16 µg/ml)
3. Carrier (+/− Hepes)
4. Brefeldin A (20 µM)
5. Anti-CD25 PE/Cy5
6. Fixation
7. Flow cytometer (10 ml)
8. Linear array
9. Post-treatment
10. Brefeldin A (10 µM)
11. Anti-IgG2a antibody
12. Flow cytometer plate
13. Flow cytometer
14. Brefeldin A (10 µM)
15. Post-treatment
16. Magnification
17. Flow cytometer
18. Flow cytometer
19. Flow cytometer
20. Post-treatment
21. Linear array
22. Pipette

Work package 1;
...Milestone plan

Initiation & protocol
Training
Reference method
Patient recruiting
Macrophage isolation
ELISA
Data QC & Reporting
Project management

2Q14  3Q14  4Q14  1Q15  2Q15  3Q15  4Q15  1Q16
Preliminary positive results using a conventional ELISA immunoassay
- Same monoclonal antibodies
- Linear standard curve
- Highly sensitive

Work package 2;
...Milestone plan
Top Level plan pharma and clinical use in EU

Proof of concept immune-PCR 2010-2011
Proof of concept additional patients 2012
Assay & blood sample handling development 2013
Verification and validation 2014
Approval EU 2015

Ongoing clinical trials conducted by AHUS, EU sample access program ongoing
New blood sampling/handling included in clinical trials
Assay ready for pharma phase 1-2
Assay ready for pharma phase 3
Assay ready for clinical use EU

Building technical & clinical documentation to support pharma development and later on clinical diagnostic use in EU

Aβ 21-31 (mid domain)
...Potential new immunoassay

Macrophage isolation

ELISA immunoassay
The importance of diagnosing Alzheimers Disease early

- Although no efficacious disease modifying drugs are currently available;
  - Intervention and life change delay disease progression
  - Early diagnosis is cost effective
  - Patients and relatives want to know
  - Pharma is moving towards the pre-dementia stage in new clinical studies

Currently AD diagnosis involves invasive and expensive procedures, no convenient, affordable nor invasive blood tests available

The Alzheimer’s Disease Market

... most growth is in Diagnostics and Biomarkers.

- “The exponential increase in the expected number of patients presenting with Alzheimer’s disease not only represents a major area of unmet medical need, it represents a significant market opportunity for therapeutics and diagnostics as there is currently no fully effective method of treating the disease.”
- “The market segment expected to see the most growth is Diagnostics and Biomarkers.”

“The stage is set for savvy market players to capture significant market share.”
Blood based Alzheimer tests ....so far

- No solid clinical documentation or applicability
  - The two hallmark proteins (beta Amyloid & Tau) in blood have no diagnostic applicability in AD (Zetterberg AAIC 2014)
  - Multiple independent assays (protein/RNA) and complex multivariate bioinformatrics
    - Unstable diagnostic tools with too optimistic diagnostic models
    - Limited links to known disease pathology
  - Cultivated blood cells, various approaches: no convincing clinical documentation.

Pre Diagnostic will produce a new ELISA-test using conventional technologies and instrumentation, directly linked to the Alzheimer pathology

- an attractive position in the future of AD management

Substantial unmet medical need within Alzheimer’s Disease
Affordable blood test for very early AD detection
Partnership with leading pharma and diagnostic companies
Strong IP & experienced team