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Development of imaging technologies for therapeutic interventions in rare diseases

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MultISyn

Multimodal Imaging of rare Synucleinopathies

Instrument: Collaborative Project

Publishable Executive Summary

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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 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.

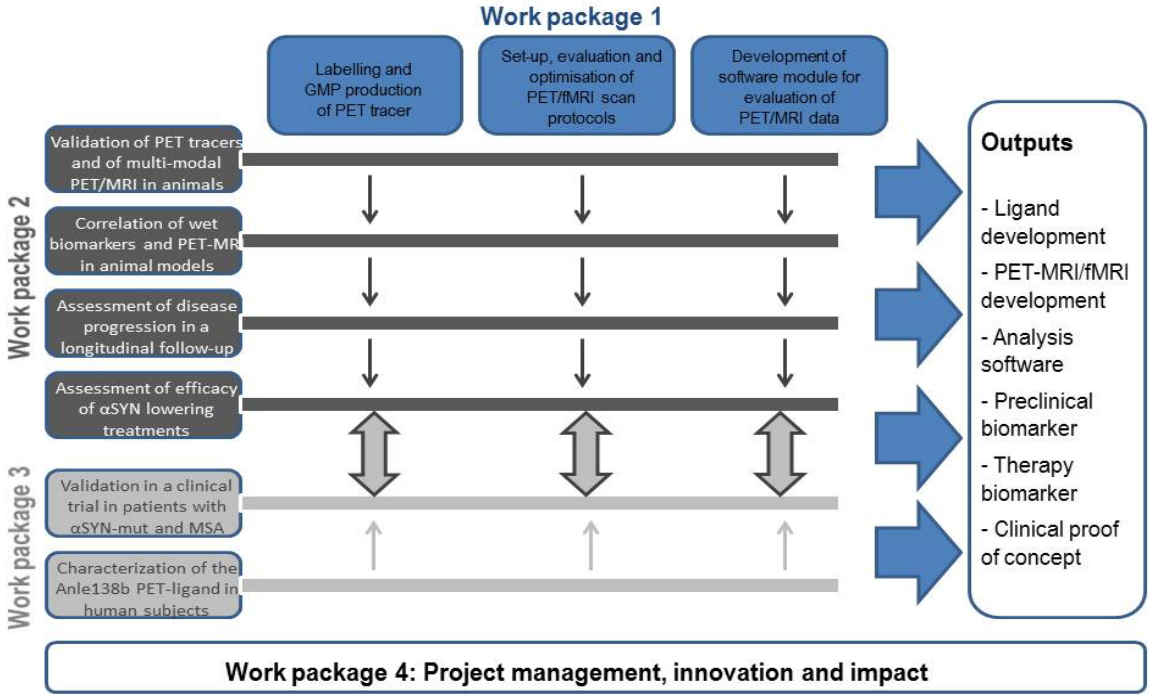

Figure 1: Scheme showing the work flow and interaction within MultiSyn

2. Description of the work performed since begin of the project and the main results achieved

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
   <u>Development of labelling strategies for novel PET tracer</u>
   - Synthesis of novel compound library
   - Approach for three 11C-labelled and one 18F-labelled PET tracer candidates developed
   - Compounds anle138b as well as four derivatives were tritiated and used for autoradiography and other binding studies.

   <u>Characterization of binding affinity and specificity of PET compounds in vitro</u>

- Preparation and provision of fibrils from recombinant αSyn and hTau 46.
- Successful quantification of binding affinities for all tritiated compounds
- Demonstration of specific binding of 11C-PIB to αSYN aggregates and competition experiments revealed highest blocking for PIB, anle138b and two candidate compounds.
- Filter binding experiments revealed very high binding affinity for one promising tritiated candidate compound to asyn fibrils and a mixture of fibrils, oligomers and monomers
- Competition binding experiments using this compound revealed a further compound as another interesting candidate with Ki values < 0.1 nM
- Development of a software tool for PET to histology coregistration for AR

PET tracer evaluation
- Set up and evaluation of a blood sampling device to accurately measure tracer and metabolite activity in plasma
- Demonstration of high binding of an 11C-labelled candidate compound to αSYN fibrils in comparison to monomers and oligomers.
- Using human brain tissue, autoradiography studies revealed binding of all tritiated reference compounds to Abeta in AD brain
- One tritiated candidate compound binds diffusely to the frontal cortex of LBD patients more than to control brain tissue.

Set-up of dedicated PET/fMRI scan protocols for 18F-FDG and 11C-raclopride in rats

Development of a dedicated PMOD software module
- Inclusion of parametric mapping into PNEURO for synergistic PET/MR analysis
- 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.
- R scripts for the statistical analysis of multiparametric outcome data in longitudinal studies.
- Extension to integrated analysis of the multi-modal image data

Further development of one promising candidate compound derived from this project in a Michael J Fox Foundation funded project.

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.
   - Injection of one promising candidate compound into wild type mice revealed a good BBB penetration, a SUV > 1.5 and fast tracer kinetics in healthy control animals
   - Metabolite analysis of this compound revealed one metabolite , which enters the brain
   - Injection of the fluorinated candidate compound into mice revealed a small uptake into the brain and high signal in the skull, likely due to defluorination making it unsuitable for PET experiments

   - Evidence that AAV-mediated rat model suitable for testing a various PET tracer and imaging protocols
   - AAV-αSYN rat model: 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.
   - AAV-αSYN rat model: Preliminary MR spectroscopy data show reductions of glutamate concentrations
   - Application of the [^{11}C]raclopride PET/BOLD fMRI protocol to the AAV-aSYN rat model of PD using a pharmacological D-amphetamine challenge revealed small differences between the healthy and aSYN overexpressing CPu (more animals needed for a statistical analysis).
   - PD01 and PD03 AFFITOPEs are immunogenic in Sprague Dawley's rats, induced antibodies are able to cross-react with the αSYN original epitope and the recombinant human αSYN protein.
   - Successfully immunization of Sprague Dawley rats against human asyn using two affitope antibodies developed by Affiris AG for use in the clinics.

- Animals with circulating Abs to human alpha-synuclein were not protected from neurodegeneration induced by overexpression of the transgenic protein using AAV viruses.
- There was however a clear reduction in detergent (Triton) insoluble, i.e., aggregated, form of a-syn in the brain suggesting that the Abs reached the target in the brain tissue. Given that the PET imaging agent we sought out to develop in this project would bind specifically the aggregated a-syn species, we think that this model would be suitable to show changes in a-syn load in the brain using non-invasive imaging tools.
- CMA induction via LAMP2A up-regulation represents an effective strategy to mitigate established ASYN pathology in BAC-hu-ASYN rats.

- A30P mouse model: An 11C-labelled candidate compound and 11C-PIB PET showed good uptake kinetics, highest binding in the brain stem and was higher in 73 weeks old tg mice compared to 20 weeks controls.
- The MSA PLP-ASYN tg mouse model demonstrates an increased ASYN burden throughout its lifespan (3- 18 mo) compared to WT, including the accumulation of relatively insoluble oligomeric and phosphorylated species, and is thus a suitable model to assess ASYN-lowering treatments.
- Characterization of the efficacy of anle138b to stop the disease progression in the PLP-α-syn mouse model of MSA
- Characterization of the efficacy of AFFITOPEs to stop the disease progression in the PLP-α-syn mouse model of MSA

- Use of PET imaging to validate a novel AAV-aSYN pig model.
- An 11C-labelled candidate compound showed increased uptake in the striatum and ventral midbrain of pig models on the side of injection of AAV-aSYN.

- Test-retest experiments using a simultaneous [^{11}C]raclopride PET/fMRI protocol showed low between scan variability of 2% (fMRI) and 8% (PET) and a within scan variability of 1-2% (fMRI) and 6-9% (PET)

3. Translate the workflow including the therapeutic modality (i.e. immunotherapy with PD01A, NCT01568099) to the clinical setting:
   - Multimodal PET/MRI protocols in humans have been established and tested in a pilot study in patients with MSA; GBA-PD and controls
   - A53T-PD cohort characterized: longitudinal clinical assessments showed prominent motor decline and deterioration of autonomic and cognitive function during the study period, providing baseline values to inform on future clinical trials.
   - MSA cohort characterized: The midbrain and putaminal volume as well as the cerebellar gray matter compartment were identified as the most significant brain regions to construct a prediction model for the diagnosis of MSA.
   - GBA cohort characterized: Natural history of PD caused by GBA-mutations (GBA-PD) focusing particularly on non-motor symptoms established, as a basis for designing clinical interventional trials and power calculations.
   - *As of April 2016 WP3 ceased to work due to the fact that a novel αSYN binding PET tracer could preclinically not be validated.*

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 was planned to obtain.

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 planned 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 anle138b-PET-compounds and PIB, as it evolves over time in the brain of human patients with MSA and inherited PD. Secondly, we planned to define the relationship between αSYN deposition and functional connectivity of the brain and the function of the dopaminergic synapse, as visualized by PET/fMRI co-registration cross-sectionally and longitudinally. However, as of April 2016 WP3 ceased to work due to the fact that a novel αSYN binding PET tracer could preclinically not be validated.

MultISyn web site

www.multisyn.eu

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MULTISYN

4. Participants involved in MultISyn

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