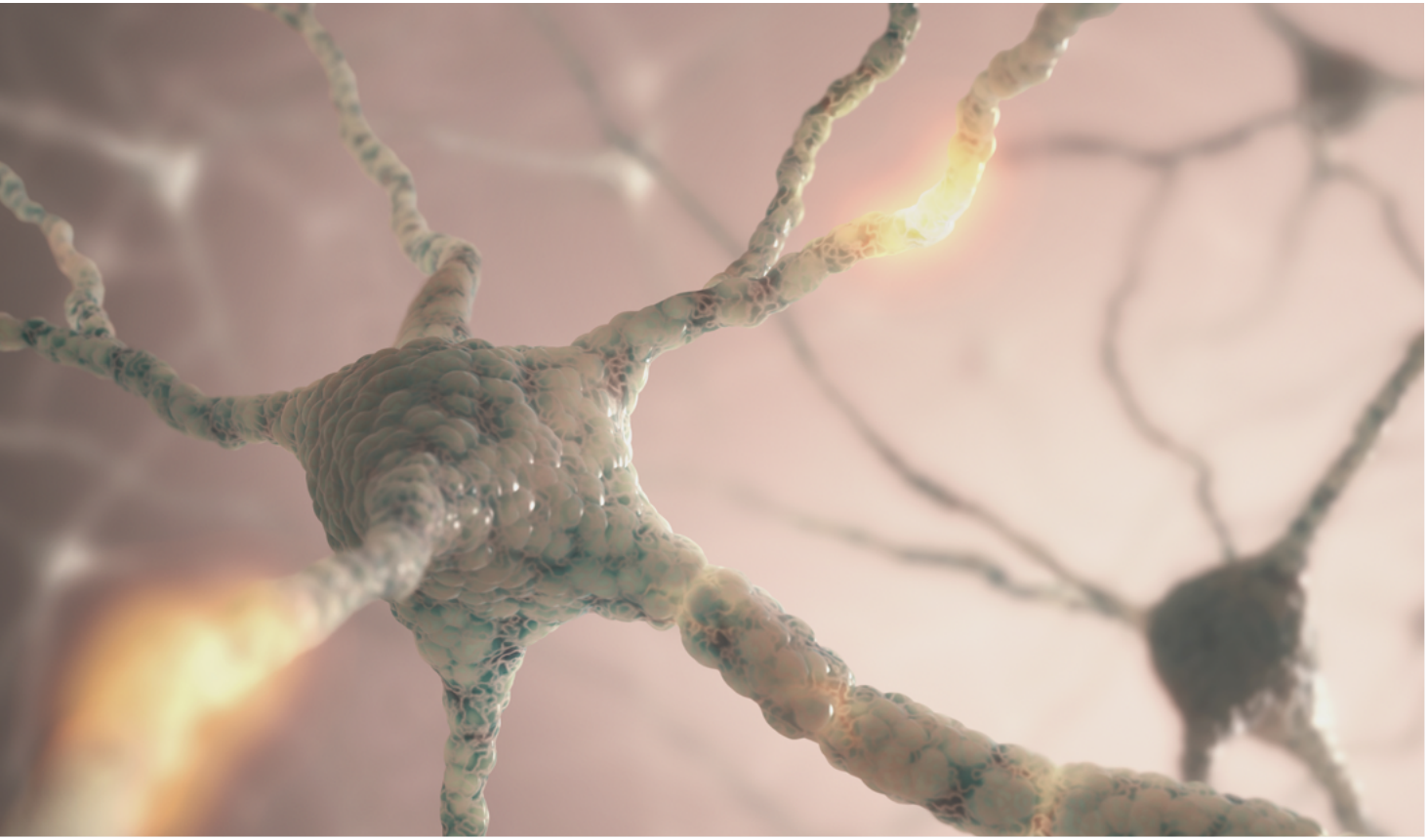
NEW MECHANISMS AND MODELS FOR IMPROVED BRAIN THERAPEUTICS



IM2PACT CONSORTIUM

imi innovative medicines initiative

efpia

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PROJECT OBJECTIVE

The blood-brain barrier (BBB) remains a major obstacle for biologics[1]. Transport receptors have been exploited to ferry drugs across the BBB but there is a strong need for receptors with better brain specificity. Several neurodegenerative diseases such as Alzheimer's or Parkinson's originate from initial BBB leakiness, suggesting that specific targets at the BBB could be used for therapy of such diseases.

IM²PACT objective is to identify targets[2] at the BBB that could either transport biologics or be modulated to treat neurodegenerative diseases.

The blood-brain barrier (BBB) is a protective layer between the brain's blood vessels and the cells that make up brain tissue.

1 Biologics are novel, very specific molecules, which however are huge compared to more classical medicine, often called Small Molecules.

2 Molecules that can interact with the molecular elements of therapeutics.

SUMMARY

The brain is protected by a structure called the blood-brain barrier (BBB), preventing substances present in the blood crossing into the brain. This barrier is made from a special type of cell, called brain capillary endothelial cells which are joined very closely together. The BBB means that trying to get treatments into the brain is not easy. New types of treatments like antibody therapies are even more challenging because antibodies are very large molecules. One way to get across the BBB is to use proteins found at the surface of the endothelial cells. These proteins might already have the job of carrying molecules across the BBB and are called transporters. Other possible proteins that could be used are the ones found on the surface of certain viruses that are able to cross the blood-brain barrier.

The IM²PACT project is about trying to find better ways to get treatments into the brain. In order to do that, we need to first better understand what genes and proteins are present in the brain endothelial cells.

We are doing that using a technique called single cell transcriptomics and another technique called mass spectrometry proteomics. We apply these techniques to brain tissue obtained after death from people who have had brain disorders such as Alzheimer's disease. We can then compare this brain tissue with tissue obtained from healthy people. This way, we can know whether the BBB is affected by disease.

Once we have found proteins that might be used to get treatments into the brain, we need to see if they really do work. We think the best way of checking that, is by using brain endothelial cells we can grow in a dish. We use a technology called induced pluripotent stem cells. This is a method where we can take an adult human cell (such as blood cell) and turn into a stem cell. The stem cell can then be turned into any cell type we want to study - in our case, this will be brain endothelial cells.

Once we have human brain endothelial cells growing in a dish we can test how well certain proteins can transport treatments. We can make quite sophisticated artificial BBB to test this. However, we also want to confirm the ability to transport treatments in whole living things because many factors might be important to consider. This is why our project is also using mice with advanced brain imaging techniques to see how treatments might be carried across the BBB by the proteins we have selected.

For many brain disorders such as Alzheimer's disease and even brain cancers - there are new exciting treatments being developed. The IM²PACT project is important because unless we find better ways of getting treatments across the BBB, the treatments may turn out not to be effective.

PROJECT STRUCTURE AND ACTIVITIES

To address this objective, the IM²PACT research activity has been organized in different workpackages (WP). WP1 covers differential proteomic[1] and transcriptomic studies[2] of human brain tissue to identify specific targets at the BBB. WP2 develops brain endothelial cells (BEC's) from human pluripotent cell progenitors (iPS[3]). These cells, from healthy and neurodegenerative disease patients will then be used to generate *in vitro* models mimicking BBB function in health and disease. In WP2, the research is focused on two-dimensional cellular model systems with one cell type, while in WP3 more complex, three-dimensional models composed of different brain cells are investigated. Another potential source of brain transport targets are used by neurotrophic viruses[4] and their investigation is the research in WP4. The targets identified in WP1 and WP4 are validated both in WP2 and WP3.[5]

Finally, WP5 will build tools and models for final *in vivo* validation of the prioritized targets. All WPs will be supported by WP6 for project management and results dissemination.

The consortium consists of 16 academic groups, 4 biotechs and 7 big pharmas from 9 EU member states. The total budget is €17.4 M with an €9 M EC contribution.

1 Assessment of which proteins are present in certain cells or subcellular compartments of the brain vasculature.

2 Assessment where, when and to what extent certain proteins are produced in the tissue. From those data researchers are able to deduce what the cell is currently doing (or not), what is its function and what receptors (potential drug targets) are currently expressed (present inside the cell or outside on the cell surface).

3 Cells that are as early in their development that, with specific treatment regimens, they can develop into a cell of choice, eg liver, brain or cells of the BBB.

4 Viruses that can enter and replicate in the central nervous system.

5 The targets are built into the models developed by WP2 and WP3 and tested whether the expected properties can be seen in those models as well.

WORK PACKAGE 1

Identifying genes that are specific for brain versus peripheral tissues in health and disease.

WORK PACKAGE 2

Constructing a human brain endothelial cell line from human pluripotent cells.

WORK PACKAGE 3

iPS-BBB models transport.

WORK PACKAGE 4

Identifying membrane proteins involved into brain penetration of virus.

WORK PACKAGE 5

Validating final targets for their ability to reach the brain in vivo.

Abbreviations used:

BBB: *Blood-Brain Barrier;* ***iPS:*** *Induced pluripotent stem cells;*

SCIENTIFIC IMPACT

Better translational tools and models to assess efficacy of novel drugs, interacting with newly identified targets. Biomarkers[1] for diseases clearly linked to clinical relevance. Better models (including in silico models) for predicting BBB permeability and pharmacokinetic in health and disease states. Development of new delivery systems into the CNS and/or therapies for diseases of the CNS.

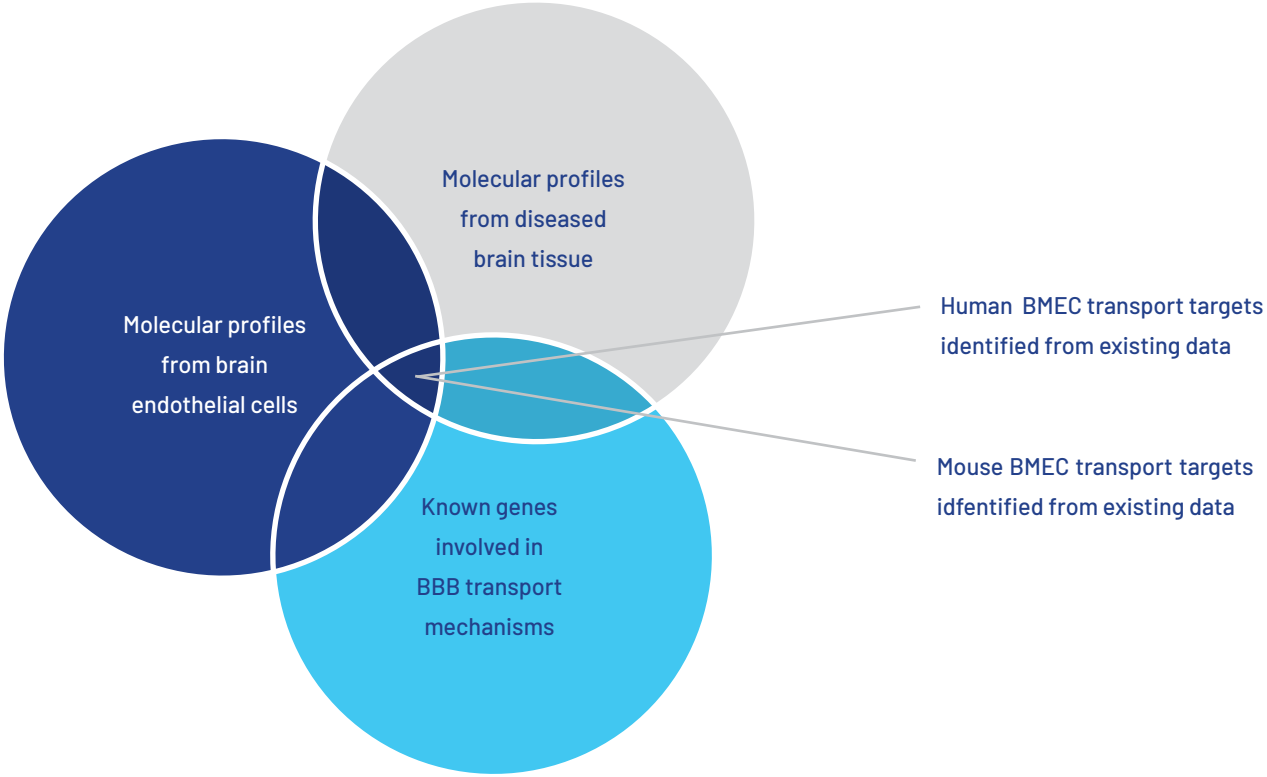
EDUCATION IMPACT

To train and develop talented researchers through a multidisciplinary cooperative approach in connection with industry.
To disseminate knowledge, in connection with other fields, to the general public, suggesting that specific targets at the BBB could be used for therapy of such diseases. IM^{2}PACT objective is to identify targets at the BBB that could either transport or be used for treating the disease.

ECONOMIC IMPACT

Strengthen the competitiveness and industrial leadership of Europe. Reducing time to clinical proof of concept. Increase success rate of clinical trials. Improve treatments.

WORKPACKAGE 1 (WP1)



Leveraging existing datasets

All existing data from mouse and human tissue from previous studies have been re-evaluted to identify potential new targets for drug transport across the blood-brain barrier.

Abbreviations used:

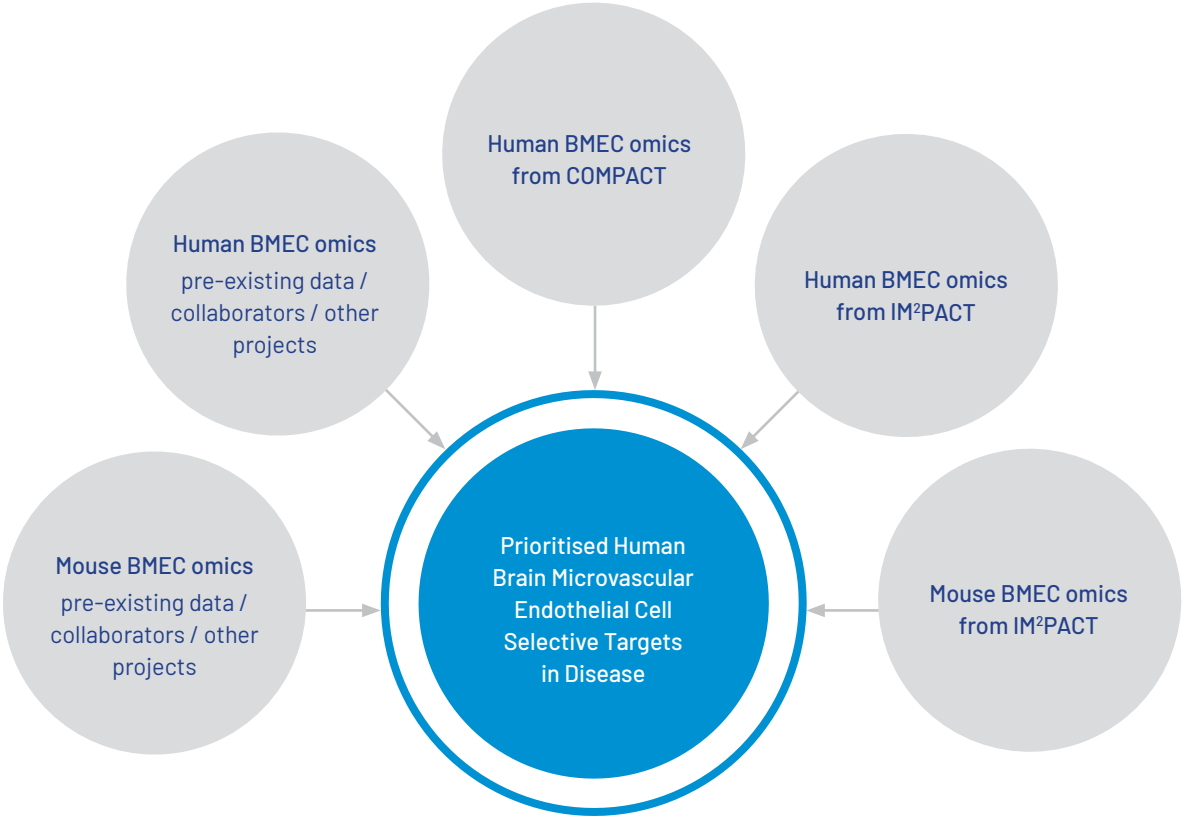
BMEC: *Brain Microvascular Endothelial Cells;*

Brain Microvasulature Endothelial Cell Omics

IM²PACT has undertaken single nuclei transcriptomic and proteomic studies from both pathological and healthy tissue. The main pathologies studied are Alzheimer's disease and Multiple Sclerosis. Using mouse brain tissue, IM²PACT is also investigating mouse obesity and diabetes models to identify brain targets that are specifically modified by metabolic diseases.

Abbreviations used:

ROI: *Regions of Interest;*



Integrating datasets

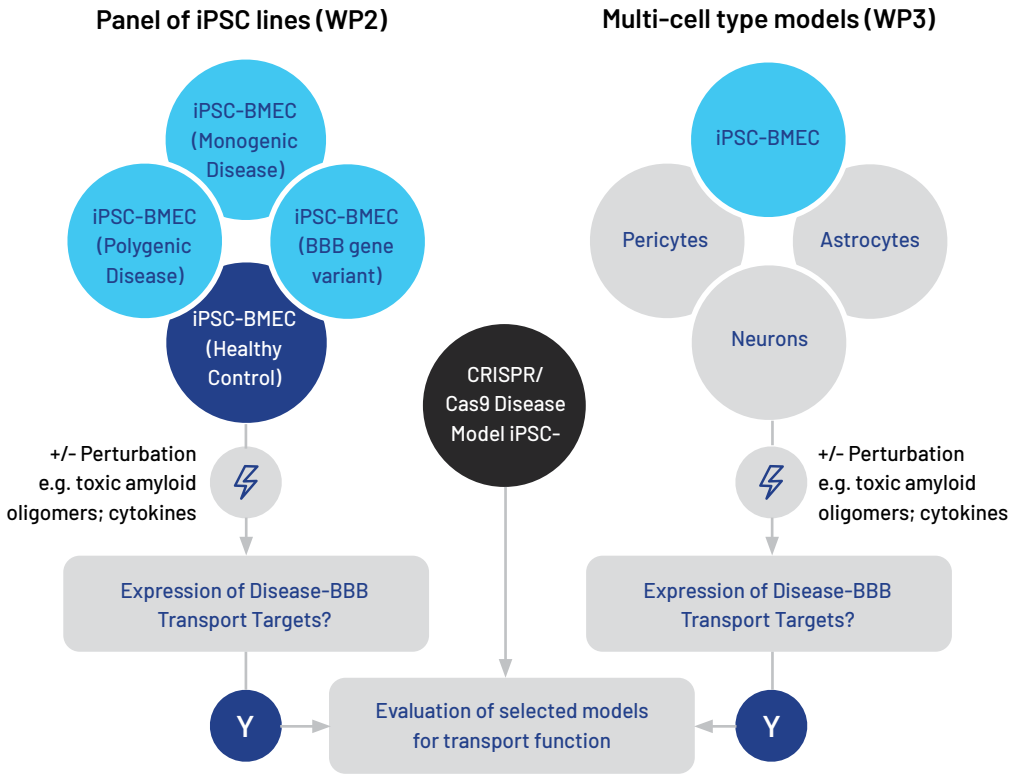
All these datasets, along with a privileged access to omic data from the IMI COMPACT[1] consortium, are integrated and analyzed to identify targets showing high differential expression, membrane localization, selective expression of the protein in brain microvessels, homology with orthologs, as well as availability of structural data to select the best transport or therapeutic brain targets.

1 A previous consortium on the topic, which are the grounding of the investigations in IM²PACT.

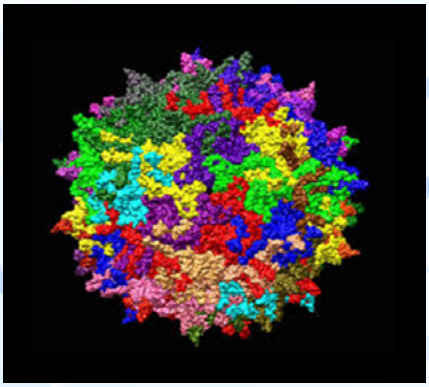
WORKPACKAGES 2 AND 3 (WP2 & 3)

In order to have the best chance of succesful translation to humans, a protocol to produce an iPS-derived brain endothelial cell line[1] has been prepared and fully characterized. These cells are further used to build in vitro blood-brain barrier models. Several models have been evaluated from the classical transwell, to microfluidic or organoid models. They have been geno- and phenotypically validated with a comprehensive range of markers and benchmarks to ensure optimal predictability. The best models are then used to validate and characterize new brain transport or therapeutic targets that have been discovered in WP1.

1 A cell line in which the genetic information can be adapted in specific ways so that this cell line has the optimal conditions for the testing.



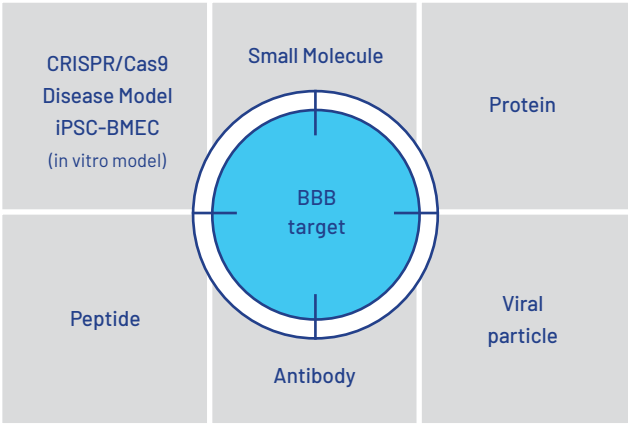
WORKPACKAGE 4 (WP4)

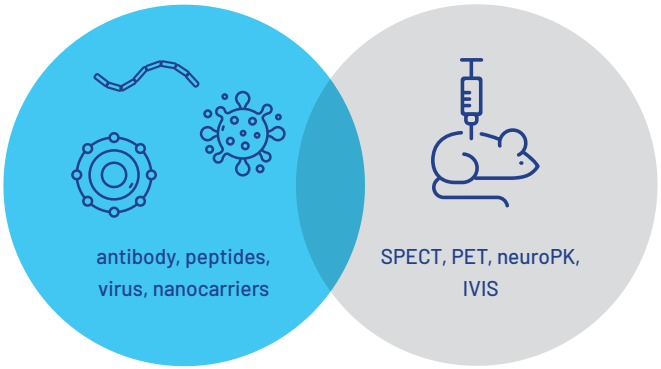


Some virus or particles can penetrate the brain but the mechanisms they use are not necessarily known. WP4 is concentrating their efforts on cellular, molecular and biochemical characterization of such mechanisms to then apply them on brain transport of biotherapeutics. After *in vitro* and *in vivo* validation of their brain penetration, genetic and proteomics analyses of the viral genes, proteins and protein fragments for their interactions with human cells and proteins are undertaken.

WORKPACKAGE 5 (WP5)

The targets identified in WP1 &WP4, and validated in vitro in WP2 & 3 are then transferred to WP5 for in vivo validation.





Target Toolbox

To this end, and as a function of the target itself, tools will be developed, ligands such as small molecules, peptides, antibodies or even nanoparticles will be identified or generated. These tools are used , eg. to block an identified target and to check whether the effects observed are the same as the predicted.

Pre-Clinical Disease Model POC CNS Therapeutic Delivery

In parallel, several specific *in vivo* imaging methods such as SPECT, PET or IVIS[1] have been developed, along with all necessary procedures for brain quantitation. Using these tools, the targets will be validated in adhoc *in vivo* models and fully demonstrate their potential to transport therapeutics to the brain or prevent or treat some of the neurodegenerative diseases they are associated with.

1 Technologies with which you can see the localization or function of certain brain targets in living animals.

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