

Topic: Discovery and characterization of blood-brain barrier targets and transport mechanisms for brain delivery of therapeutics to treat neurodegenerative & metabolic diseases

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Topic details

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

Specific challenges to be addressed

The **blood–brain barrier** (BBB) acts as a strict control point for what can enter the brain, and is created by drug efflux transporters (transport barrier) expressed on cerebromicrovascular endothelial cells and by tight junctions and adherens junctions between those endothelial cells (biophysical barrier) supported by basement membrane, astrocytic end-feet, pericytes, and neuronal innervation. The barrier functions of the BBB lie in the integrity and physiological regulation of the neurovascular unit (NVU). The BBB facilitates the passage of nutrients and metabolic necessities to the brain but restricts the entry of most blood-borne drugs and neurotoxic agents into the brain. The ability to cross the BBB must be considered for neurotherapeutics administered peripherally. In particular the BBB remains a major obstacle for biopharmaceuticals (e.g., antibodies, peptides) and restricts the chemical properties of passively brain-permeable small moleculesⁱ. While there are examples of actively transported central nervous system (CNS) drugs (e.g. Lyrica®) the state of transporter substrate specificity understanding makes development of these largely dependent on luck rather than design. This also explains why no centrally acting biopharmaceuticals (e.g. antibodies, peptides, proteins, oligonucleotides) are currently on the marketⁱⁱ. Transport receptors or carriers, mostly mediating receptor- or carrier-mediated transcytosis (such as transferrin and insulin receptors, LRP 1, GLUT1, CD98hc) triggered by antibodies or peptides, have been reported to ferry biopharmaceuticals across the BBBⁱⁱⁱ. However, these systems have not totally proven their safety and efficacy yet and no development of transferrin receptor antibody-enabled biopharmaceutical has been reported to-date. Insulin receptor antibody has been recently employed to deliver iduronate-2-sulfatase to the brains of MPS-II (Type II mucopolysaccharidose or Hunter syndrome) patients in a phase II clinical trial (NCT02262338). It appears to be safe, tolerable and improve cognitive scores in the patients. In addition to RMT and CMT mechanisms, liposomes^{iv}, nanoparticles, and more recently exosomes^v have been explored to enhance brain delivery of therapeutics. These have targeted both passive and active uptake mechanisms and have shown mixed results to date. Studies have also explored approaches of employing viral vectors/particles-vesicles or protein fragments to deliver genes or biopharmaceuticals into the brain. Other approaches of drug delivery, such as intranasal delivery of therapeutics across the olfactory epithelia into the brain, still remain to be explored further. While all these results seem promising, a major challenge in this field is validation of the various transport mechanisms and drug delivery systems by independent researchers and further understanding challenges to advancing into clinical drug development by biotech/pharma. A goal of the action to be generated by this topic is to work precompetitive to validate targets and transport mechanisms at the BBB and provide additional insight into any developmental challenges.

One of the central hurdles in driving structure-activity relationship (SAR) for brain uptake and in identifying new mechanisms of brain delivery is the lack of **blood brain barrier models** truly predictive of *in vivo* exposures of biologics as well as lack of selective BBB targets for brain transport. Even if some reports in the literature present human inducible pluripotent stem cell (hiPSC)-derived BBB models^{vi}, their robustness and predictability remain to be assessed, and no fully reconstituted human model convincingly mimicking the

neurovascular unit has been successfully developed to-date^{vii}. To this end, 3D- or spheroid models and microfluidics could be ideally suited and a few interesting directions are starting to emerge in the literature^{viii}.

A **compromised or altered permeability of BBB** has been reported in brain tumors and for several **neurological and metabolic diseases**^{ix}. Even though it is still a matter of debate, it seems increasingly evident that this BBB dysfunction might be at the very root and pathogenesis of some of these neurological diseases (such as multiple sclerosis and vascular dementia)^x. And even though the pharmacological understanding of many of these diseases has identified attractive potential therapeutic targets, most of these are currently not believed to be developable due the hurdle of the BBB and the lack of predicted brain penetration based upon general understanding of BBB characteristics. Availability of *in vitro* and *in vivo* models of the BBB representative of those present in these diseases would allow much more aggressive testing of hypotheses around therapeutic delivery and potentially lead to greater investment in targeting these diseases due to the improved tools and mechanistic understanding to explore novel delivery strategies and to develop therapeutic agents. Both of these outcomes would improve the probability of developing successful therapeutic agents to treat these diseases. Moreover, it would provide a more expansive suite of experimental tools with which to further develop an understanding of the fundamental biology, which underpins the absorptive-/receptor-mediated processes across the BBB. **Thus, the physiology of the BBB and the transport mechanisms in health and diseases play a critical role in the development of brain delivery technologies for the treatment of neurodegenerative diseases.**

Human iPSC-derived cell models hold great promises for human *in vitro* BBB and disease modelling and could be used to understand the pathogenesis of neurodegenerative disorders, the roles of BBB in the pathogenic process, and to identify new potential improved screening tools for new drugs^{xi}. Thus iPSC cell-derived BBB models might represent a promising tool to link human neuropathology to BBB dysfunction and a screening tool for permeability, mechanistic and functional studies. However, there is no report on patient-derived human iPSC's BBB models or disease/genetic models generated by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-cas9 technology. In addition there is a general lack of a consensus on the clinical characteristics of such disease models and on what successful validation would be required.

Although results reported in the literature describing efforts to profile brain endothelium via microarray analysis, transcriptomics and proteomics approaches^{vii} are in principle useful, they do not necessarily resemble the disease situation. In this situation, the composition of the surface proteome of brain endothelial cells, the organization and interaction between cells and cell types and permeability in this barrier may be altered. This could strongly impair the efficacy of a brain delivery system if the employed **transport** protein/receptor is down-regulated **in disease**. As a consequence, the therapeutic efficacy of such a delivery system would be greatly reduced. The identification of transport mechanisms which remain stably expressed or, even better, upregulated in diseases, would greatly improve the chances for a successful delivery of therapeutics for treatment of CNS diseases. There is also lack of computational or *in silico* models for pharmacokinetics of drugs and biopharmaceuticals in penetration of the BBB (levels and capacity of relevant receptors and carriers at the BBB for receptor/carrier-mediated transcytosis for drug delivery) and the distribution and clearance of drugs/biopharmaceuticals in different compartments of CNS under normal and disease conditions (such as interstitial fluid ISF, neurons, and cerebro spinal fluid (CSF)). *In vitro* and *in vivo* data from published sources or pharma industrial database may be collected to build such an *in silico* model. It is known that neurotropic viruses can selectively penetrate the BBB and CNS or infect nerve and neurons. However, the mechanisms of those viruses in penetrating BBB and CNS have not been fully characterized. Understanding the mechanisms of the viral mediated processes would generate useful knowledge to inform potential approaches for the development of brain selective delivery technologies.

Thus several challenges have yet to be addressed to better understand the role and alterations of the BBB and transport mechanisms in health and diseases. Relevant diseases are neurodegenerative diseases (e.g. Alzheimer and Parkinson's diseases, Amyotrophic Lateral Sclerosis (ALS)), vascular dementia, multiple sclerosis and metabolism-related central diseases (diabetes and obesity). It will be also important to understand the mechanisms of neurotropic virus-mediated BBB and CNS penetration, and to be able to apply this knowledge for the development of innovative drug delivery systems, especially for biopharmaceuticals, and the identification of novel drug targets.

Need and opportunity for public-private collaborative research

In light of the above, the magnitude and complexity of the BBB in health and diseases is beyond the reach of a single company or institution, such that it can better be addressed by a major public-private-partnership involving a variety of stakeholders and expertise.

Shared understanding of measurable attributes of disease-specific BBB models combined with successful development of both the methodologies and technologies to identify validated predictive human models is necessary to enable significant advances in strategies to expand the brain-accessible pharmacopeia and to encourage renewed investment to develop treatments for these disorders. Specific areas of immediate focus include:

1. establishment and characterization of disease or genetic models for neurodegeneration and BBB better amenable to evaluate disease-modifying agents;
2. identification of translational readouts closer to the pathogenesis of neurodegeneration and mimicking altered BBB under disease conditions;
3. in-depth understanding of the biology of the BBB and characterization of various transport mechanisms across the BBB (including virus-mediated BBB and CNS penetration);
4. discovery and development of innovative and efficacious brain delivery systems. Because of the scale and scope of this endeavor, success will require the collaboration of a cross-functional/cross-institutional consortium of academic, SME/biotech and industrial scientists.

The engagement of leading pharmaceutical companies with detailed understanding of pre-clinical and clinical consequences of disease-modified BBB and with the chemical/analytical resources necessary to both validate and implement these models will enable the partnership to capitalise on the knowledge and innovation generated. The role of industry in this endeavor is crucial as they benefit from state-of-the-art equipment not always available to universities or academia (such as NGS technologies or high throughput and robotized material for cell culture) and experienced people to run them along with powerful and connected bioinformatics with a direct link into the clinic.

Biotech/SME companies would be very valuable in contributing with innovative technologies and tools and know-how in iPSC- or progenitor-derived cells and/or defined extracellular matrix hydrogels and/or human BBB models.

Academic groups will be necessary to provide strong know how on BBB and disease models (neurodegenerative/metabolic) and to contribute on characterizing the mechanisms of brain transport or virus-mediated transport. A few iPSC-based BBB models have been reported in recent years with good barrier properties and transport of various known brain-penetrating agents; however, their robustness and predictability needs to be put to the test^{viii-ix}. In addition, these models are based on 'healthy' iPSC clones and not based on iPSC cells from patients. The expertise of such academic partners in establishing iPSC-based endothelial cultures/models and in characterizing brain transport mechanisms will be important for the successful conduction of the program. Even more so, the ideal situation would be to be able to develop a full BBB neurovascular unit with all cell types derived from patients and understand the mechanisms of brain transport under health and disease conditions. Successful collaboration and integration in a public private partnership of all these diverse stakeholders will be key for success in implementing the objectives of this topic.

Scope

The **objectives** of this proposal are to:

1. select specific genes and pathways expressed in endothelial cells of normal and/or diseased human brains or preclinical models;
2. validate in vitro and in vivo that these genes or pathways are responsible for normal/deficient/ altered transport at the BBB and the impacts of disease development and progression on these genes or pathways, and to generate improved BBB models for neurodegenerative/metabolic diseases predictive for the disease situation with optimized in vitro-in vivo correlation compared to established models;

3. develop in silico models for predicting BBB penetration and PK of therapeutics in CNS;
4. identify and validate novel targets for brain delivery (main focus) and/or for the therapy of these diseases;
5. understand the mechanisms of neurotropic virus-mediated BBB and CNS penetration to inform innovative ways of brain-selective delivery.

By **diseases** here we mean neurodegeneration (in particular, Alzheimer and Parkinson's diseases), ALS, vascular dementia, multiple sclerosis, and metabolism-related central diseases (diabetes and obesity).

Metabolic disorders such as type II diabetes (T2D) and **Alzheimer's Disease** (AD) were conceptually considered as two independent disorders. Recent evidence points to a link between impaired insulin signalling and dementia. This has even led researchers to propose the term "type III diabetes" for AD to capture the connection between these diseases. Impaired insulin signalling in the brain will cause neurodegenerative changes in cerebral glucose metabolism and can lead to mitochondrial dysfunction, excitotoxic damage to neurons, reactive oxygen species production, neuroinflammation etc., which can trigger apoptotic cell death and ultimately lead to dementia. This link is not only supported by impaired insulin signalling but also from other mechanistic pathways which are altered in obesity such as adipocyte secreted proteins, hormones as well as inflammatory cytokines which, when crossing the BBB, may be involved in the pathophysiological changes leading to dementia. For example, a meta-analysis has shown that people with obesity ($BMI > 30 \text{ kg/m}^2$) have an increased risk factor for AD, while there are several yet unclarified possible mechanisms for the obesity-AD connection ranging from changes in amyloid transport and clearance to alterations in lipid metabolism^{xii}.

The **potential deliveries** of the proposal are several: The use of 'healthy' and patient-derived specimens, iPSC clones and other types of progenitors offers compelling approaches due to the direct connection to patients with the underlying disease. The impacts of these new models could include (1) yielding novel insights into currently identified BBB transport mechanisms for drugs, especially biopharmaceuticals, (2) using comparative assessment between "healthy" and "diseased" BBB, including in silico models, to prioritize some approaches for specific disease(s) because the transport mechanism is modified in the disease state, (3) leading to the identification and characterization of novel transport mechanisms that are unaffected or upregulated in the disease or neurotropic virus-mediated, making them even more interesting, and (4) discovery and characterization that may lead to novel targets addressing the vascular aspect of neurological disorders like AD and thus open up novel routes for therapy.

This topic plans to create a project that will deliver a set of well-defined and reproducible **cell-based in vitro BBB tools/models and in vivo models** to improve the development of therapeutics and enable delivery of those therapeutics for Neurodegenerative (NeuroD) and metabolism-related central diseases by: (1) establishing iPSC- or progenitor-based human BBB models that accurately replicate the clinical behaviour of a range of passively permeable and actively transported/excluded molecules (low molecular weight, mixed modality, and biopharmaceuticals), (2) establish and validate BBB models derived from iPSC cells or progenitors derived from human patients with NeuroD and metabolism-related central diseases, (3) identify and characterize novel potential candidate mechanisms for targeted brain delivery of therapeutics in NeuroD and metabolism-related central disease models, and characterize the mechanisms of neurotropic virus-mediated BBB and CNS penetration for potential selective brain delivery, and generate novel potential candidate targets to address the neurovascular hypotheses of neuro/metabolic disorders.

Expected key deliverables

The overall aim of the proposed research topic is to further the understanding of the BBB in health and disease states towards the development of innovative brain delivery systems, especially for biopharmaceuticals (e.g., peptides, antibodies, etc) and the identification of novel disease drug targets (Alzheimer's Disease, PD, etc). The related key deliverables would be as follows:

- **Identification and validation of specific genes and/or mechanisms** which are altered in brain endothelial cells of the diseases of interest in this topic, namely neurodegeneration (AD/PD), vascular dementia, MS, ALS, central metabolic disorders, and which modify the BBB properties in vitro and in vivo.
- Generation, validation and characterization of **robust and predictive iPSC-derived BBB models**: The developed models should be more reflective of the in vivo situation than existing models, in the healthy as well as in the disease state. The validation employing existing preclinical disease models should make them more predictable for the human clinical pathology. The use of defined media and hydrogel matrices will add to the robustness (reproducibility) and predictability of the BBB models.

- **New, efficacious and safe mechanisms and technologies of brain delivery.** Capitalizing on the findings in particular from the IMI COMPACT consortium, namely several potential new targets for brain delivery identified through an -omics approach, could be a key asset in this endeavor^{xiii}, if this data becomes available at the time the consortium gets formed. The output of this topic should also result in an expanded and deepened understanding of the fundamental processes that underpin drug-trafficking across the BBB, which in turn can further support endeavors to elucidate novel and more efficacious brain delivery mechanisms.
- Characterized **new genetic models for the diseases** of interest in this topic which are better amenable to evaluate disease-modifying agents. Findings from the –omics studies on patient- or preclinical model-derived endothelial cells may give novel insights into disease pathways which may also lead to the development of new models that are more disease relevant.
- Characterized mechanisms of neurotropic virus-mediated BBB and CNS penetration for development of selective brain delivery systems.
- Established in silico/mathematical models in predicting BBB penetration of therapeutics (such as receptor- or carrier-mediated transcytosis for delivery across the BBB) and pharmacokinetics (PK) of biopharmaceutics in different compartments of CNS.
- Identification of relevant translational readouts which are better amenable to elucidate the role of the BBB in the pathogenesis of neurodegeneration and could eventually lead to **new targets for the treatment of the neurovascular causes of the diseases**. The vascular hypotheses of some neurological diseases involve BBB dysfunction in their pathogenesis. However, to-date no clear evidence allows to clearly assess whether these neurovascular dysfunctions are cause or consequence of the neurodegenerative disease. Identification of specific readouts common to preclinical models and human pathologies would be a great advance for the field.

Expected impact

The IMI2 action generated from this topic (“the project”) is expected to deliver new state of the art in vivo and in vitro validated models as well as validated new neurovascular targets to address the BBB and tools required to predict efficacy and safety of new therapeutic approaches. These achievements will benefit the biomedical research community and will rapidly accelerate the pace of research in the development of new therapies and new delivery technologies for diseases for which there is a high unmet medical need, such as Alzheimer’s disease. As the project learnings might eventually enable brain access for large molecules, the project will facilitate academics/SMEs/pharma to open new ways for treatments and delivery systems, encouraging a renewed investment in developing drugs for neurodegenerative & metabolic disorders where the brain is the target. In particular Biotech/SME companies will be able to stress-test their technologies in a non-competitive open innovation environment which will help them to bridge the “valley of death” for turning these into products ready for market.

Thus, it can be anticipated that the results of the project will benefit patients and society through the accelerated discovery of new drugs targeting the brain and new delivery technologies which will provide effective therapies for neuro-related diseases.

Altogether, the results generated from the implementation of this topic hold promise in many of the most important aspects of pharmaceutical R&D and therefore have an impact on the objectives of IMI2:

- improving the current drug research process by providing better translational tools and models to assess efficacy;
- improving the drug development process by providing biomarkers for diseases clearly linked to clinical relevance; better models (including in silico models) in predicting BBB permeability and PK of therapeutics in CNS;
- reducing the time to reach clinical proof of concept in the area of neurological and neurodegenerative diseases;
- increasing the success rate in clinical trials of highly challenging diseases such as those of the CNS;
- developing new delivery systems and/or therapies, based on characterization and understanding of novel transport mechanisms and/or neurotropic virus-mediated transport, for diseases for which there is a high unmet need, such as Alzheimer’s disease and Parkinson’s disease;
- reducing the failure rate candidates in phase III clinical trials through new biomarkers for initial efficacy and safety checks.

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 capitalize on past achievements, available data and tools/models and lessons learnt where possible, thus avoiding unnecessary overlap and duplication of effort.

The project generated from this topic in particular should, among others, build strongly on reported achievements and knowledge from other relevant IMI projects such as COMPACT (<http://www.compact-research.org/> and <http://www.compact-research.org/publications/>).

As the current proposal focusses heavily on iPSC technology, it could have strong synergies with other iPSC-focused efforts like the IMI projects Stembanc (http://www.stembanc.org/) and EBiSC (<https://www.ebisc.org/>) which have established, characterized and banked Alzheimer's and Parkinson's Disease patient-based iPSC clones. These clones could be a valuable tool for the identification of interesting clones for the establishment of BBB and/or disease models in this consortium and thus provide 'added value'.

The action generated from this topic should also consider relevant findings from the FP7 projects JUSTBRAIN, (<http://www.justbrain-fp7.eu/index.php?id=779>)

EURIPIDES (http://cordis.europa.eu/project/rcn/88178_en.html),

NEUROBID (<http://www.neurobid.eu/>)

Industry Consortium

The industrial consortium is expected to provide benchmarks biopharmaceuticals to validate the BBB models, access to iPSC's from patients, high capacities in transcriptomic and proteomic studies, disease models of neurodegeneration and knowledge on translational clinical design.

Indicative duration of the project

The indicative duration of the action is 60 months

Applicant Consortium

The applicant consortium will be selected on the basis of the submitted short proposals. The applicant consortium (in which it would be of value to also include SMEs having relevant know-how and technologies) is expected to address all the objectives and 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.

The applicant consortium should be able to demonstrate the full scope of expertise in order to address effectively and meet all goals outlined in this topic. This may require mobilising, as appropriate: expertise ranging from translational medicine, in vivo models of neurodegeneration, biomarker development to data and knowledge management, project management and professional communication expertise. In particular the following expertise and resources are highly relevant:

- Know-how on state-of-the-art BBB model (iPSC or progenitor-based would be high priority but any other cell model are acceptable), including 3D models, microfluidics or spheroids. Experience in this field would allow generation of innovative approaches to in vitro BBB modelling, from classical Transwell® models to more sophisticated, more in vivo like models.
- Expertise in mathematical/in silico modeling of BBB/blood-CSF-barrier and PK of therapeutics in CNS
- Expertise and access in/to iPSC- or progenitors-derived endothelial cell models in mono- and co-cultures

- Expertise in the biology of molecular transport systems of the BBB (endocytosis, receptor- or absorptive-mediated transcytosis, endosomal trafficking etc.), in discovery and characterization of novel targets/mechanisms more specific for brain delivery, and in the design and development of delivery systems, such as antibodies, bispecific antibodies, liposomes/nanoparticles, aptamers, affimers, etc.
- Expertise and access to disease models in particular models of neurodegenerative diseases such as AD, PD, vascular dementia, MS, ALS, neuropathic/chronic pain, metabolic diseases of central mechanisms. In order to be able to assess the translatability of the developed in vitro models and to establish an in vitro-in vivo correlation, state-of-the-art disease models are needed.
- Expertise and know-how in the study of neurotropic viruses and their brain-penetrating mechanisms

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 industry participation including their contributions and expertise.

The final architecture of the full proposal will be defined by the full proposal applicants in compliance with the IMI2 rules and with a view to the achievement of the project objectives.

In the spirit of the partnership, and to reflect that IMI2 Call topics are built upon identified scientific priorities agreed together with EFPIA beneficiaries/large industrial beneficiaries, it is envisaged that IMI2 proposals and projects may allocate a leading role within the consortium to an EFPIA beneficiary/large industrial beneficiary. Within an applicant consortium discussing the full proposal to be submitted at stage 2, it is expected that one of the EFPIA beneficiaries/large industrial beneficiaries may elect to become the coordinator or the project leader. Therefore to facilitate the formation of the final consortium, all beneficiaries are encouraged to discuss the weighting of responsibilities and priorities therein. Until the roles are formally appointed through a consortium agreement the proposed project leader shall facilitate an efficient negotiation of project content and required agreements.

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

It is suggested to organize the work-plan into six main themes (each corresponding to a specific work package, see chart at the end of the document):

WP1: Selection of genes or pathways candidates associated with neurodegenerative diseases, expressed in brain endothelial cells and/or the neurovascular unit (NVU)

Targets identified by different approaches like:

- Genetic analyses of existing data (GWAS, other published databases)
- Transcriptomic and proteomic profiling of patient primary brain endothelial cells, cells from the neurovascular unit or tissues
- Transcriptomic and proteomic profiling of preclinical disease models primary brain endothelial cells, cells from the neurovascular unit or tissues
- Glycomics of BBB cells and/or cerebral vasculature of diseased brains

Deliverables: disease-associated or differentially expressed genes and/or pathways which play roles in the alteration of BBB integrity and transport mechanisms in endothelial cells/cells of the NVU of potential importance to brain delivery.

EFPIA contribution: Patients primary cells, Omics, genetic analyses, preclinical disease models
Applicant consortium contribution: Genetic analyses, omics

WP2: Phenotypic validation of the identified genes and/or pathways in brain endothelial cells/NVU:

This could be achieved in four steps:

- Generation of endothelial cells from iPSC or Progenitors.
- Generate iPSC cells from primary cells from patients
- Induce mutations of genes/pathways involving BBB permeability and transport by genome editing (such as CRISPR cas9 technology)
- Evidence phenotypic or transport differences in monocultures or 3D/co-cultures.

Many parameters could be analyzed such as glucose and amyloid transport, immune cell migration, permeability to other specific proteins or toxics. The clones displaying phenotypic differences between healthy and disease situation might be prioritized for further work.

Deliverables: validated disease-specific or differentially expressed genes and/or pathways of potential relevance to brain transport.

EFPIA contribution: iPSC cells or progenitors, differentiation into endothelial cells and other cell types (astrocytes, pericytes, neurons...), monocultures, 3D/co-cultures, CRISPR,

Applicant consortium contribution: iPSC or progenitor cells, CRISPR, Benchmark tools and methods for transport analysis and other phenotypic investigations (IgG's, TfR Ab, InsR Ab ...)

WP3: Develop best state-of-the-art (e.g.hiPSC- or progenitor-derived) BBB models (mono- or co-cultures, 3D, etc) by differentiation into endothelial cells and barrier formation characterization

This could be done using mono- or co-cultures, 3D-setting, microfluidics or other settings by differentiation into brain endothelial cells and barrier formation characterization. Full characterization such as apical/basolateral receptor activity would be essential. The model would be considered as validated if it is able to predict *in vivo* exposures of biopharmaceuticals in the various disease or normal state. A last step would be the employment of validated models to further elucidate mechanistic studies pertaining to BBB absorption biology and transport mechanisms.

Mathematical/in silico modeling of receptor-/carrier-mediated transcytosis across the BBB (the capacity of each receptor in mediating transcytosis and brain delivery), and PK of biopharmaceuticals in the brain (particularly the PK and clearance of antibodies/proteins in ISF, neurons, and CSF) should be also a part of this characterization, including disease conditions (such as the expression levels of relevant receptors, carriers and proteins).

Deliverables: Characterize apical/basolateral receptor activity, validate model with a set of reference compounds with known *in vivo* BBB transport data, validate candidates *in vitro*; a more in-depth understanding of the fundamentals and principles of absorption-/receptor-mediated processes of transcytosis across brain capillary endothelial cells and validate candidates *in vitro*. At least one *in vitro* BBB-model and an *in silico* model reproducing/predicting disease features and BBB permeability *in vivo* are expected

EFPIA contribution: BBB models, microfluidics, organ on a chip, spheroid technologies

Applicant consortium contribution: Benchmark tools for transport analysis (IgG's, TfR Ab, InsR Ab, small molecules with available *in vivo* neuro PK data); *in silico* modelling; complex 3D cell systems

WP4: Characterization of neurotropic virus-based BBB and brain penetration mechanisms

A number of neurotropic viruses are capable of entering the CNS to infect neurons and/or glial cells, such as rabies virus, JC (John Cunningham) virus, West Nile virus, adeno-associated virus (AAV) variants. However, the mechanisms by which those viruses either penetrate the BBB or retrograde transport from peripheral nerve to CNS are not fully characterized. Understanding the mechanisms may help in the development of drug delivery technologies selective or specific to CNS.

Different approaches may be employed to characterize the mechanisms and/or to identify the targets/proteins/peptides for brain penetration:

- Genetic and proteomics analyses of the viral genes, proteins and protein fragments for their interactions with human cells and proteins
- Cellular, molecular and biochemical characterization of viral interactions with cellular proteins and/or receptors and virus-mediated penetration of BBB or peripheral nerve/neuronal cells
- Preparation and testing of viral particles (empty viral vesicles) for interactions and penetration across the BBB in vitro or in vivo animal models
- Viral proteins or protein fragments if identified for BBB penetration may be employed to functionalize liposomes and/or nanoparticles for crossing the BBB in vitro and/or in vivo animal models.

Deliverables: Viral proteins and protein fragments and/or viral mechanisms and human proteins/receptors which play roles in virus-mediated BBB and CNS penetration.

EFPIA contribution: human cells, Omics/genetic analyses.

Applicant consortium contribution: Genetic analyses, omics, virology, in vitro and in vivo models.

WP5: Follow-up on identification and characterization of new potential targets from WP1/WP2/WP4 for brain delivery.

These targets could be investigated as new mechanisms of brain delivery . Building and providing tools and models for validation of the new mechanisms would be full part of this package (Ab's, ligands, cell lines). Testing tools against these novel targets in vivo will be an important aspect of the validation strategy as well. This could be done in disease models as well as in healthy wild-type model systems.

Deliverables: Tools for validation and characterization of the new mechanisms and targets (Ab's, ligands, cell lines). In vivo set ups for validation (including e.g. imaging). Validated new brain-delivery targets (by demonstration of increased in vivo brain exposure of Ab or ligand of the target). Validated new neurovascular target with potential for brain delivery in a neurodegenerative disease in disease models or validated such virus-based targets.

EFPIA contribution: Preclinical disease models

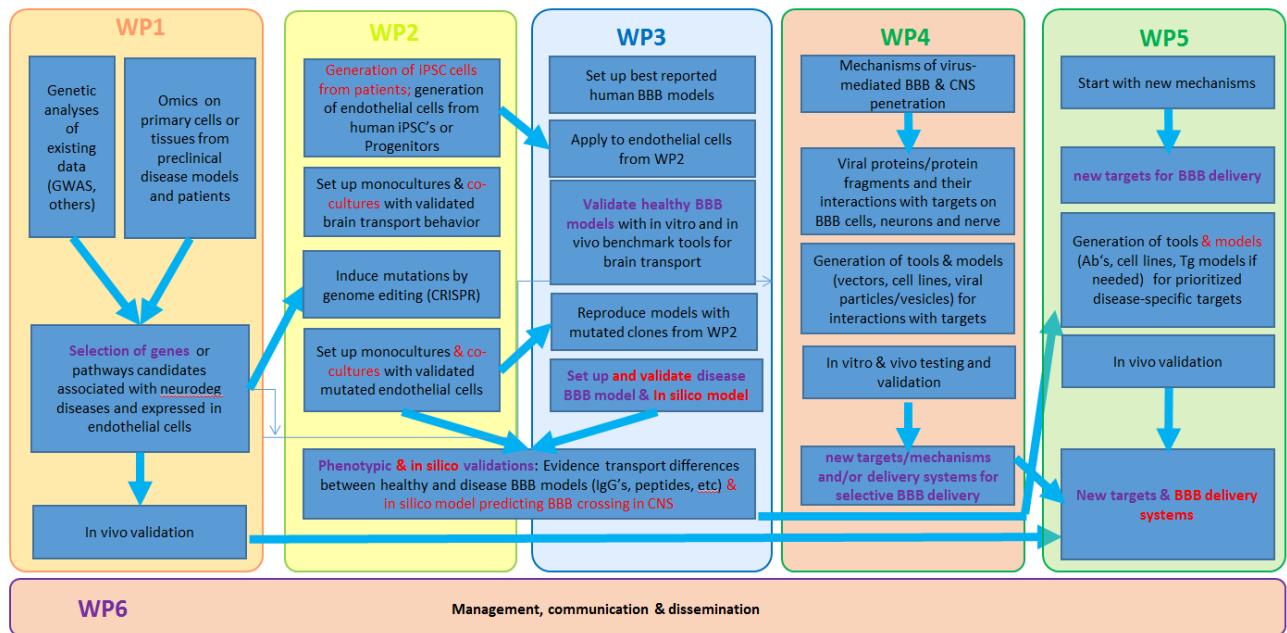
Applicant consortium contribution: tools for validation of the new mechanisms (Ab's, ligands, cell lines); in vivo PK; disease models

The new targets identified in WP1 WP2 and WP4 should be fully characterized.

WP6: Management, communication & Dissemination

This work-package should be designed to be fit for purpose to govern and implement the project as a successful public-private partnership

Flow Chart of Proposed Project



Indicative

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