A new in vivo gene therapy system to treat temporal lobe epilepsy.

I. Contexte, positionnement et objectif(s) de la proposition

Epilepsy is the most frequent neurological disorder after stroke and headache. Temporal lobe epilepsy (TLE), the most common form of partial epilepsy is defined by the periodic occurrence of seizures, which alter normal brain function. Dynamic balance between neuronal excitation and inhibition breaks down under epileptic conditions. Altered GABAergic signaling is a major feature of both epileptic disorders and cognitive decline, providing a compelling rationale for targeting exacerbated excitation to improve cognitive function and restrain epilepsy. Despite treatment with currently available antiepileptic drugs, one third of the TLE patients remain pharmaco-resistant, making it crucial to discover novel interventions that will ultimately improve the quality of life of the patients. Recently, we have demonstrated that bumetanide given during the asymptomatic time-period, constrained chronic seizures by reversing aberrant GABAergic signaling in adult experimental epilepsy. Thus, we propose to develop a new approach to specifically target GABAergic signaling in granular cells of the hippocampus in the rat pilocarpine model of TLE. The final aim of the project is to unveil novel therapeutic strategies to restore cognitive performance in temporal lobe epilepsy by blocking DG cells sprouting in a non-viral, specific and efficient way.

Our results together with others have highlighted the role of chloride transporters, neurotrophin receptor p75NTR and hyperexcitability in the settling up of hippocampal network changes happening during epileptogensis. Up to now, all strategies trying to reduce or block seizures have been either surgically- or pharmacologically-based with the aim of reducing the network hyperexcitability after seizures appearance. Thanks to our pharmaceutical approach we prove the role of early chloride-mediated changes in GABAergic neurotransmission in network alteration through modification of the two main proteins expression, namely p75NTR, a pan receptor for neurotrophins responsible for neuronal branching plasticity and cell death both in central nervous system and spinal cord and KCC2 for potassium-chloride co-transporter, the main neuronal-specific chloride extruder, impaired in epileptic condition.

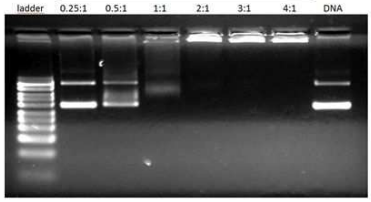
By restoring the GABAergic function in the asymptomatic phase, we show a 50% reduction in seizure severity. In this application we will use an active consortium between INMED and OZ Biosciences **to develop a new method based on biodegradable nanoparticles to genetically modify DG cells in vivo** in order to block either the p75NTR up-regulation or KCC2 calpain-mediated down regulation by using DNA encoding shRNA or with a CRISPR/cas9 method. We will target these two pathways identified as main contributors in generating epileptic-like event. More precisely the aim will be to express a chimera plasmid coding for the mature and active form of KCC2 protein to prevent the aberrant sprouting of DG cells that is responsible for epileptogenesis and/or to use a gene editing method for re-expressing KCC2. This protein sequence will be inserted in a plasmid that will be conditionally expressed and repressed. **The gene delivery system will be based on a new proprietary generation of harmless lipid- and polymer-based nanoparticles** own by the SME.

Objectives and novelty. We focus on elucidating the early cellular and molecular events that drive the hippocampal structural reactive plasticity and subsequent cognitive disabilities in temporal lobe epilepsy with regard to GABAergic transmission. Using in vivo model of TLE, telemetric EEG and behavioral processing we will investigate the impact of functionally restored chloride homeostasis onto granular neurons and derived structural hippocampal reactive plasticity changes on memory retrieval. The goal is to develop novel gene delivery nanoparticles suitable for in vivo use, without side effects such as inflammatory processes and that could be used to deliver specifically DNA, RNA or CRISPR/Cas9 nucleoprotein complexes by stereotaxic approach. This procedure increases accuracy, high efficacy, low volume. Our final aim is then to transpose this innovative tool to human tissue thanks to our active collaboration with the group of Gilles Huberfeld in Paris. Collected tissues from patients suffering from TLE will be cultured, then processed for ex

vivo transfection and tested for seizures detection using extra-cellular field recording and multi electrodes array approach.

Risks. The protocol of TLE is already in use in the lab, we already get preliminary results on the efficacy of the compounds used to deliver DNA and the OZ Biosciences SME is a well-known company developing new nanoparticles that will be used in the study. All the patents on this newly generated nanoparticles-based delivery system belong to the company and we already designed and tested candidate formulations. Our data showed a strong expression of reporter gene in vivo that is detectable by immunocytochemistry and biochemistry. The SME already launch commercially this new in vivo gene delivery reagent (BrainFectIN). The risky part is to constructs the desired plasmid, to design the proper CRISPR/Cas9 system and to insure a sufficient delivery for blocking the expression or inversely for re-expressing the targeted protein in the granular cells of the hippocampus. The expertise of Pellegrino C, INMED and the SME team significantly reduce this risk. Furthermore, our active collaboration with Paris neurosurgeons is active and fruitful.

A



B

	Size		Zeta Potential	
Conditions	Size	SD	Zeta	SD
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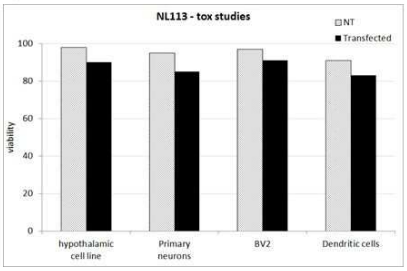
2:1	135,9	9,2	7,3	2,6
3:1	118,3	18,8	6,6	2,8
4:1	108,5	18,0	4,5	5,9

C



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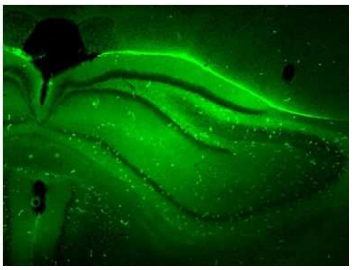

Figure 1: In vivo transfection using nanoparticle approach: A) Examples of experimental procedures aiming at defining the optimal ratio of nano-particle based compound (NL-113) to bind plamidic DNA, results are visualized on agarose gel. C) Toxicity assay of NL-113 on different cell types from neuronal and non-neuronal cells. Our results clearly show that there is no induced toxicity after transfection. D) Example of brain transfected by the stereotaxic injection of 1ul of NL-113, cells expressing GFP antibody, fluorescence is clearly visible in the entire hippocampus. 4X magnification.Unpublished data.

II. Organisation du projet et moyens mis en œuvre

OZ Biosciences is a French corporation founded in 2003 that creates and develops new research tools focused on delivery technologies of bioactive materials (nucleic acid, protein, peptide…) into living organisms intended for the life sciences research world and pharmaceutical industries. The company is particularly specialized in nanotechnologies and transfection. The proprietary technologies of OZB are based on new lipids, polymers, liposomes and magnetic nanoparticles. OZB major expertise is in non viral gene therapy, drug delivery systems and nanosciences. The current research activities of OZB R&D department are: development of DNA and RNA delivery systems for in vitro and in vivo applications, design of transfection technology for haematopoietic, immune, stem and IPs cells, ex-vivo gene therapy for neurosciences or regenerative medicine applications, development of new adjuvant formulation and targeted delivery

Claudio Rivera's team, from INSERM represented by INMED UMR901 institute, has a huge experience in modifying cells either in vitro and in vivo through viral- and non-viral based approaches and is known for his work on chloride homeostasis impairment, his group at INMED Institute is fully equipped to perform such project, the collaboration between the two structures is already a fruitful collaboration and leads to publications. The lab contribution will consist in managing the post-doctoral candidate for all in vivo studies, making experiment and analyzing data. The experience of Rivera's lab in molecular biology, behavioral analyses and EEG analysis will be essential. The lab has already a patent application in the early treatment of aberrant sprouting of DG cells using bumetanide, making the project relevant to the field.