IMPRiND Publishable Summary

March 2017 – February 2022

Summary of the context and overall objectives of the project

Over seven million people in Europe suffer from neurodegenerative diseases and this number is predicted to double by 2040 due to our increasingly ageing population, with a dramatic impact on social services and potentially unsustainable financial burden on healthcare providers. Such urgent and currently unmet clinical need requires an unprecedented research effort that can only be achieved through a coordinated approach across leading European laboratories, the pharmaceutical industry and other international initiatives.

Work performed over the duration of IMPRiND

A growing body of data indicates that the propagation of pathogenic protein aggregates across neural systems could be mediated by misfolded protein seeds that are released and taken up by anatomically connected neurons causing disruption of their function. Therefore, blocking this process may help arrest the progression of Parkinson's (PD) or Alzheimer's (AD) disease. The Consortium **IMPRiND**, funded by the Innovative Medicine Initiative (IMI), is a group of European academic laboratories and members of the European Federation of Pharmaceutical Industries and Associations (EFPIA) that aims to delineate and target critical steps in the propagation of α-synuclein (α-syn) and tau assemblies between neurons. Our programme is collaborative and mobilises diverse expertise in order to deliver disease-relevant phenotypes suitable for high throughput screening and validation models using iPSC, organotypic cultures and animal models.

Over the fifth and final year, we have made significant progress in delivering on our objectives. This has been facilitated by regular teleconferences and one virtual Consortium meeting.

The main research efforts of IMPRIND are focused on the following areas: (a) Generation of brain-derived or amplified assemblies and their characterisation using biochemical techniques, (b) cryo-EM structure of tau and α-syn assemblies, (c) conduction of CRISPR screens in cell lines followed by cross-validation in primary neuronal screens, (d) bioinformatic integration and analysis of screens based on druggability and gene expression data, (e) development of advanced cell-based models or animals for validation of selected targets and (f) data dissemination. Over the duration of the project we have achieved the following objectives:

- LMB, Lilly and Janssen have optimised and cross-validated protocols to isolate and quality control brain-derived tau assemblies from Alzheimer's disease brain. CNRS has established methodologies for amplification of proteopathic assemblies from brain homogenates of α-synucleinopathies.

- LMB has published the cryo-EM structures of tau filaments isolated from Alzheimer's disease, Pick's disease and chronic traumatic encephalopathy (Fitzpatrick et al., 2017; Falcon et al., 2018; Falcon et al., 2019; Shi et al., 2021) as well as cryo-EM structures of MSA filaments (Schweighauser et al., 2020). CNRS has published the cryo-EM structure of de novo generated (Guerrero-Ferreira et al., 2019) and brain amplified α-syn fibrils (Burger et al. 2021).

- Development of assays suitable for high throughput screening and completion of primary screens were completed by UOXF, Novartis and HLU. These include focused CRISPR/Cas9 proteostasis screens for α-syn and tau, genome-wide CRISPR screens for tau, primary neuronal screens using siRNA for 300 targets for tau and the Deubiquitylase (DUB) screen for α-syn.

- Hits from these screens were further analysed in an integrated fashion by Lilly to prioritise targets for further validation in primary neurons.

- Validation of prioritised hits was performed by Novartis and HLU in primary neurons using siRNA or shRNA for tau and siRNA for α-syn. Janssen also assessed selected

hits using CRISPR/Cas9 knockout in primary neurons. Further assessment of selected hits and additional targets for tau was performed at Janssen using CRISPR/Cas9 knockout in primary neurons and for α-syn by UOXF using a shRNA library prepared at DZNE.

- A number of advanced models have been fully characterised and their suitability for target validation has been established. These include the iPSC-based dopaminergic neuronal model (UOXF, Tanudjojo et al., 2021), organotypic cultures (DZNE, Barth et a., 2021) and in vivo mouse (BRFAA and HLU) models for α-syn. Tau seeding mouse models have been established by Lilly and Janssen. The transplantation of iPSC-derived neurons for in vivo assessment of tau propagation and the tau drosophila model (both VIB) have also been completed. iPSC-based tau organoids (UCAM) and hNP-based tau neuronal model (DZNE) have also undergone initial characterisation. UBx has developed a model of tau propagation in primates using AD brain derived fibrils.

- Different Adeno-associated virus (AAV) mediated silencing approaches were tested and characterized in vivo. Janssen, BRFAA and HLU have optimised the delivery method for effective knockdown of hits from the genetic screens using SNCA and microtubule-associated protein tau (MAPT) RNAi in proof of principle studies. Knockdown of targets is on-going.

- We generated an on-line registry for critical reagents and initiated the deposition of data in public repositories to enable sharing of datasets or dissemination following publications. Website updates and news, social media messages, a number of conference presentations and posters and a collection of research highlights have completed the dissemination activities. We have also organised two international symposia during the biennial ADPD meeting in 2019 and 2021.

Progress beyond the state of the art, expected results until the end of the project and potential impacts

New scientific consensus has been established by the cryo-EM characterisation of tau and α-syn filaments isolated or amplified from human brains. This work has led to a structure-based stratification of tauopathies and improved standards for the development of ligands or therapeutics (e.g. PET ligands, antibody immunotherapies or anti-aggregation molecules).

Importantly, the consortium has used highly characterized seeds and readouts of seeded aggregation to complete CRISPR-based and siRNA screens and identified modifiers of α-syn and tau aggregation in cell lines and primary neurons. These screens will become publicly available upon completion of the project and provide a rich source of data for Industry and academic partners interested in drug target discovery and development.

References

Barth, M., Bacioglu, M., Schwarz, N., Novotny, R., Brandes, J., Welzer, M., Mazzitelli, S., Häsler, L. M., Schweighauser, M., Wuttke, T. V., Kronenberg-Versteeg, D., Fog, K., Ambjørn, M., Alik, A., Melki, R., Kahle, P. J., Shimshek, D. R., Koch, H., Jucker, M. & Tanriöver, G. Molecular Neurodegeneration 16, 54 (2021). doi: 10.1186/s13024-021-00471-2.

Burger, D., Fenyi, A., Bousset, L., Stahlberg, H. & Melki, R. Preprint. doi:10.1101/2021.07.08.451588.

Falcon, B., Zhang, W., Murzin, A. G., Murshudov, G., Garringer, H. J., Vidal, R., Crowther, R. A., Ghetti, B., Scheres, S. H. W. & Goedert, M. (2018). Nature 561, 137–140. doi:10.1038/s41586-018-0454-y.

Falcon, B., Zivanov, J., Zhang, W., Murzin, A. G., Garringer, H. J., Vidal, R., Crowther, R. A., Newell, K. L., Ghetti, B., Goedert, M. & Scheres, S. H. (2019). Nature 568, 420–423. doi:10.1038/s41586-019-1026-5.

Fitzpatrick, A. W. P., Falcon, B., He, S., Murzin, A. G., Murshudov, G., Garringer, H. J., Crowther, R. A., Ghetti, B., Goedert, M. & Scheres, S. H. W. (2017). Nature 547, 185–190. doi:10.1038/nature23002.

Guerrero-Ferreira, R., Taylor, N. M., Arteni, A.-A., Kumari, P., Mona, D., Ringler, P., Britschgi, M.,

Lauer, M. E., Makky, A., Verasdonck, J., Riek, R., Melki, R., Meier, B. H., Böckmann, A., Bousset, L. & Stahlberg, H. (2019). eLife 8, e48907. doi:10.7554/eLife.48907.

Schweighauser, M., Shi, Y., Tarutani, A., Kametani, F., Murzin, A. G., Ghetti, B., Matsubara, T., Tomita, T., Ando, T., Hasegawa, K., Murayama, S., Yoshida, M., Hasegawa, M., Scheres, S. H. W. & Goedert, M. (2020). Nature 585, 464–469. doi:10.1038/s41586-020-2317-6.

Shi, Y., Zhang, W., Yang, Y., Murzin, A. G., Falcon, B., Kotecha, A., van Beers, M., Tarutani, A., Kametani, F., Garringer, H. J., Vidal, R., Hallinan, G. I., Lashley, T., Saito, Y., Murayama, S., Yoshida, M., Tanaka, H., Kakita, A., Ikeuchi, T., Robinson, A. C., Mann, D. M. A., Kovacs, G. G., Revesz, T., Ghetti, B., Hasegawa, M., Goedert, M. & Scheres, S. H. W. Nature 598, 359–363 (2021). doi:10.1038/s41586-021-03911-7.

Tanudjojo, B., Shaikh, S. S., Fenyi, A., Bousset, L., Agarwal, D., Marsh, J., Zois, C., Heman-Ackah, S., Fischer, R., Sims, D., Melki, R. & Tofaris, G. K. Nature Communications 12, 3817 (2021). doi: 10.1038/s41467-021-23682-z.

IMPRiND

University of Oxford (UOXF)	UK
University of Cambridge (UCAM)	UK
University Medical Center Göttingen (UMG-GOE)	DE
Université de Bordeaux (UBx)	FR
Aarhus Universitet (AU)	DK
University of Dundee (UNIVDUN)	UK
MRC Laboratory of Molecular Biology (LMB)	UK
Biomedical Research Foundation of Athens (BRFAA)	GR
Centre national de la recherche scientifique (CNRS)	FR
VIB Center for Brain & Disease Research (VIB)	BE
Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE)	DE
H. Lundbeck A/S (HLU)	DK
Janssen Pharmaceutica NV (Janssen)	BE
AbbVie Ltd (AbbVie)	DE
Novartis Pharma AG (Novartis)	CH
Institut de Recherches Servier – IdRS (Servier)	FR
Eli Lilly & Co Ltd (Lilly)	UK
SCIPROM Sàrl (SCIPROM)	CH

This project receives funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 116060. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

This work is supported by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00038.

The opinions expressed and arguments employed herein do not necessarily reflect the official views of these funding bodies.

WWW.IMPRIND.ORG