# Topic: Mitochondrial Dysfunction in Neurodegeneration

**All information regarding future IMI Call topics is indicative and subject to change. Final information about future IMI Calls will be communicated after approval by the IMI Governing Board.**

## Topic details

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| Action type | Research and Innovation Action (RIA) |
| Submission & evaluation process | 2 Stages |

## Specific challenges to be addressed

Neurodegenerative diseases such as Parkinson's disease (PD) are of growing public health concern in developed countries and the need for novel effective treatments continues to increase. Neurodegenerative diseases take many forms, reflecting the degeneration of different populations of neurons at different times from distinct causes, but many of them also share common features. Amongst the commonalities are bioenergetic failure and oxidative stress, both of which reflect the dysfunction of mitochondria within neural and glial cells [1][2][3][4]. As such, a detailed understanding of mitochondrial dysfunction in the brain in the context of ageing, injury by misfolded protein toxicity and genetic factors associated with neurodegeneration holds much promise for the development of therapeutic interventions that could impact multiple neurodegenerative disease states.

While mitochondria are found in most cell types, they have a distinct involvement in the brain and in neurodegeneration and especially in neuronal cells. Indeed in diseases caused by mutations in the mitochondrial genome (thus in principle systemic, in all cells), the typical disease phenotype is dominated by neuronal dysfunction [5]. In addition, many mitochondrial toxins (such as rotenone or 3-nitroproprionic acid) delivered systemically result in selective injury to the central nervous system [6][7]. Thus, while the brain may be selectively vulnerable to mitochondrial dysfunction, this also implies that the nervous system may preferentially benefit from mitochondrial-targeted therapeutics.

Mitochondria are not static organelles. They constantly undergo fission and fusion, their genome replicates also in post-mitotic cells, and they move to different cellular locations up to the end of the neuronal axon [8][9][10]. Neuronal injury has been associated with cessation of mitochondrial movement and sometimes dramatic alterations in mitochondrial morphology [11] but the impact of altered mitochondrial structure and function on neurodegenerative disease is yet to be fully elucidated. Some of the key modifiers of mitochondrial dynamics have been identified, such as fission and fusion promoting proteins [10][12] as well as proteins that

regulate mitochondrial movement [13][14], but the specific role of these proteins in the context of neurodegeneration has not been established.

Mitochondrial dysfunction may be due to abnormal respiratory function, biogenesis, dynamics (axonal transport, fission, fusion) or mitophagy and can affect several different cellular activities, including abnormal cellular energy generation encompassing oxidative phosphorylation, the citric acid cycle and beta-oxidation. On the other hand, dysfunction can emerge from perturbations in key functions which are energetically distinct yet intimately related to mitochondrial function as they ensure overall cellular metabolic health. Examples are iron-sulfur cluster biogenesis, organisation of the Endotelial Reticulum (ER)-mitochondria network, mitochondrial quality control (mitophagy) and synthesis of mitochondrial proteins and lipids [1]. It is widely believed that neuronal health is reliant on the proper positioning of the mitochondrial network within long axonal projections, and therefore insults to the ever adapting nature of mitochondrial networks is seen as a key turning point in the aetiology of many neurodegenerative indications [15].

The overall aim of this topic is to develop an unprecedented appreciation of the evolution of mitochondrial dysfunction in models of PD in order to understand if dysfunction is a driver of disease progression. A key goal is to develop an unprecedented appreciation of mitochondrial function in an in vivo model of neurodegenerative disease, which is currently lacking. Other challenges to be addressed within this topic are to quantitatively dissect changes in mitochondrial function in *in vitro* and *in vivo* PD disease models, and to understand the impact on the degeneration of neurons and/or glia. There is a growing appreciation of the impact of glial cells (astrocytes, oligodendrocytes and microglia) in neurodegeneration, so this topic may include investigations of mitochondrial dysfunction in several cell types [16]. There is also the opportunity to investigate mitochondrial function in neural cells derived from human sources, both from patients and unaffected individuals [17]. Identification of the key molecular drivers of mitochondrial dysfunctions in the disease models will provide a unique scaffold to enable the discovery and the development of new therapeutics to halt neurodegenerative disease progression. It is anticipated that the topic will lead to the identification of key molecular drivers which will provide a foundation for the identification and validation of new drug targets, facilitating innovative therapeutic approaches within the neurodegeneration field. Moreover, mitochondrial abnormalities serve as a connecting theme between several neurodegenerative diseases, with a direct link to several processes known to be impaired in neurodegeneration such as bioenergetics and misfolded protein toxicity [18]. Therefore, the learnings are anticipated to also feed into the understanding of the role of mitochondrial dysfunctions in other neurodegenerative diseases such as Alzheimer's Disease.

## Need and opportunity for public-private collaborative research

Neurodegenerative diseases are complex entities that demand cross-disciplinary investigation. Successful development of methodologies and technologies will require quantitative assessment of mitochondrial dysfunction *in vitro* and *in vivo*, identification of mitochondrial dysfunction in robust neurodegenerative disease models and the understanding of the impact this dysfunction has on disease progression. These insights will enable significant advances in strategies to expand the repertoire of targets and to encourage renewed investment to develop treatments for neurodegenerative disorders.

It is beyond the reach of a single company or institution to fully understand the magnitude and complexity of the roles of mitochondria in health and diseases. Because of the scale and scope of this endeavour, success will require the collaboration of a cross-functional/cross-institutional consortium of academic, Small- and Medium-sized Enterprises (SMEs)/biotech and industrial scientists covering a large variety of scientific expertise.

State of the art *in vitro* and *in vivo* models (transgenic and models based on the "prion-like hypothesis") developed in industry and already used in the Research and Development (R&D) process, along with creative new disease models and novel methodologies to quantify mitochondrial dysfunction from academia and innovative SMEs, will create synergies that would otherwise likely be unobtainable.

## Scope

The overall scope of the project generated by this topic is to identify and understand the impact of mitochondrial dysfunction in *in vitro* and *in vivo* models of neurodegenerative diseases, incorporating core characteristics of neurodegeneration such as protein misfolding. Understanding if dysfunction is a driver of disease progression, and the detailed mechanisms responsible for it, will enable exploration of novel targets for therapeutic approaches to neurodegenerative diseases.

The scope will be reached by a scientifically robust strategy building on established PD models and the appropriate technology experience within the consortium. More specifically, this will include addressing the following objectives:

***In vitro***

- In established *in vitro* models of PD in neurons, microglia, oligodendrocytes and/or astrocytes, understand the impact of mitochondrial dysfunction (such as respiratory function, biogenesis, trafficking, fission, fusion and mitophagy) on the development/severity of the disease phenotype and identify key molecular drivers of these dysfunctions. Assessment of correlation between morphology and function should be included to ease later interpretation of morphological observations *in vivo*.
- Among others, the *in vitro* phenotype would ideally include a demonstration of mitochondrial dysfunction induced by α-synuclein or tau in a humanised model system such as inducible Pluripotent Stem cells (iPSCs). These cellular models would then be further developed into a robust model for therapeutic target identification. Models could potentially include organotypic slice cultures *in vitro*.
- Neurodegeneration is a phenomenon directly associated with ageing, while most *in vitro* cell-based models use neonatal tissues as a source of primary cells. Moreover, iPSCs essentially have their biological clock reset, thus eliminating elements of ageing in the model. Incorporating a component affecting mitochondrial ageing as a model variable would be a valuable addition to the *in vitro* approach.

***In vivo***

- In a well characterised robust *in vivo* model PD, investigate if mitochondrial dysfunction can be identified. Understand the impact of these changes on disease progression such as neuronal and synaptic health, as well as the potential for their therapeutic modulation. While many *in vivo* models of PD exist, convenient models using transgenic animals already aged before the start of the project or injection of fibrillary forms of disease associated proteins as a seeding mechanism to trigger neurodegeneration would be the most appropriate [19]. These models typically develop disease pathology over a time frame suitable for the studies proposed here.

## Key deliverables

The applicants should develop a translational framework for the study of mitochondrial dysfunction *in vitro* and *in vivo* that will provide mechanistic insight into the role of mitochondria on disease pathology progression. This should be achieved by the delivery of:

- Development of robust tools and assays to study and quantitatively address mitochondrial dysfunction in well characterised *in vitro* and *in vivo* models of PD;
- Identification of mitochondrial dysfunction in established and well characterised PD models using *in vitro* and *in vivo* approaches;
- Understanding the role of the identified mitochondrial dysfunction on disease progression/severity;
- Validation of the experimental robustness of the identified mitochondrial dysfunction and the quantitative detection of the endpoint. This is a pre-requisite for application of the model system in pharmaceutical research;
- Understanding of the role of misfolded proteins associated with PD on mitochondrial dysfunction, in vitro and in vivo;

- Identification of key molecular drivers of mitochondrial dysfunction promoting neurodegenerative diseases. This will provide an unprecedented foundation for the pharma industry to identify and validate innovative drug targets in the field of neurodegeneration;
- Establishment of a European multidisciplinary research platform of excellence of mitochondrial dysfunction in neurodegeneration facilitating the understanding of neurodegenerative disease aetiology, , thus ensuring the sustainability of project outcomes.

## Expected impact

Progressive neurodegenerative diseases represent a large and growing burden. Despite a considerable investment in research aimed at understanding and treating neurodegeneration, the lack of disease modifying therapies remains notable. Recognizing this gap, the treatment of neurodegenerative disease is a clearly identified goal of IMI2, and the expected impact of the project to be generated by this topic is closely aligned with the overall goal.

There is considerable evidence implicating mitochondrial dysfunction in the pathogenesis of a number of progressive neurodegenerative diseases, including Parkinson's disease, but no efficacious treatments have been developed based on this knowledge.

By developing a set of validated cellular assays and *in vivo* tools, the project will remove an important barrier that has limited the systematic exploration of mitochondrial dysfunction in neurodegenerative disease. A clear identification of the specific mitochondria dysfunctions (such as respiratory function, biogenesis, trafficking, fission, fusion or mitophagy) contributing to neurodegeneration will enable the discovery of novel targets for intervention.

By taking advantage of recent advances in the understanding of mechanisms that control mitochondrial dynamics and using innovative technologies to access mitochondrial dysfunction (e.g. axonal transport and fusion/fission in highly relevant model systems), this approach should provide unprecedented insight into the causal link between mitochondrial dysfunction and neurodegeneration.

The project learnings will strongly aid neurodegenerative disease understanding and identification of novel targets giving academics/SMEs/pharma new options for treatments of diseases with mitochondrial dysfunction, such as PD. Moreover it would encourage a renewed investment in developing drugs for neurodegenerative disorders for which there is a high unmet medical need. In particular biotech SMEs will be able to 'stress-test' their technologies in a non-competitive open innovation environment which will greatly facilitate the development of novel and important therapeutics.

Thus, it can be anticipated that the results of the project will benefit patients and society through the accelerated discovery of new drugs and therapies for neurodegenerative diseases.

## Potential synergies with existing Consortia

Applicants should take into consideration, while preparing their short proposal, relevant national, European (both research projects as well as research infrastructure initiatives), and non-European initiatives. Synergies and complementarities should be considered in order to incorporate past achievements, available data and lessons learnt where possible, thus avoiding unnecessary overlap and duplication of efforts and funding.

The project generated from this topic in particular should, among others, consider:

- IMI: **IMPRiND**: Inhibiting Misfolded protein Propagation in Neurodegenerative Diseases (https://www.imprind.org/)

  Relevant with regard to both in vitro and in vivo model systems for spreading and seeding processes in PD and AD
- IMI: **EBiSC**: European Bank for induced pluripotent Stem Cells (https://www.ebisc.org/)

Relevant with regard to IPSCs lines from patients

- MJ Fox foundation: **LRRK2 Cohort Consortium** (https://www.michaeljfox.org/page.html?lrrk2-cohort-consortium

  Relevant for samples, cellular and animal models

- MJ Fox foundation: **Parkinson's Disease Research Tools Consortium** (https://www.michaeljfox.org/page.html?tools-consortium)

  Relevant for cellular and animal models of protein misfolding in PD

- **MIND MAPS**: Molecular Imaging of Neurodegenerative Disease – Mitochondria, Associated Proteins & Synapses consortium (https://mitochondrialdiseasenews.com/2017/04/05/imanova-mrc-funding-mind-maps-study/)

  Relevant for imaging mitochondrial dysfunction in patients with AD and PD

- H2020 funded project **SYSMEDPD**: Systems Medicine of Mitochondrial Parkinson's Disease (http://sysmedpd.eu/)

  Relevant for cellular and animal models of protein misfolding in PD

- H2020 funded project **MEFOPA**: European Project on Mendelian Forms of Parkinson's Disease (http://cordis.europa.eu/result/rcn/149388_en.html)

  Relevant for cellular and animal models of protein misfolding in PD

## Industry consortium

The industry consortium will contribute the following expertise and assets:

- Access to *in vivo* and *in vitro* disease models:
  - Well established *in vivo* α-synuclein seeding models in Wild Type (WT) rodents including: SNCA-OVX Tg mice model (expressing human α-synuclein (SNCA) locus WT α-synuclein at disease-relevant levels) [20], pre formed fibrils (PFF) mouse model [21][22] and an α-synuclein rat model (AAV-A53T-α-synuclein rats; showing protein aggregation, dystrophic axonal morphology and progressive loss of dopaminergic neurons in the Substantia nigra pars compacta) [23][24], hereunder assay protocols, seed material based on recombinant α-synuclein fibrils, and α-synuclein pathology endpoint analysis.
  - Well established α-synuclein seeding models in WT or F28 (human α-synuclein expressing mice) [25][26] primary neurons, assay protocols, seed material based on recombinant α-synuclein fibrils, pregnant F28 mice for establishment of the cultures and α-synuclein pathology endpoint analysis.
- Access to iPSC lines, iPSC neuronal progenitors and protocols for differentiation into neurons. Protocols and tools for viral transduction and siRNA knockdown of proteins in iPSC neurons.
- Access to human tissue sample for validation studies from a collection of ~1000 PD cases and 200 controls from which formalin fixed and flash frozen brain tissue is available.
- Evaluation of consistency and robustness of mitochondrial dysfunction key molecular endpoints to ensure future application for target identification/validation.
- Industry will also support communication, dissemination and project management.

## Indicative duration of the action

The indicative duration of the action is 36 months.

## Applicant consortium

The applicant consortium will be selected on the basis of the submitted short proposals.

The applicant consortium is expected to address all the research objectives and to make key contributions to the defined deliverables in synergy with the industry consortium which will join the selected applicant consortium in preparation of the full proposal for stage 2.

Participation of SMEs with relevant knowhow and standardised technologies and assays is strongly supported.

This may require mobilising, as appropriate, the following expertise and resources:

- Expertise in using *in vivo* models of PD, and the capability to enable easy transfer of Industry models to the applicant laboratories. This includes necessary animal facilities and handling experience;
- Expertise using *in vitro* models of PD, including access to models which exhibit a robust and well characterised disease phenotype with a strong link to the pathology in the patient brains, i.e. protein aggregation. The applicant laboratories must have relevant cell culture facilities and strong knowhow on the proposed model systems (primary cultures or iPSCs) regardless of whether the model system is already running in their laboratories or they will be transferred from an Industry partner;
- The use of *in vitro* and/or *in vivo* PD models that involve introduction of seeding proteins to trigger disease processes may be an advantage;
- Expertise in evaluation of key elements of mitochondrial function *in vitro*, including bioenergetics, ROS production, biogenesis, fission, fusion and mitophagy;
- Expertise in and tools for *in vitro/in vivo* imaging for the investigation of mitochondrial morphology and trafficking. This could include expression of mitochondrial-targeted fluorescent proteins in relevant cell populations;
- Knowhow and tools for manipulation of mitochondrial function. For morphology this could be through the expression of proteins such as DRP1, mitofusin 2, OPA1 or Miro or other tools. Small molecules would also be helpful;
- Knowhow and innovative mind-set for development of new tools and assays to study and quantitatively address mitochondrial dysfunction in *in vitro* and *in vivo* models of PD;
- Expertise in approaches to model mitochondrial ageing in the *in vitro* models;
- Expertise in communication, dissemination project management and coordination of research activities.

## Suggested architecture of the full proposal

The applicant consortium should submit a short proposal, which includes their suggestions for creating a full proposal with an effective and simple architecture, taking into full consideration the deliverables, and the contributions and expertise of the industry consortium.

In the spirit of the partnership, and to reflect how IMI2 JU call topics are built on identified scientific priorities agreed together with EFPIA beneficiaries/large industrial beneficiaries, these beneficiaries intend to significantly contribute to the programme leadership and project and financial management.

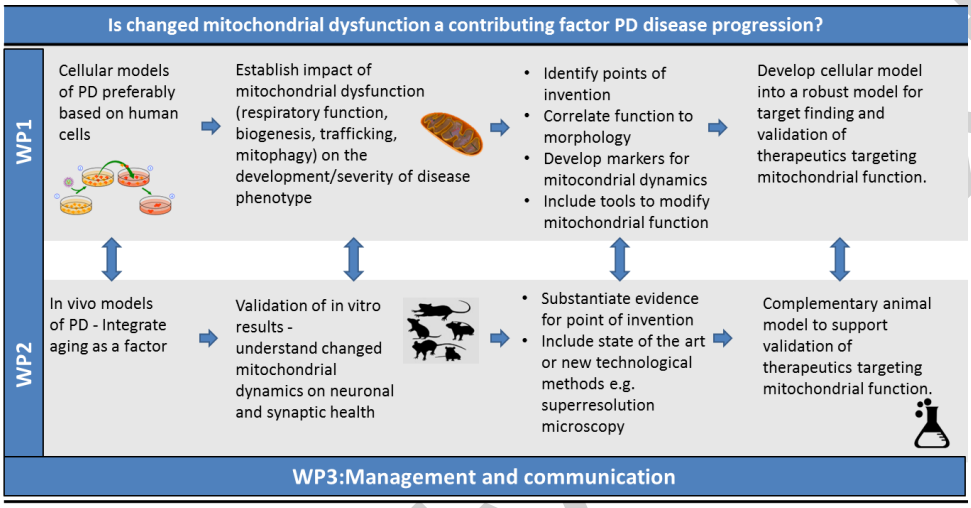
The final architecture of the full proposal will be defined by the participants in compliance with the IMI2 JU rules and with a view to the achievement of the project objectives. The allocation of a leading role within the consortium will be discussed in the course of the drafting of the full proposal to be submitted at stage 2. To facilitate the formation of the final consortium, until the roles are formally appointed through the consortium agreement, the proposed project leader from among EFPIA beneficiaries/large industrial beneficiaries shall facilitate an efficient negotiation of project content and required agreements.

All beneficiaries are encouraged to discuss the project architecture and governance and the weighting of responsibilities and priorities therein.

A plan for aspects related to sustainability, facilitating continuation beyond the duration of the project should also be proposed.

The architecture outlined below for the full proposal is a suggestion. Different innovative project designs are welcome, if properly justified.

It is suggested to organise the work-plan into three main themes (each corresponding to a specific work package):




**Work package 1 – Evaluation of mitochondrial dysfunction in cellular models of Parkinson's disease**

- Identification of specific mitochondria dysfunctions (respiratory function, biogenesis, trafficking, fission, fusion or mitophagy) in established *in vitro* PD models which exhibit a robust and well characterised disease phenotype with a strong link to the pathology in the patient brains, i.e. ex. protein aggregation;
- Establishment of quantitative detection of the mitochondrial dysfunction endpoints and demonstration of robustness of the parameters. This could ideally include implementation of tools (proteins, reagents) modulating the relevant mitochondrial functions;
- Understanding the role of the identified mitochondrial dysfunction on disease phenotype progression/severity. Potentially, understand contribution of mitochondrial damage due to misfolded proteins. The latter would include establishment of relevant tools and cellular models exhibiting relevant manifestation of ageing;
- Identification of key molecular drivers of the identified mitochondrial dysfunctions;
- If relevant, transfer of model systems from Industry partner/s to applicant consortium partner/s;
- As necessary, development of new robust tools and assays to study and quantitatively address mitochondrial dysfunction *in vitro*.

With these methods in hand, the goal is to provide a detailed characterization of the contribution of mitochondrial dysfunctions to PD related degeneration of the relevant cell types. Identification of the key molecular drivers of the identified dysfunctions is of particular interest. Tools modulating the abnormal mitochondrial parameters, such as fission or mitophagy, may provide the opportunity to identify mitochondrial targets for therapeutic intervention. Moreover, it would be advantageous to have the opportunity to understand contribution of mitochondrial damage due to misfolded proteins and ageing to neurodegenerative disease progression and severity.

Industry contribution will include contribution of cellular models, tissue from animal models and protocols as well as development of mitochondria dysfunction assays and quantitative detection of mitochondrial functional

levels. Technologies to be contributed may include high content screening, bioenergetics assays and iPSC derived models.

Expected Applicant consortium contribution will include development of novel tools and models to assess the impact of ageing and misfolded protein to manifestation of mitochondrial dysfunction, as well as the development of additional novel mitochondrial dysfunction assays.

**Work package 2 – Evaluation of mitochondrial dysfunction using *in vivo* models of Parkinson's disease**

- Identification of specific mitochondria dysfunctions (respiratory function, biogenesis, trafficking, fission, fusion or mitophagy) in robust and well established *in vivo* PD models, e.g. *in vivo* seeding models. This would implicate following changes in mitochondrial function over the course of development of neuropathology to understand if a sub-acute time course could be identified and then allow mitochondrial dysfunction to be tracked before and during the development of neurodegeneration;
- Establishment of quantitative detection of mitochondrial dysfunction endpoints and demonstration of robustness of the parameters. This could ideally include development of ways to modify the parameters either genetically or pharmacologically;
- Understanding the role of the identified mitochondrial dysfunction on disease phenotype progression/severity;
- Identification of key molecular drivers of the identified mitochondrial dysfunctions;
- If relevant, transfer of model systems from EFPIA partner to applicant consortium partners;
- If required, development of new robust tools and assays to study and quantitatively address mitochondrial dysfunction *in vivo*. It could for example be methods for imaging of mitochondrial dynamics in mouse models.

Industry contribution will include the contribution of animal models of neurodegenerative diseases with a focus on seeding models of disease, together with relevant protocols and the assessment of mitochondrial functional endpoints in these models.

Expected Applicant consortium contribution will include development of tools and assays to quantitatively assess mitochondrial dysfunction endpoints in vivo and implementing them to enable longitudinal of mitochondrial function in relevant models of diseases and correlation to disease phenotype and severity.

**Work package 3 – Project and data management.**

- Define work expectations of different work streams, deliverables, dates and activities and review progress regarding adherence to budget, timelines and quality (by all consortium members);
- Ensure legal and contractual management;
- Ensure the set-up of joint governance structure (by all consortium member);
- Ensure appropriate communication/dissemination within the consortium and with the external scientific community and the public;
- Develop and manage communication via web portal and other social media tools with a repository of key document;
- Quality assessment of documents;
- Define project interdependencies, stakeholders and risks;
- Ensure ethics management.

Industry contribution will include co-leading this work package, including management of legal, contractual, ethical and QA aspects and developing and executing a detailed communication and dissemination plan, all to

be achieved in partnership with all remaining consortium members, who will also work together to define governance structure and full work plan.

Expected Applicant consortium contribution will include co-leadership and input to all structures and activities needed for decision making, monitoring and project management, and jointly develop governance structure and full work plan.

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