**Acronym**: JUSTBRAIN

**Title**: Blood-brain barrier junctions as targets for paracellular drug delivery to the brain

**Contract number**: HEALTH-F2-2009-241861

**EC contribution**: 2,989,845 € (Two million nine hundred ninety-eight thousand Euros)

**Duration**: 48 months

**Starting date:** 01/11/2009

**Instrument**: Collaborative Project: small-medium scale focussed

**Summary**

To maintain homeostasis of the central nervous system (CNS), the blood-brain barrier (BBB) prevents the free transcellular passage of hydrophilic molecules from the blood into the CNS. Because of this, the BBB is now recognized as the major obstacle to the treatment of most neurological disorders, as it hinders the delivery of many potentially important therapeutic and diagnostic substances to the CNS.

Previous approaches in improving drug delivery across the BBB, which have primarily aimed at hijacking the transcellular transport machinery that is dedicated to the selective transport of specific molecules across the BBB, have had limited success, especially with regard to large molecular weight drugs. Lack of knowledge on the molecular composition and function of cerebrovascular cell-to-cell junctions has hampered the development of safe strategies for paracellular drug delivery across the BBB until recently.

**Background**

There is a lack of therapies for most neurological disorders ranging from rare, however, fatal diseases such as lysosomal storage diseases and primary brain tumors to major public health concerns such as brain metastases, multiple sclerosis, stroke or Alzheimer's disease. In addition to the development of effective drugs, the delivery of diagnostic or therapeutic compounds into the brain has become a major challenge in successfully treating these diseases. To maintain homeostatsis of the brain, passage of potentially harmful substances from the blood-stream into the brain is restricted by the highly specialized vascular barrier, **the blood-brain barrier (BBB).** In this neuroprotective role, the BBB also hinders the delivery of many potentially important diagnostic and therapeutic drugs to the central nervous system (CNS). Current estimates are that only 2% of small molecule drugs and even lower amounts of large molecule drugs in clinical use cross the BBB. This has dramatically hampered the development of pharmacotherapies and immunotherapies in brain diseases. Because most drugs cannot penetrate the BBB, the treatment of i.e. primary brain tumors or brain metastases is presently reduced to symptomatic and palliative measures.

**Aim**:
Members of the JUSTBRAIN consortium have accumulated knowledge on the structure and function of BBB cell-to-cell junctions, identified endothelial signals controlling the expression of individual junctional proteins and have begun to develop approaches, which may either open or close BBB junctions. JUSTBRAIN will

- Define the best known molecular targets of BBB junctions by applying available inhibitors and therapies in health and disease
- Characterize novel junctional adhesion proteins and/or their intracellular binding partners in health and disease
- Characterize the transcriptional control of BBB junction maintenance in health and disease.
- Transiently increase or decrease expression of other/novel junctional proteins (JAMs, L1) at the BBB *in vitro* and *in vivo*
- Modulate the extracellular homophilic or heterophilic interaction of tight junction proteins, i.e. claudins at the BBB *in vitro* and *in vivo*
- Choose feasible approaches for paracellular drug delivery to be further developed for potential clinical development

JUSTBRAIN consortium aims at offering a specific, controlled and transient opening of BBB junctions and unraveling the molecular architecture of endothelial BBB junctions and their brain specific regulation to modulate delivery of large drugs to the brain.

**Expected results**:
The cutting-edge knowledge created within the JUSTBRAIN consortium combined with the unique set of experimental tools including sophisticated transgenic mouse and *in vitro* BBB models puts the JUSTBRAIN consortium in a exceptional position to explore the possibilities of transiently opening the of BBB cell-to-cell junctions for the paracellular delivery of large molecular hydrophilic drugs into the brain.

Using *in vitro* and *in vivo* BBB models and animal models of neurological disorders, where BBB opening may be therapeutic, JUSTBRAIN is dedicated to translate this basic knowledge into identifying an entire novel platform of drugable molecular targets that could be functionally modulated to allow bypassing the BBB via the paracellular route. By these means JUSTBRAIN expects to improve efficient delivery of large molecules into the CNS and thus to expand on diagnostic and therapeutic possibilities for neurological disorders.

JUSTBRAIN is expected to pave the way towards the development of novel strategies to specifically and transiently open BBB junctions, thus allowing the safe and effective delivery of large molecular drugs across the BBB into the brain.

**Potential applications**:
The global CNS pharmaceutical market, worth about 80 billion € a year with about 8% growth in 2007, is poised to become the number one target for drug development during the next decade. In Europe and US it has been calculated that the number of patients suffering of Alzheimer's Disease will peak up to more than 13 million by 2050; a 300% increase on numbers today. One might foresee that JUSTBRAIN findings would facilitate the development of drugs that help to close the BBB in disorders such as stroke and also brain tumors, where concomitant brain edema adds significantly on patient morbidity and mortality. On the other hand, controlled opening of the BBB would allow access of large molecules in neurodegenerative disorders such as Alzheimer's disease, psychiatric disorders like depression and also therapeutic targeting of brain tumors. In fact these problems are faced daily in the clinical routine and therefore the outcome of the proposed work in JUSTBRAIN will have major impact on the clinical routine and finally on patients treatment, healthiness and survival.

**Project web-site**: www.JUSTBRAIN-FP7.eu

**Key words**:
Blood-brain barrier, tight junctions, claudins, junctional adhesion molecules, brain tumors, Alzheimer's disease, drug delivery

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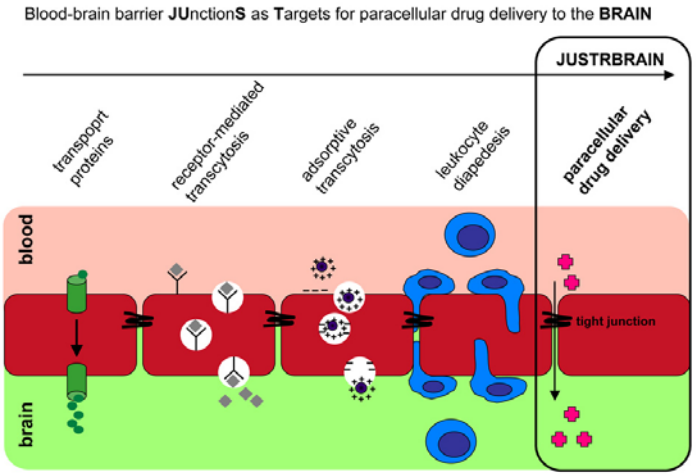


**Figure 1: Strategy of the JUSTBRAIN consortium to target BBB junctions for paracellular drug delivery into the brain**
Recent discoveries on the molecular composition and regulation of endothelial junctional complexes combined with the knowledge that leukocytes can pass through endothelial junctions provide the basis of the JUSTBRAIN consortium to develop strategies to **transiently open BBB junctions** for the delivery of large molecule drugs into the brain.

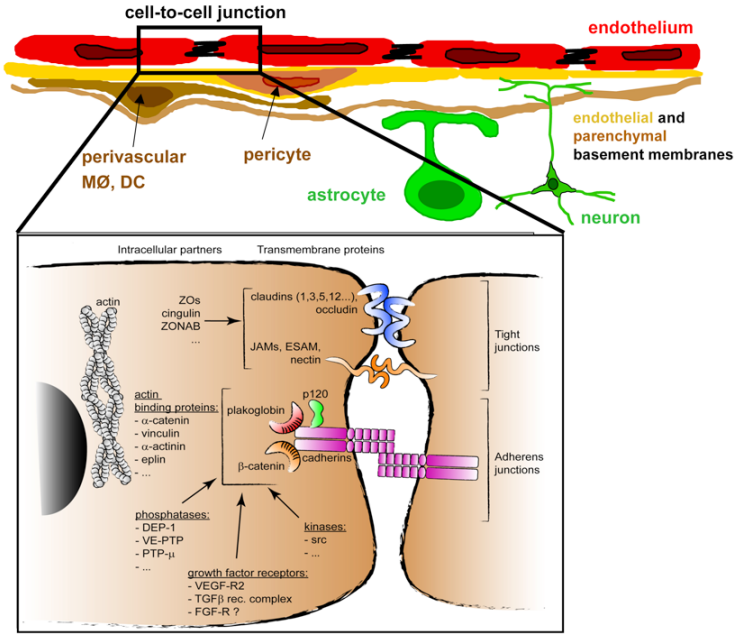


**Figure 2: Molecular composition of the BBB tight junctions targeted by the JUSTBRAIN strategies for paracellular drug delivery across the BBB**

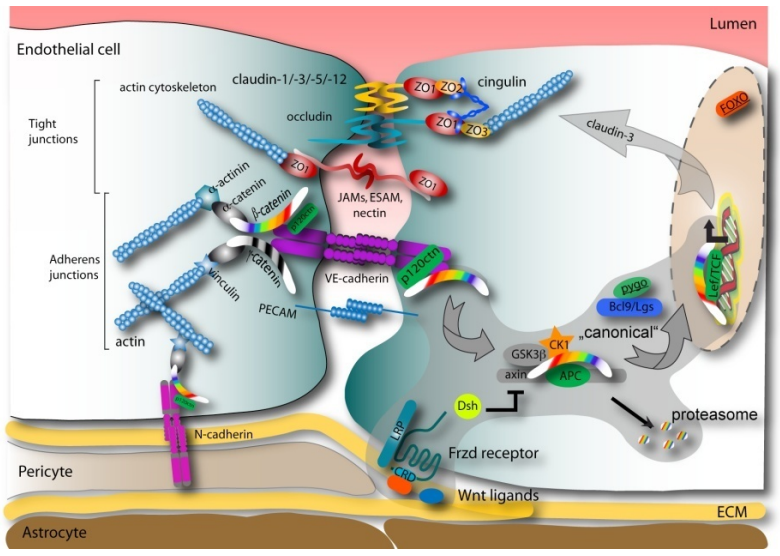


**Fig. 3: Schematic junctional organization and Wnt/β-catenin signaling in endothelial cells.** In the absence of Wnt growth factors, β-catenin is marked for degradation in the proteasome by a complex formed by Axin, adenomatous poliposis coli (APC), glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α). Upon Wnt stimulation of frizzled (Frzd) receptors a cascade of phosphorylations of *dishevelled* (Dsh) and Axin leads to the inhibition of the degradation complex. β-catenin then binds to the transcription factors lymphoid enhancer factor-1 (Lef1)/T-cell factor (TCF) and starts transcription of genes like claudin-3 that are important in BBB TJ formation. See the grey supported area and arrows for the "canonical" Wnt pathway (Liebner et al., J Cell Biol., 2008).