MultISyn: Multimodal Imaging of rare Synucleinopathies

PUBLISHABLE EXECUTIVE SUMMARY FOR PERIODIC REPORT

1. Summary description of project context and main objectives

Neurodegenerative diseases, such as Alzheimer’s disease (AD) or Parkinson’s disease (PD) are progressive, devastating and incurable neurologic conditions. In the majority of cases, they are thought to be caused by a complex interplay of genetic and environmental factors. The aggregation of disease-specific abnormally folded proteins, such as the A-beta protein in AD or α-synuclein (αSYN) in PD, and probably their spreading from cell to cell throughout the brain, appears to be the central driver of pathogenesis. The removal of these aggregates holds considerable promise as a therapeutic strategy. However, the relationship between protein misfolding and aggregation on the one hand, and neuronal dysfunction and cell death, on the other hand, is far from being well understood, particularly in the common sporadic forms of these disorders. The consequences of this lack of understanding are dramatically illustrated by the experience from failed vaccination studies in AD that resulted in a reduction of aggregate load but did not translate into clinical improvement. As a consequence, rare genetically defined forms of neurodegenerative disorders are now generally considered to offer substantial advantages for drug development such as a clear cause-effect-relationship and the possibility of diagnosis at an early stage of the disease process when it is still possible to modify its course.

Non-invasive imaging methods such as PET and MRI can be valuable tools to aid diagnosis of neurodegenerative diseases, provide input to differential diagnosis and follow disease progression or therapeutic effects of disease modifying treatments. However, and in contrast to the situation in AD, for many important neurodegenerative diseases including the synucleinopathies, tauopathies, and TDP-43 proteopathies, specific PET tracers for the underlying cellular pathology, which would allow tracking the disease burden are still lacking. Moreover, there has been no systematic work performed to link neuronal function as detected by fMRI with molecular information as provided by PET-imaging of aggregates in these diseases. Novel PET/MR imaging systems which could fill this knowledge gap for rodents and humans are available but require the establishment and validation of sensitive and aggregation specific tracers as well as of disease-specific imaging protocols and data analysis tools.

Furthermore, the research community faces a major challenge in generating evidence to support that animal models have predictive validity in development of disease modifying therapies for humans. This is especially true for neurodegenerative diseases, including those that involve abnormal handling and deposition of conformationally altered toxic species of proteins. Again, rare genetic forms of these proteopathies, caused for example by mutations in genes for the aggregating proteins Amyloid precursor protein (APP), α-Synuclein (αSYN) or microtubule-associated protein Tau (MAPT), are generally considered to be valuable model diseases for the common neurodegenerative disorders of Alzheimer’s disease, Parkinson’s disease or Frontotemporal dementia, respectively, and animal models overexpressing the mutated genes have been generated. Among others, the models developed by Partners 2 and 3 of this consortium have now been well characterized and replicate key components of the disease in humans.

From these considerations it becomes evident, that there is an urgent need

(i) to develop novel and to re-evaluate existing PET-tracers that allow to track aggregated proteins and to develop multimodal imaging protocols to link protein aggregation pathology to functional read-outs on the systems level in animal models and in the corresponding human patient populations with rare, but well characterized model diseases, such as genetic proteopathies,

(ii) to validate these tracers in suitable animal models and to test their value as markers to monitor progression in disease-modifying treatment studies, and finally

(iii) to use these tracers to define the distribution and evolution of protein-aggregation pathology in human patients and to provide proof-of-concept that multimodal imaging protocols are suitable for monitoring disease-modifying individualized treatments.

In order to overcome the critical road-blocks described above, we have assembled an interdisciplinary consortium, consisting of world-leading experts in structural biology and ligand development, multimodal neuroimaging, animal models and clinical trials. With this consortium, we are in a unique position to develop a novel imaging system for combined simultaneous molecular and functional imaging (PET-MRI/fMRI) for two rare subtypes of parkinsonism caused by excessive accumulation of misfolded alpha-synuclein (αSYN): multiple
system atrophy (MSA) and parkinsonism caused by mutations in the alpha-synuclein gene (αSYN-mut-PD), which will serve as proof-of-principle models for the more common and heterogeneous NDD like AD and PD. These rare synucleinopathies are uniquely suited for this approach, as they are (i) characterized by a high αSYN load and therefore offer a favorable signal-to-noise ratio, (ii) have a rapid progression and therefore a relatively easily defined endpoint for therapeutic interventions, and (iii) can be modeled in many aspects in rodents. With the PET/MR technology and novel imaging biomarker development we will pioneer the monitoring of protein aggregation as a surrogate marker for therapeutic effects in the framework of individualized causative treatment. The central aspects of the work-flow including ligand design, software development and drug trials will be driven by three highly specialized SMEs, while imaging workflow, translation to animal models and clinical use will be implemented by top academic centers.

In this consortium we have the ability to achieve ground-breaking progress and aim to

1. Establish a multimodal imaging workflow based on specific PET tracers and embracing structural and functional MRI methods to yield a tool that sensitively and specifically detects αSYN pathology and associated changes
2. Test this multimodal, molecular neuroimaging methodology in animal models with regard to its potential for diagnosis, monitoring the natural disease course and response to therapy, as well as guide and optimize therapeutic interventions.
3. Translate the workflow including the therapeutic modality (i.e. immunotherapy with PD01A, NCT01568099) to the clinical setting.

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**Figure 1:** Scheme showing the work flow and interaction within MultiSyn

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2. Description of the work performed since the beginning of the project and the main results achieved so far

Broken down to aims, the work performed and results achieved can be summarised as follows:

1. Establish a multimodal imaging workflow based on specific PET tracers and embracing structural and functional MRI methods to yield a tool that sensitively and specifically detects αSYN pathology and associated changes (WP1):
   - Successful radiochemical synthesis of $^{18}$F-sery363b, $^{11}$C-sery392b and $^{11}$C-sery512a under conditions easily adoptable to GMP compliant manufacture for in vivo PET imaging of alpha-synuclein aggregates
• Compounds showed good blood brain barrier penetration, but specific binding does not reach the required significance level in the brain yet (observed in autoradiography and PET experiments).
• Optimization of the synthesis of anle253b as a PET tracer
• Successful synthesis and biophysical analysis of further undisclosed potential PET compounds by partner MODAG
• Tritiation of five most promising compounds to speed-up tracer development
• The first two tritiated compounds (\(^{3}H\)-anle138b, \(^{3}H\)-compound 4) did not reach specific binding levels in human brain slices of MSA and LBD yet but gave a cortical staining pattern in AD patients. Tritiated anle138b labels protein aggregates in tissue sections from animal models and human patients. However, it binds to Abeta in Alzheimer tissue so is not selective enough to be a marker for aSYN. It is possible that it is also detecting tau in frontal areas of PSP and DLB patients. Immunostaining is required to clarify the nature of the frontal binding.

Characterization of binding affinity and specificity of PET compounds in vitro
• Preparation and provision of fibrils from recombinant asyn
• Development of a dot blot assay for competition studies
• Fibril binding assay for \(^{11}C\)-PIB was set up but requires further optimization

Set-up of dedicated PET/fMRI scan protocols in rodents
• Dedicated PET/fMRI scan protocols were being set up with \(^{11}C\)-raclopride PET experiments and with BOLD fMRI imaging protocols at resting state with a simultaneous dynamic PET acquisition for 60 min using the tracer \[^{18}F\]FDG for mice and rat models

Development of a dedicated software module enabling the handling and quantitative voxelwise evaluation of simultaneously acquired PET-MRI data
• Inclusion of parametric mapping into PNEURO for synergistic PET/MR analysis of structural and functional data.
• Extension of PNEURO from human to animal brains.
• Addition of a perfusion mapping plug-in for ASL MRI data and diffusion mapping and tensor calculation plug-in for DTI MRI data.
• Implementation of a palette of fiber tracking algorithms based on tensor results.
• 3D visualization of fibers together with planar images.
• R scripts for the statistical analysis of multiparametric outcome data in longitudinal studies.
• Implementation of a non-invasive kinetic model for D2 Receptor PET Scans with \(^{11}C\)-Raclopride.

2. Test this multimodal, molecular neuroimaging methodology in animal models with regard to its potential for diagnosis, monitoring the natural disease course and response to therapy, as well as guide and optimize therapeutic interventions (WP2).

Specific characterisation of mouse, rat and pig models
• Triton insoluble human asyn as well as ProteinK resistant aggregates of asyn were found in the striatum of rats injected with rAAV vectors into the substantia nigra which makes this rat model suitable for testing a novel asyn PET tracer.
• We successfully expressed for the first time human asyn and GFP in the pig substantia nigra.
• Aged (10-15 month old) PLP-ASYN mice have an overall increased ASYN load which includes oligomeric species and insoluble forms.
• Increased aSYN could be confirmed in whole brain lysates of the PLP-aSYN mouse model of MSA by Western blot analysis.
• Total ASYN levels in CSF of 8-9 month old PLP-ASYN mice were higher than WT controls.
• Initiation of preparations for immunotherapy experiments in the AAV-a-syn rat model

PET imaging of rat and pig models
In the AAV-αSYN overexpression rat model, we found a positive correlation between 11C-PIB binding and αSYN load as well as dopaminergic cell loss, measured with 11C-methylphenidate and postsynaptic D2 receptor expression changes, measured with 11C-raclopride.

We used PET imaging to validate a novel AAV-aSYN pig model of PD. We found decreased striatal VMAT2 binding on the side of aSYN injection 12 weeks after inoculation along with increased microglial activation compared to GFP animals. [11C]Sery512a and b PET showed no significant aSYN binding.

In an initial study, the [11C]Sery392 tracer showed increased uptake in the striatum and ventral midbrain of pig models on the side of injection of AAV-aSYN.

PET/MRI methodology

- Bolus plus constant infusion experiments with 11C-raclopride were established to measure DA release in rats and mice and future experiments will be performed using simultaneous 11C-raclopride PET/BOLD fMRI
- Simultaneous BOLD fMRI/18F-FDG PET experiments were successfully established

3. Description of the expected final results and their potential impacts and use

Upon completion of WP1 we aim to have established and validated labelled tracers, PET/MR imaging workflows and data analysis tools available to utilize the full potential of temporally correlated functional, molecular and morphological in vivo data to monitor progression of pathology in αSYN aggregation disorders. These tools and workflows will be available for preclinical imaging studies in animals as well as in clinical studies. In addition, approval for clinical studies will be obtained.

WP2 is going to establish the experimental basis and present the proof-of-concept for simultaneous PET/MRI imaging as a powerful tool for assessing disease related changes in animal models and provide proof of concept for the use of this method to monitor disease modifying treatments for rare forms of synucleinopathies. At the completion of the work, we will not only be leading the field internationally in how such imaging biomarkers can be implemented to benefit disease staging and define therapeutic benefits in animal models of a specific class of neurodegenerative diseases but will also have established the data set required to take the next step into clinical testing.

In WP3, we expect to fully characterize the signal distribution which, based on our results in animal models, most likely reflects the distribution of the αSYN aggregates, as imaged by using specific tracers such as anle138-PET-compounds (such as sery363a and sery392b) and PIB, as it evolves over time in the brain of human patients with MSA and inherited PD due to αSYN mutations. Secondly, we expect to define the relationship between αSYN deposition and functional connectivity of the brain and the function of the dopaminergic synapse, as visualized by PET/MRI co-registration cross-sectionally and longitudinally. We finally expect to validate this multimodal imaging strategy as surrogate endpoint in a clinical trial with an αSYN lowering agent in two rare synucleinopathies, MSA and PD caused by αSYN-mutations.
4. Participants involved in MultiSyn

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<th>No</th>
<th>Participant</th>
<th>Country</th>
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<tbody>
<tr>
<td>1</td>
<td>Eberhard Karls Universitaet Tuebingen (EKUT)</td>
<td>Germany</td>
<td>Thomas Gasser, Bernd Pichler, Holm Graessner</td>
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<tr>
<td>2</td>
<td>Lund University (ULUND)</td>
<td>Sweden</td>
<td>Deniz Kirik</td>
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<tr>
<td>3</td>
<td>Medical University Innsbruck (MUI)</td>
<td>Austria</td>
<td>Gregor Wenning</td>
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<tr>
<td>4</td>
<td>Biomedical Research Foundation, Academy of Athens (BRFAA)</td>
<td>Greece</td>
<td>Leonidas Stefanis</td>
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<tr>
<td>5</td>
<td>Aarhus Universitet (AU)</td>
<td>Denmark</td>
<td>David Brooks</td>
</tr>
<tr>
<td>6</td>
<td>AFFiRiS (AFF)</td>
<td>Austria</td>
<td>Achim Schneeberger</td>
</tr>
<tr>
<td>7</td>
<td>MODAG</td>
<td>Germany</td>
<td>Armin Giese, Christian Griesinger</td>
</tr>
<tr>
<td>8</td>
<td>PMOD Technologies (PMOD)</td>
<td>Switzerland</td>
<td>Cyril Burger</td>
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</tbody>
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**Address of the Coordinator**

Prof. Dr. Thomas Gasser  
Centre of Neurology  
University of Tübingen  
Hoppe-Seyler-Str. 3  
D 72076 Tübingen, Germany

Phone: +49-7071-2986529  
Email: thomas.gasser@uni-tuebingen.de