In vivo molecular diagnostics in Alzheimer Disease

The LeARN (Leiden-Alzheimer Research Nederland) project
Age-related cognitive impairment and dementia impose an increasing burden to the aging Western societies. According to the report of the Health Council of the Netherlands (2002), at least 1% of individuals 65 years of age suffer from dementia, a figure that rises to more than 20% for the age group of 80+. This burden comprises loss in quality of life of affected patients and their environment and high healthcare costs (approx. 3 billion Euro yearly in the Netherlands).

Alzheimer’s disease (AD) is the most frequent cause of dementia in old age (approximately 50%), and forgetfulness is often its first manifestation. Memory loss and related cognitive dysfunctions, however, are not specific for AD and occur in many elderly not suffering from this disease. In a large number of subjects, memory loss and related cognitive dysfunctions will remain an isolated finding. Still, the widespread awareness of memory loss as an early sign of AD make elderly with memory complaints (and their relatives) fear that dementia will follow. This situation and the high prevalence of memory complaints in the growing elderly population lead to a high and further increasing demand for medical help and increasing health care costs. The main question for patients with memory complaints is whether they will develop dementia or not. Consequently, for physicians dealing with these patients, an important challenge is to differentiate those who will not develop dementia (and who can thus be comforted) from those who will develop full-blown AD with dementia (and for whom healthcare should be provided). Currently, reliable tests for a definite diagnosis of AD in living patients are absent. The final diagnosis is based on histological findings at autopsy.

In this project, the aim was to develop and evaluate new instruments, based on molecular markers, that allow for 1) an earlier and more reliable diagnosis of AD during life, and 2) an effective evaluation of novel therapies for AD patients. In work package 1 novel PET radiotracers were developed that targeted the NMDA receptor, and that can help stratification of AD patients for individualized treatment and for development of new generations of drugs. In this work package two new compounds have been developed and identified as promising; they have been tested in human volunteers and patient studies have been planned. In work package 2 novel diagnostic imaging techniques exploiting the recently launched ultra-high field MRI technology were developed. In this work package new contrasts were discovered in brain tissue of AD patients based on iron accumulation. This observation sheds new light on the pathophysiology of AD and may lead to a new biomarker for the disease. The diagnostic value of this observation is now being evaluated in human studies. Another approach in this work package was to develop molecules that are visible with MRI and that allow for detection of molecular hallmarks of AD in vivo. Promising agents based on a specific type of antibodies have been developed and are awaiting further evaluation. The aim of work package 3 was the development of novel techniques for the detection of minute amounts of AD molecular markers in cerebrospinal fluid. However, the new techniques that were developed in this work package have not proven to be superior to existing methods. Finally, in work package 4 the clinical and economic value of existing and newly developed diagnostic tests was assessed. The basis for this work package was the rich set of patient data that has been acquired in this project. These data are being analyzed and results will continue to be revealed.
Clinical Need

Tests that contribute to making the diagnosis AD in vivo, in a specific way and at an early stage, by detecting the molecular hallmarks of AD (currently the diagnosis is only made post-mortem).

Tools

For this project, it was chosen to focus on techniques that are particularly promising and that already shown sound proof-of-concept. This enables a fair likelihood of knowing the clinical and economic value at the conclusion of the project. Approach:

a. Development of PET tracers for the NMDA receptor.
b. Development of novel diagnostic imaging techniques at ultra high field MRI.
c. Development and validation of analytical techniques for novel biochemical markers.
New innovative (molecular) imaging and molecular diagnostic (CSF) tests for early diagnosis of Alzheimer Disease
Organization and Partners

**Advisory board LeARN ISAC CTMM**

**Workpackage leaders**
- WP1: Dr. B. van Berckel (VUmc)
- WP2: Prof. Dr. M.A. van Buchem (LUMC)
- WP3: Dr. M. Verbeek (Radboudumc)
- WP4: Prof. Dr. F. Verheij (MUMC)

**Partners**
- Coordination
- Finance
- Publications

**SteeringCie**
Partner Representatives
CTMM

**Project Team**
PI: Prof. Dr. M.A. van Buchem
Co-PI: Dr. B. van Berckel (VUmc)
IP manager: Andrea Hall (LUMC)
WP leaders
Industrial partners
Dr. Ir. Henny Bruinewoud (CTMM)

**Coordination Finance Publications**

**CTMM**

**ADVICE**

**DECISIONS**

**OPERATIONS**
Budget: CTMM manages the flow of funds

**Funding:**
- 25% Academia
- 25% Industrial
- 50% Government Subsidy

**Project costs:**
- Personnel
- Materials
- Use of existing equipment
- Investments
- Third parties
- Management (5%)
Facts & Figures

Distribution of the LeARN budgets to the partners in order to perform the R&D activities

Budget 14 M €
Start 2008
End 2014
Partners 12

CASH COSTS

<table>
<thead>
<tr>
<th>Category</th>
<th>Academic cash costs</th>
<th>Total industrial cash costs</th>
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<tbody>
<tr>
<td>PhD student</td>
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<td>0</td>
</tr>
<tr>
<td>PostDoc</td>
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</tr>
<tr>
<td>Senior Staff</td>
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<td>1.000.000</td>
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<tr>
<td>Supporting Staff</td>
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<tr>
<td>IT-staff</td>
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<td>Materials &amp; Services</td>
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<tr>
<td>Investments</td>
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KIND COSTS

<table>
<thead>
<tr>
<th>Category</th>
<th>Academic kind costs</th>
<th>Total industrial kind costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhD student</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PostDoc</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Senior Staff</td>
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<td>0</td>
</tr>
<tr>
<td>Supporting Staff</td>
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<td>0</td>
</tr>
<tr>
<td>IT-staff</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Materials &amp; Services</td>
<td>2.000.000</td>
<td>2.000.000</td>
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Academic Partners
Industrial Partners Large
Industrial Partners SME
CTMM investments
## Facts & Figures

<table>
<thead>
<tr>
<th>Category</th>
<th>No</th>
<th>Details</th>
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<tr>
<td><strong>Output</strong></td>
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<tr>
<td>Papers</td>
<td>69</td>
<td>6 papers in submission - mean impact factor all published LEARN papers: 5.9</td>
</tr>
<tr>
<td>Theses</td>
<td>10</td>
<td>6 planned</td>
</tr>
<tr>
<td>Personal grants</td>
<td>3</td>
<td>2014 Marie Curie International Outgoing Fellowship (Rik Ossenkoppele, VUmc); 2014 Internationale Stichting Alzheimer Onderzoek: Identification of novel Aβ-associated proteins involved in early AD pathogenesis (NM Timmer, Radboudumc); 2014 VIDI grant Louise van der Weerd, LUMC.</td>
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<tr>
<td>Patent application</td>
<td>6</td>
<td>P.J. Klein (Vumc); P.J. Klein (Vumc); J.A.M. Christiaans (Vumc); L. van der Weerd (LUMC); J. Schooleman (Virtual Proteins) J.A.M. Christiaans (Vumc, aborted)</td>
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<td>Spin-off activities</td>
<td>1</td>
<td>Collaboration with MVI-project ‘Responsible (early) diagnostics for Alzheimer’s Disease’. PI Marianne Boenink, University of Twente.</td>
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<tr>
<td>Raising Capital (&gt; 1 M€)</td>
<td>3</td>
<td>CVON grant 2013: Heart-brain access - Prof M. van Buchem (LeARN) &amp; Prof M. Daemen (PARISK); ZonMW- Memorabel Programma 2014: MM Verbeek (PI), MA van Buchem, M Vernoij, A Rozemuller et al.: “CAVIA: Cerebral Amyloid Angiopathy: Vascular Imaging and fluid markers of Amyloid deposition”; Internationale Stichting Alzheimer Onderzoek (ISAO) 2014: “Aβ clearance and barrier dysfunction in CAA, MM Verbeek”</td>
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<tr>
<td>Awards</td>
<td>2</td>
<td>2014 Internationale Stichting Alzheimer Onderzoek (ISAO) Postdoc Price (Rik Ossenkoppele, VUmc) 2012 Internationale Stichting Alzheimer Onderzoek (ISAO) fellowship (Stephanie Vos, MUMC)</td>
</tr>
</tbody>
</table>

### Budget
- **14 M €**
- Start 2008
- End 2014
- Partners 12
- Charity 0
- Persons 63
- FTE 90 (5 years period)
PET tracers development:
- > 100 fold selectivity of GMOM and PK209 for NMDA receptor ion channel (screened against 79 CNS targets)
- Successfull synthesis and labeling of 4 new compounds for the PCP site with higher affinity compared to GMOM
- Radiolabeled HACH242 shows in-vitro a 2-fold higher affinity for the NR2B site, compared to σ sites, and >100-fold selectivity against all other targets examined
- First in humans studies of PET tracers 11C-GMOM and 18F-PK209
- Development of tracer kinetic models for 11C-GMOM and 18F-PK209

MRI imaging:
- During the application of the developed MRI protocols a considerable number of scans was significantly affected by motion artifacts deteriorating the quality of the images. Further investigation into this problem demonstrated that these artifacts were not induced by regular physiological movements (heart, respiration) but by movements of arms or heads, which is not unexpected considering the cognitive status of our patients. LUMC investigators developed a method to correct for these artifacts, using navigator techniques, that considerably improved the quality of our clinical data.
- Phase difference between gray and white matter in the neocortex is increased in AD patients compared to controls, which most likely indicates a higher amount of iron deposition in the gray matter.
- A novel and robust loading and purification method was developed for the post-hoc labeling with 111In inside of the VHH-formulated GSH-PEG liposomes. With this system, almost 100x higher levels of VHHs have been obtained in the brain (in-vitro) by the GSH-PEG liposomal formulation as compared to free VHHs

CSF – in-vitro diagnostics:
- Low-molecular weight oligomers in CSF are not suitable as diagnostic biomarkers for AD, but may be related to progression
- Levels of several glutamate-related proteins (vGlut1, GLUD1 and PSD95) may be regarded as proteomic biomarkers in AD for the relation between Aβ accumulation and glutamatergic neurotransmission.
- A novel, potentially interesting, Aβ-binding protein has been discovered (dkk3). Its affinity to Aβ, colocalization in amyloid plaques and its potential as a biomarker has been investigated.
- A novel assay for glutamine synthetase has been developed and offers potential as biomarker for discrimination of dementia syndromes. After clinical validation in 100 dementia patients and controls it revealed no differences, however clinical validation in 44 NMO, 68 MS, 6 ON, and 37 control CSF revealed significantly elevated levels in NMO and MS patients.
- Development of antibodies specific for high-molecular weight oligomers
- Aβ/ ApoE complexes can be detected in the circulation
- The micro-RNA’s miR29a and miR146a were identified as novel biomarkers for AD
- Levels of many miRNAs in CSF are modified by cell count

Clinical epidemiology & HTA
- Preclinical AD is common and strongly associated with future cognitive decline and mortality. This makes preclinical AD an important target for therapeutic interventions.
- Headroom analysis indicated that 0.39 possible QUALYs to be gained and €30,081 possible savings per subject if a perfect test was simulated in all subjects.

Highest Impact Papers – mean 14,3

Mean Impact Factor

<table>
<thead>
<tr>
<th></th>
<th>International - neurology</th>
<th>CTMM - neurology</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>Mean impact factor based on 106 papers from the CTMM cardiovascular first call projects.</td>
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Scientific Value Creation - Theses

<table>
<thead>
<tr>
<th>Thesis</th>
<th>Partner</th>
<th>Year</th>
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<tbody>
<tr>
<td>Petra Spies</td>
<td>Radboudumc</td>
<td>2012</td>
</tr>
<tr>
<td>Rik Ossenkoppele</td>
<td>VUMC</td>
<td>2013</td>
</tr>
<tr>
<td>Lyzel Elias – Sonnenschein</td>
<td>MUMC+</td>
<td>2013</td>
</tr>
<tr>
<td>Rob Nabuurs</td>
<td>LUMC</td>
<td>2014</td>
</tr>
<tr>
<td>Lies Clerx</td>
<td>MUMC+</td>
<td>2014</td>
</tr>
<tr>
<td>Ron Handels</td>
<td>MUMC+</td>
<td>2014 <em>cum laude</em></td>
</tr>
<tr>
<td>Stephanie Vos</td>
<td>MUMC+</td>
<td>2014 <em>cum laude</em></td>
</tr>
<tr>
<td>Megan Herbert</td>
<td>Radboudumc</td>
<td>2014</td>
</tr>
<tr>
<td>Wesley Jongbloed</td>
<td>VUMC</td>
<td>2014</td>
</tr>
<tr>
<td>Sanneke van Rooden</td>
<td>LUMC</td>
<td>2015</td>
</tr>
<tr>
<td>Pieter Klein</td>
<td>VUMC</td>
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<tr>
<td>Mareike Mueller</td>
<td>Radboudumc</td>
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<td>Sofie Adriaanse</td>
<td>VUMC</td>
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<td>Kim Bruggink</td>
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<tr>
<td>Thalia Doef</td>
<td>VUMC</td>
<td>Planned</td>
</tr>
<tr>
<td>Marissa Zwan</td>
<td>VUMC</td>
<td>Planned</td>
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</table>
Scientific Value Creation - Infrastructure

- Radioligand in/vitro binding assays, including saturation, competition and kinetics.
- Various competition assays were set up, using [3H]MDL 105,519, [3H]infenprodil, and [3H]MK-801 as the reference ligands.
- Quantitative autoradiography to visualize radioligand distribution in the rodent brain.
- Dynamic PET scans in rhesus monkeys.
- Transgenic AD mice models for evaluating BBB passage of labeled compounds.
- APP/PS-1 and Tg-SwDI mice models for parenchymal and vascular accumulation of Aβ proteins.
- Animal models to study acute effects of the NMDA-receptor antagonists L-701,324 and MK-801 have been developed. Read-outs: behavioral analysis, protein and metabolite analysis (targeted and "–omics")

251 AD patients included in the LeARN cohort:

<table>
<thead>
<tr>
<th>Centre</th>
<th>BL</th>
<th>1 year FU</th>
<th>2 year FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUMC</td>
<td>77</td>
<td>83 %</td>
<td>66 %</td>
</tr>
<tr>
<td>Radboudumc</td>
<td>50</td>
<td>72 %</td>
<td>66 %</td>
</tr>
<tr>
<td>VUmc</td>
<td>124</td>
<td>82 %</td>
<td>70 %</td>
</tr>
<tr>
<td>LUMC</td>
<td>39</td>
<td>7 %</td>
<td>9 %</td>
</tr>
</tbody>
</table>

- Data available:
  - MRI (DTI, rsMRI): for all 251 patients
  - CSF (beta-A, (P)-tau): for all 132 patients
  - PET (FdG, PIB-PET): for all 112 patients
- Decision analytic model (Excel)
- Assay development:
  - to quantify Aβ aggregates in tissue and CSF
  - to quantify complexes of Aβ with other proteins. Such as Aβ / α1-antichymotrypsin, αβ-crystallin and Aβ / ApoE complex. But also novel Aβ-binding proteins.
  - Assays for glutamatergic neurotransmission.
- Hybrid assays with increased sensitivity for immunodetection of proteins: expertise obtained with Bio-barcode assay and immuno-PCR assay
- Technology platforms:
  - Proteomics and Metabolomics (LC-MS/MS and 1H-NMR-spectroscopy) of brain tissue and CSF
  - Developed pipeline for the identification of novel Aβ-binding proteins, which can be applied to any protein of interest
  - Methods to quantify miRNAs in tissue and CSF
- PET protocols were developed using arterial sampling to generate a metabolite corrected input curve. This includes displacement studies with intravenous administration of ketamine.
- 7T MRI protocols: data acquisition and data processing
- Two libraries of 19F based compounds: backbone bisstyrylbenzenes and pyridine.
- Pre-clinical SPECT protocols
- Fluorescent and radioactive labeling protocols of VHH's
Clinical and Economic Value Creation of LeARN

New ‘products’ for clinical care
# Main Product Pipelines – in-vitro diagnostics

### Discovery Biomarkers / assay development

<table>
<thead>
<tr>
<th>Hybrid assays (technology platform)</th>
<th>bio-barcode assay Immuno PCR</th>
<th>Both hybrid assay designs failed to increase sensitivity in several immunoassays.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ oligomers</td>
<td>2 assays developed</td>
<td>Limited diagnostic value, but relation shown with progression. To be validated in LeARN cohort.</td>
</tr>
<tr>
<td>Heteromeric Aβ aggregates</td>
<td>αB-crystallin: failed, technical problems</td>
<td>Not financed by CTMM, follow up initiated</td>
</tr>
</tbody>
</table>

### CSF biomarkers

<table>
<thead>
<tr>
<th>Proteomics: targeted approach</th>
<th>Glutamine synthetase: clinical validation in 100 dementia patients &amp; controls revealed no differences. Novel Aβ–associated protein: “dkk3”. clinical validation in 30 patients with AD, 17 with DLB, 18 with FTD and 30 non-demented controls revealed no differences in either CSF or serum.</th>
<th>Not financed by CTMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteomics: untargeted approach</td>
<td>87 targets identified; 6 proteins selected but not reproduced in validation</td>
<td>Not financed by CTMM</td>
</tr>
<tr>
<td>Metabolomics: targeted approach</td>
<td>Glycine and aspartate</td>
<td>Not financed by CTMM</td>
</tr>
<tr>
<td>Metabolomics: untargeted approach</td>
<td>Chemometry analysis abandoned due to lack of identification of novel biomarkers</td>
<td>Not financed by CTMM</td>
</tr>
<tr>
<td>Modulators of NMDA receptors: targeted metabolomics</td>
<td>Abandoned due to lack of identification of novel biomarkers</td>
<td>Not financed by CTMM</td>
</tr>
<tr>
<td>Modulators of NMDA receptors: untargeted proteomics</td>
<td>Tg mice did not reveal novel protein biomarkers associated with glutamergic neurotransmission</td>
<td>Not financed by CTMM</td>
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</table>
PET tracers aiming at the NMDA receptor

The PET tracers developed in LeARN aim at in vivo detection of the neurotransmitter glutamate and one of its receptors (NMDA receptor) in the brain. A schematic overview of this receptor is given in the Figure below:

The most obvious clinical applications for a NMDA receptor PET ligand in AD are:
1: effective dosing of NMDA antagonists for therapeutic purposes
2: identifying distinct phenotypes of AD
3: prediction of treatment response of drugs targeting the NMDA receptor
4: neuroprotective treatment.

**Product**
PET tracer [11C]GMOM (PCP site)

**Leading partners**
VU medical center Amsterdam, Cyclotron BV

**Current strategy**
Clinical diagnosis with deteriorating course; confirmation of clinical diagnoses only post-mortem. No effective treatment currently available.

**Prevalence:** 260,000 in NL (2014), 35.6 Million global (2012)
**Yearly costs:** approx. 4,8 M€ yearly in NL (2011), approx. 604 US$ global (2010)

**Expected impact of this product:**
Both the PCP site and the NR2B site ligand can be used to assess in-vivo occupancy of novel drugs targeting these sites. In addition, NMDA receptor bioavailability can be related to cognitive function, especially memory function both in healthy and diseased states, like AD, depression and schizophrenia.

The combination of NR2B imaging and PCP site imaging is also very interesting as binding to the PCP site is depending on activity of the NMDA receptor while NR2B binding depends on the number of receptors.

**PET tracers aiming at the NMDA receptor**

**Main achievements in CTMM:**

**PCP site of NMDA receptor:**
[11C]GMOM
The first NMDA receptor ligand assessed in humans. Preclinical evaluation of this compound was published before. In addition to that, the project team labeled GMOM with [3H] and tested it extensively in pharmacological assays to set up a proper in vitro binding assay and to replicate the published binding characteristics of [3H]GMOM.

The GMP compliant synthesis of [11C]GMOM is performed in the VUmc. Microdosing toxicity studies were successful and the compound was cleared for human application. Ten healthy volunteers underwent 90 minutes dynamic PET scans. A plasma input tracer kinetic model for quantification of [11C]GMOM kinetics has been developed.

**Future outlook**
Test retest reliability study and phase I clinical study in patients with AD.

**Progress obtained in translational pipeline**

**Discovery**
Chemical synthesis & labeling
**Selection**
Preclinical Development
**Clinical Development**
GMP, Phase I, II & III
**Market access**

**PET tracers aiming at the NMDA receptor**

**PET tracers aiming at the NMDA receptor**

**PET tracers aiming at the NMDA receptor**

**PET tracers aiming at the NMDA receptor**
PET tracers aiming at the NMDA receptor

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1: effective dosing of NMDA antagonists for therapeutic purposes
2: identifying distinct phenotypes of AD
3: prediction of treatment response of drugs targeting the NMDA receptor
4: neuroprotective treatment.

Product
PET tracer [18F]PK209 (PCP site)

Leading partners
VU medical center Amsterdam, Cyclotron BV

Current strategy
Clinical diagnosis with deteriorating course; confirmation of clinical diagnoses only post-mortem. No effective treatment currently available.

Main achievements in CTMM:
PCP site of NMDA receptor: [18F]PK209
This NMDA receptor ligand has been developed from the drawing board in the VUmc within WP 1. Preclinical evaluation in rodents showed appropriate blood brain penetration and washout. Metabolism in blood was found to be very fast but there is no evidence that metabolites enter the brain. The GMP compliant synthesis of [18F]PK209 is set up in the VUmc. Primate studies confirmed data found in rodent studies. Microdosing toxicity studies were successful and the compound was cleared for human application. Six healthy volunteers underwent 120 minutes dynamic PET scans. A plasma input tracer kinetic model for quantification of [18F]PK209 kinetics has been developed.

Expected impact of this product:
Both the PCP site and the NR2B site ligand can be used to assess in-vivo occupancy of novel drugs targeting these sites. In addition, NMDA receptor bioavailability can be related to cognitive function, especially memory function both in healthy and diseased states, like AD, depression and schizophrenia. The combination of NR2B imaging and PCP site imaging is also very interesting as binding to the PCP site is depending on activity of the NMDA receptor while NR2B binding depends on the number of receptors.
PET tracers aiming at the NMDA receptor

**Main achievements in CTMM:**

**NR2B site of NMDA receptor:**

Compound HACH242 has been radiolabeled with carbon-11, in good yields and high purity. Initial *in vitro* blockade by the selective antagonist Ro25-6981 indicated high ligand selectivity for the NR2B subunit of NMDA receptors. Full selectivity profiling has been completed at Cerep. HACH242 shows 2-fold higher affinity for the NR2B site, compared to σ sites, and >100-fold selectivity against all other targets examined. *In vivo* brain uptake of [11C]HACH242 is one of the highest reported in literature (forebrain:cerebellum ratio=3.2±0.7). *In vivo* blocking studies in rats and mice show variable results, which have been attributed to the use of anesthesia. Microdosing toxicity of HACH242 were completed at Advinus in June 2013. This compound has been cleared for human use and GMP compliant synthesis has been implemented.

**Expected impact of this product:**

Both the PCP site and the NR2B site ligand scan be used to assess in-vivo occupancy of novel drugs targeting these sites. In addition, NMDA receptor bioavailability can be related to cognitive function, especially memory function both in healthy and diseased states, like AD, depression and schizophrenia. The ... to the PCP site is depending on activity of the NMDA receptor while NR2B binding depends on the number of receptors.
7T MRI protocols: native contrast

Currently, the role of MRI in the diagnostic work-up of patients with memory loss or dementia, in whom AD is suspected, is limited. MRI is used a) to exclude other causes than AD for the symptoms (e.g. brain tumors), and b) to gain positive evidence for the presence of AD. 7T imaging opens new frontiers in biomedical imaging, allowing for higher anatomical resolution and increased sensitivity for tissue iron.

**Product**
Clinical protocols for detection of AD-specific cortical changes exploiting increased sensitivity of 7T imaging for tissue iron.

**Leading partners**
LUMC and Philips

**Current strategy**
Clinical diagnosis with deteriorating course; confirmation of clinical diagnoses only post-mortem. No effective treatment currently available.

**Main achievements in CTMM:**

**MRI protocols: data acquisition**
T2*-weighted sequences for 7T SWI MRI (motion artefacts reduced) have been tested in 300 subjects (60 late onset AD, 20 early onset AD, 20 MCI, 20 subjective memory complaints, 50 HCHWA-D, 130 controls).

The data demonstrated that phase changes in the cortex selectively occur in AD patients and not in controls. The underlying process of this can be found in the combination of altered iron metabolism with differences in myelin structures in subjects with AD.

**MRI protocols: data processing**
The developed processing tools include tools for phase unwrapping from native T2*-weighted MR sequences, cortical segmentation and parcellation tools based on ultra-high field T2*-weighted data, and tools to segment the hippocampus on ultra-high field T2*-weighted images.

**Expected impact of this product:**
This clinical tool is capable to clearly distinguish specific pathological processes, i.e. differences in iron metabolism, between early and late onset AD, indicating a more severe cortical iron deposition in early AD compared with late onset AD. Currently, the translational aspect of our data, which is the analysis and implementation of this technique at lower field strength (3T) is being investigated.
Amyloid-beta (Aβ) related stroke and cognitive dysfunction is affecting many patients globally. Related diseases range from rare, hereditary diseases with frequent strokes (HCHWA-D), cerebral angiopathies with severe vascular damage (CAA), to progressive dementia in a generalized ageing population (Alzheimer’s disease; AD). In contrast to AD, the more specific occurrence of CAA in patients, characterized by highly localized Aβ deposits within cerebral blood vessels, has an established pathophysiological link between Aβ accumulation and vascular damage. There is a high unmet need for patient selection in this disease population. Using labeled probes that target to amyloid provides tools for patient stratification. This requires specific drug delivery approaches to allow sufficient amounts in the brain without destroying the blood-brain barrier (BBB).

**Pre-clinical Product**
Liposomal formulation loaded with labeled VHH & SPECT protocols

**Leading partners**
2BBB, BAC and LUMC

**Current strategy**
Clinical diagnosis with deteriorating course; confirmation of clinical diagnoses only post-mortem. No effective treatment currently available.

**Expected impact of this product:**
These labeled VHHs allows detecting and differentiating two important disease entities: parenchymal amyloid in AD and vascular amyloid in cerebral amyloid angiopathy (CAA), which is currently not possible. These probes could also be used to study the pathogenesis of AD and CAA. In particular probes that allow for detecting CAA could help to unravel the presumed vascular contribution to dementia in general and AD in particular.

**Main achievements in CTMM:**
- In vivo pharmacodynamic studies using 99mTc-labeled VHHs, observing fast renal clearance. Brain uptake of VHH-pa2H was significantly higher in AD mice compared to wild-type controls 24 hours after injection.
- DMPC and EYPC-based formulations of the nanoparticles were stable and had sufficient circulation time in combination with VHH.
- Since SPECT isotopes have a sufficiently long radioactive half-life (e.g. 111-Indium: 67 hours) a SPECT labeling method for the VHH was developed, based on DTPA chelation. Active loading methods to radiolabel the VHH with 111-Indium after encapsulation in the nanoparticles were prepared, which allows radioactive labeling at the time of imaging.
- In vivo biodistribution studies with the radiolabeled particles show > hundred-fold improvement of targeted brain uptake of the VHH using liposomal delivery.

**Future outlook:**
Translation towards clinical studies.
CSF in-vitro diagnostics

The CSF is closely connected to the brain and its composition may reflect pathological processes occurring in the brain. In detecting Alzheimer’s disease, and differentiating them from other dementia disorders, CSF markers such as amyloid-β-42, tau and phospho-tau are often measured. However, these biomarkers have a limited diagnostic accuracy. The measurement of proteins in CSF that are specific for one disease, would be of great value. However, such proteins are often present at very low concentrations, that are undetectable by conventional ELISA. Therefore, more sensitive techniques are required to obtain a low detection limit.

Potential Product
Novel assays to quantify:
1. Glutamine synthetase (GS) assay
2. microRNAs in CSF
3. Aβ oligomers in CSF
4. ApoE/Aβ complexes in serum

Main achievements in CTMM:
- A novel technique for the quantification of multimeric proteins has been developed and applied to the quantification of Glutamine synthetase in CSF. GS naturally occurs as a decameric protein. A specific sample preparation was developed to convert decameric GS into monomeric GS which allows for the subsequent quantification by a newly developed ELISA.
- Novel biomarkers that were identified for AD: miR146a and miR29a
- We observed that cell number in CSF is an important confounder of microRNA quantification if CSF
- Identification of dkk3 as a novel Aβ-binding proteins provides new insights into the pathogenesis of AD
- Novel proteomic biomarkers (vGlut1, GLUD1 and PSD95) for the relation between Aβ accumulation and glutamatergic neurotransmission.
- Establishment of animal models for the evaluation of effects of modulators of NMDA receptor function

Future outlook
- Extensive study of the value of miRNAs as biomarkers for dementia syndromes
- Study of ApoE/Aβ complexes as potential biomarkers for dementia syndromes
- Application of the technique to quantify multimeric proteins in CSF to other proteins than GS
- More extensive study on role of dkk3 in AD

Expected impact of this product:
The identification of specific miRNA’s as potential biomarkers for Alzheimer’s disease may be instrumental in defining an (early) diagnosis of the disease. Furthermore, identification of novel Aβ-associated proteins such as dkk-3 and the development of assays for the detection of protein complexes in biological fluids, may open a new field of biomarker research in Alzheimer’s disease and other neurological disorders that are characterized by accumulation of abnormally folded proteins.
New research criteria for the diagnosis of Alzheimer’s disease (AD) have recently been developed to enable an early diagnosis of AD pathophysiology by relying on emerging biomarkers. To enable efficient allocation of health care resources, evidence is needed to support decision makers on the adoption of emerging biomarkers in clinical practice. The following aspects were taken into account:

1) diagnostic test accuracy of current clinical diagnostic work-up and emerging biomarkers in MRI, PET and CSF,
2) cost-consequence analysis and
3) long-term cost-effectiveness

The uniqueness of this approach was the assessment of resource utilization and quality of life to enable an economic evaluation. Furthermore, the decision analytic model enables the evaluation of techniques to be developed during the study in sub-cohorts of the study population.

**Product**
Protocol to establish golden standard in diagnostic accuracy studies.
Decision analytic model to enable the evaluation of CSF in future clinical practice from a health-economic perspective.

**Leading partners**
MUMC

**Current strategy**
Clinical diagnosis with deteriorating course; confirmation of clinical diagnoses only post-mortem. No effective treatment currently available.

**Main achievements in CTMM:**
In a cohort design 241 consecutive patients suspected of having a primary neurodegenerative disease were approached in four academic memory clinics and followed for two years. Clinical data and data on quality of life, costs and emerging biomarkers were gathered.

Diagnostic test accuracy is determined by relating the clinical practice and new research criteria diagnoses to a reference diagnosis. Both the clinical practice diagnosis and the CSF based diagnosis at baseline were reflected by a consensus procedure among experts assessing the clinical information. The reference diagnosis is determined by a consensus procedure among experts based on clinical information on the course of symptoms over a two-year time period.

CSF markers had no added value to the standard diagnostic workup for predicting the 2-year course of cognitive or functional status. This can be explained by the extensive clinical diagnostic information available in standard practice, which enabled the experts to predict short-term decline fairly accurately. Future research should focus on the application of CSF markers in specific, rather than routine, situations such as in younger patients or those with atypical complaints or for predicting long-term course of symptoms.

**Expected impact of this product:**
The research results are important to decide what biomarker tests are valuable for patients. Furthermore, the results can be used to optimize the standard diagnostic workup and to promote an efficient use of medical resources.
The rapides

Main achievements in CTMM:

- Test sequence analyses suggest that CSF Aβ1-42/tau ratio has added value for short-term prediction of AD-type dementia in addition to hippocampal volume (HCV) assessment. Vice versa the added value of HCV is limited to subjects with normal CSF.
- In a memory clinic setting, combined [(11)C]PIB and [(18)F]FDG PET are of additional value on top of the standard diagnostic work-up, especially when prior diagnostic confidence is low.
- A decision analytic health economic model was developed to evaluate biomarkers in a future scenario in which disease modifying treatment is available. The simulation results indicate that when a (CSF) biomarker is performed to verify non-AD (instead to verify AD) it generates the highest benefits.

Expected impact of this product:
The research results are expected to improve the clinical diagnosis and to aid in the decision in what tests should be performed in current practice. In future practice, when disease modifying treatment becomes available, the decision analytic model aids in explicitly positioning a biomarker in the clinical diagnostic workup to maximize treatment benefit and ensure cost-effective care. The results indicated more potential benefit from a biomarker positioned to verify subjects who are not expected to have AD (i.e. to prevent under treatment).
Early HTA: Potential Impact of New Technologies

Current Care setting
Current clinical guidelines advise advanced biomarkers for AD only in part of the patients with mild cognitive impairment. No pharmacological treatments are available in this pre-dementia phase.

Model development
A diagnostic test sequence model for predicting AD-type dementia in MCI using cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) biomarkers was developed. This sequential testing was validated in the LeARN cohort using clinical practice simulation by expert panel consensus meetings to determine the risk of cognitive and functional decline in each patient.

Patient stratification
The sequential risk prediction model was validated in the LeARN cohort using clinical practice simulation by expert panel consensus meetings to determine the risk of cognitive and functional decline in each patient.

New care setting
If treatment becomes available then the impact of testing with advanced biomarkers for AD is related to the treatment effects and becomes highly important. Dependent on the treatment and population characteristics, testing should be targeted at preventing over- or undertreatment.

Performance evaluation
CSF increased predictive accuracy in subjects after MRI testing on hippocampal volume. MRI increased predictive accuracy only in subjects with normal CSF.

Implications
These results provide further support for the use of CSF and magnetic resonance imaging biomarkers to identify prodromal AD.

Potential of personalized treatment
CSF markers had no added value to the standard diagnostic workup for predicting the 2-year course of cognitive or functional status. The results demonstrate the importance of future research focused on the application of CSF markers in specific, rather than routine, situations such as in younger patients or those with atypical complaints.

Commercial Headroom available
Headroom analysis indicated that 0.39 possible QUALYs to be gained, €33,622 ($43,372) possible savings, 2.0 potential beneficial treatment years, and 1.3 year delay in dementia conversion per subject if a perfect test was applied in a single patient with MCI.
Partners

Centre for Human Drug Research (CHDR) - Leiden
Leiden University Medical Center (LUMC) - Leiden
Maastricht University Medical Center (MUMC+) - Maastricht
Radboud university medical center (Radboudumc) - Nijmegen
VU University Medical Center (VUmc) - Amsterdam
BAC BV - Leiden
BBB Therapeutics - Leiden
BV Cyclotron VU - Amsterdam
Merck Sharp & Dohme BV (MSD) - Oss
Royal Philips - Eindhoven
Virtual Proteins BV - Eindhoven
Center for Translation Molecular Medicine - Eindhoven


List of Publications


List of Publications

Other Sources

B. Nationaal Kompas Volksgezondheid, RIVM, https://www.volksgezondheidenzorg.info
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Aβ</td>
<td>Amyloid-beta</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
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<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
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<tr>
<td>BL</td>
<td>Baseline</td>
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<tr>
<td>CAA</td>
<td>Cerebrale Amyloid Angiopathie</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DLB</td>
<td>Dementia with Lewis Bodies</td>
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<tr>
<td>DMPC</td>
<td>1,2-Dimyristoyl-sn-glycero-3-phosphocholine</td>
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<tr>
<td>DTPA</td>
<td>Diethylene triamine pentaacetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>EYPC</td>
<td>Egg yolk phosphatidylcholine</td>
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<td>FDG</td>
<td>Fludeoxyglucose</td>
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<tr>
<td>FTD</td>
<td>Frontotemporal dementia</td>
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<tr>
<td>GMP</td>
<td>Good Manufacturing Practices</td>
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<tr>
<td>GS</td>
<td>Glutamine synthetase</td>
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<tr>
<td>HCHWA-D</td>
<td>Hereditary Cerebral Hemorrhage With Amyloidosis–Dutch type</td>
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<tr>
<td>HCV</td>
<td>Hippocampal volume</td>
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<td>IMPD</td>
<td>Investigational Medicinal Product Dossier</td>
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<td>ISAC</td>
<td>International Scientific Advisory Committee</td>
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<tr>
<td>ISAO</td>
<td>Internationale Stichting Alzheimer Onderzoek</td>
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<tr>
<td>LCMS</td>
<td>Liquid chromatography–mass spectrometry</td>
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<tr>
<td>LeARN</td>
<td>Leiden-Alzheimer Research Nederland</td>
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<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
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<td>MVI</td>
<td>Maatschappelijk Verantwoord Innoveren</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate receptor</td>
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<td>NR2B</td>
<td>N-methyl D-aspartate receptor subtype 2B</td>
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<td>ON</td>
<td>Optic neuritis</td>
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<td>PCP</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PET</td>
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<tr>
<td>PI</td>
<td>Principle Investigator</td>
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<tr>
<td>PIB</td>
<td>Pittsburgh compound B</td>
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<tr>
<td>QUALY</td>
<td>Quality-adjusted life year</td>
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<tr>
<td>SME</td>
<td>Small and medium-sized enterprise</td>
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<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
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<tr>
<td>SWI</td>
<td>Susceptibility weighted imaging</td>
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<tr>
<td>VHH</td>
<td>Variable domain of heavy chain antibodies</td>
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Co funded by

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