The blood-brain barrier (BBB) inhibits the free transcellular passage of hydrophilic molecules by an extremely low pinocytotic activity and their paracellular diffusion by an elaborate network of complex **tight junctions (TJs)** between its endothelial cells (ECs). Specific transport or receptor-mediated transcytosis systems, selectively expressed by brain endothelial cells to ensure the effective delivery of nutrients from the blood to the brain and the rapid removal of toxic metabolites from the brain have been exploited for drug delivery, however, with limited success. In contrast, lack of knowledge on the molecular composition of cerebrovascular TJs has hampered the development of safe strategies for paracellular drug delivery across the BBB until recently.

Our **specific scientific objectives** during the second 18 months of research have been the following:

- Finalization of the definition of the transmembrane proteins and intracellular partners expressed at BBB adherens junctions (AJs) and tight junctions (TJs)
- Assessment of the role of different components in maintaining AJ and TJ organization and permeability control
- Identification of signaling pathways able to modify AJ and TJ organization and permeability properties. To define tools to reversibly increase BBB permeability to large molecular weight molecules.
- Finalization of the generation of molecular tools for BBB targeting of endothelial junctional proteins outside of organized adherens and tight junctions
- Targeting BBB junctional borders *in vivo*. Molecular definition of EC junctional stabilization by pericytes for the identification of novel targets. Screening and validation of pathways and transcription factors other than β-catenin regulating genes for BBB junctions in ECs
- Manipulation of paracellular permeability in the *in vitro* models of the BBB by effectors interfering with transcription factor function Generation and improvement of claudin targeting peptides with the potential to modulate the extracellular interactions between claudins.
- Finalize the testing of the potential of claudin-targeting peptides to manipulate the paracellular tightness for enhanced permeability *in vitro* Characterization of claudin targeting peptides found to manipulate the paracellular tightness for enhanced permeability *in vitro* Testing the potential of claudin-targeting peptides to modulate BBB permeability *in vivo*
- Transfer of modulatory peptides and co-administration procedure for practical application
- Continue to validate the potential of claudin-targeting peptides for delivery of large molecular weight drugs across the BBB *in vitro*
- Validate the role of the selected compounds targeting (novel) endothelial junctional

  proteins for the delivery of large molecules across the BBB *in vitro*
- Targeting barrier opening strategies to the BBB by using ultrasound and microbubble technologies
- Modelling paracellular delivery of large molecules across the healthy BBB in vivo
- Modelling paracellular delivery of anti-tumor antibodies across the BBB in mouse models of brain tumor/brain metastases
- Modelling paracellular delivery of anti-β-amyloid antibodies across the BBB in a mouse model for Alzheimer's disease
- Modelling paracellular delivery of neurotrophins/gliotrophins across the BBB in EAE, a mouse model for multiple sclerosis

Major achievements so far

JUSTBRAIN demonstrated that, unlike in other vascular barriers, the family of junctional adhesion molecules (JAMs) is not critically involved in regulating the integrity of BBB TJs and AJs. Therefore JAM-A, JAM-B and JAM-C are no suitable targets for regulating BBB TJs and AJs. Although this is a negative finding it emphasizes that BBB TJs are different from TJ in other vascular barriers which might allow for BBB-specific manipulations of TJ integrity.

Modulation of the localization of claudins in BBB TJs is critical for controlling BBB TJ permeability. JUSTBRAIN could demonstrate that ectopic expression of claudin-1 in BBB TJs leads to amelioration of an animal model of MS due to sealing of BBB TJs and thus reduces BBB leakiness. JUSTBRAIN also demonstrated for the first time that the first extracellular loop of claudins is directly involved in homo- and heterophilc interactions between classic claudins.

The interactions are redox-sensitive which is of relevance for pathological conditions related to oxidative stress. Inhibition of these homo- and heterophilic claudin-interactions with functional peptides allows increasing BBB TJ permeability in vitro. Unfortunately these peptides cannot be used for opening BBB TJs in vivo due to toxic effects when applied systemically. It remains to be shown if the novel antibodies produced against the extracellular domains of claudins will provide useful tools for modulating BBB TJ integrity in vitro and in vivo.

The central role of of Wnt/β-catenin signalling for BBB formation and BBB maintenance *in vivo* in mouse models and *in vitro* in mouse and human BBB models could be firmly and reproducibly established. Using this knowledge we have established a novel *in vitro* BBB model using an endothelial cell line and could demonstrate that manipulating this signaling pathway allows increasing BBB TJ permeability for large molecules in vitro. In this context it is also relevant to mention that we identified novel binding partners for β-catenin and novel targets downstream of β-catenin. These molecules open novel possibilities for developing strategies to manipulate BBB TJs.

JUSTBRAIN is dedicated to specifically explore and develop strategies targeting individual junctional proteins for a **regulated and transient – and thus safe - opening of BBB junctions allowing for the delivery of large molecular drugs into the brain**.
From the work performed so far we have identified the Wnt/β-catenin signalling pathway as the most promising to manipulate for transient opening of BBB TJs for the delivery of large molecular drugs across the BBB *in vivo*. In the remaining time of JUSTBRAIN we will therefore focus on testing a number of options to manipulate the Wnt/β-catenin signaling pathway in vivo including transgenic mouse models for transient opening the BBB TJs for the
delivery of large molecular drugs *in vivo.*

More information see www.JUSTBRAIN-FP7.eu