BrainVectors Newsletter n° 6

Marie Curie Industry-Academia Partnerships and Pathways - Contract

Dissemination of results and scientific communication outsidethe network:

Workshop: Neurotrophic factor gene delivery for neurodegenerative diseases : still a promising clinical paradigm?

CHUV, Lausanne, 2-14 September 2016.

FINAL PROGRAM

September 12th

12:00-13:00 Registration and posters hanging.
12:00-13:30 *Lunch*
13:45 -14:00 Welcome addresses:
Liliane Tenenbaum, Lausanne University Hospital, BrainVectors coordinator.
Nicole Déglon, Head of the Center for Neuroscience Research, Lausanne University Hospital.
Renaud Du Pasquier, Head of the Neurology Service, Lausanne University Hospital.

Session 1: ***Neuroprotective gene therapy for neurodegenerative diseases*** Chairman: Marc Levivier
14:00-14:45 **Mart Saarma**, Helsinki (FI), ***Potential of neurotrophic factors for Parkinson's disease***
14:45-15:30 **Krys Bankiewicz**, San Francisco (CA-USA), ***AAV-mediated neurotrophic factor gene delivery : updates of clinical trials for Parkinson's and Alzheimer diseases.***
Coffee break
16:00 -16:45 **Stéphane Palfi,** Paris (FR), ***The place of gene therapy for Parkinson's disease among existing treatments: history of the ProSavin clinical trial .***
16:45-17:15 **Pavlina Konstantinova**, Amsterdam (NL), ***Public acceptance of Gene therapy products: risk:benefit ratio.***
17:15-18:30 **Round table:** ***The future of neurotrophic gene therapy for Parkinson's disease.***
Mart Saarma, Helsinki (FI), **Krystof Bankiewicz,** San Francisco (CA-USA), **Harald Petry,** Amsterdam (NL), **Stéphane Palfi,** Paris (FR)**, José-Luis Lanciego, Pamplona** (SP).
Moderator: **Liliane Tenenbaum**.

September 13th

Session 2: ***Optimization of neurotrophic factor gene delivery*** Chairmen: Mart Saarma and Sebastian Kügler

9:00-9:30 **Tomas Gonzales-Hernandez**, Tenerife (SP) ***Dose- and time-dependent effects of GDNF on the rat mesostriatal system: a rationale for controlling GDNF release.***
9:30 -10:00 **Sebastian Kügler**, Göttingen (D) ***Discontinuous GDNF delivery by GeneSwitch vectors***
10:00-10:30 **Mikko Airavaara**, Helsinki (FI) ***Will new neurotrophic factors reveal the same limitations? The example of CDNF.***
Coffee break
Session 3: ***The BrainVectors industry-academia joint program*** Chairmen: Eric Kremer and Otto Merten
11:00– 11:15 **Liliane Tenenbaum,** Lausanne (CH) ***Presentation of the BrainVectors consortium***
11:15-11:45 **Eric Kremer**, Montpellier (F) ***Why a dog virus could be a neurobiologist's best friend?***
11:45 -12:15 **Cecilia Lundberg**, Lund (S) ***Post-transcriptional regulation of transgene expression***
12:15-12:45 **Atze Das**, Amsterdam (NL) ***Tet systems for doxycycline-controlled gene expression***
13:00-14:30 *Lunch*
Short communication by BrainVectors fellows
14:30 -14:45 **Marie Humbert-Claude**, Lausanne (CH) ***Pharmacological control of GDNF biological effects in the brain by clinically-acceptable dox doses using a sensitive inducible AAV vector.***
14:45 -15:00 **Diego Pignataro**, Pamplona, (SP) ***The development of new molecular tools for AAV-mediated CNS transduction.***
15:00-15:15: **Shelby Shrigley,** Lund (SE) ***Inducible and cell-specific expression of GDNF using lentiviral vectors.***
15:30-17:00 **Poster session** + *coffee*
18:30 – 22:00 *Social event (restricted to invited speakers).*

September 14th

Session 4: ***Preclinical evaluation of cellular and molecular therapies in the CNS .*** Chairperson: Liliane Tenenbaum
9:30– 10:00 **José-Luis Lanciego**, Pamplona (SP) ***Gene therapy approaches for the treatment of Gaucher-related synucleinopathies like Parkinson's disease and dementia with Lewy bodies.***
10:00-10:30 **Jocelyne Bloch**, Lausanne (CH) ***Preclinical evaluation of adult brain cell autotransplantation in the MPTP non-human primate model.***
Coffee break
11:00-11:30 **Nicole Déglon,** Lausanne (CH) ***Molecular therapies for Huntington's disease.***
11:30-12:00 **Bernard Schneider,** Lausanne (CH) ***Gene therapy for amyotrophic lateral sclerosis: the importance of non-cell autonomous disease mechanisms.***
12:00-12 :30 **Nicolas Serratrice,** Lausanne (CH) ***Evaluation of helper-dependent canine adenovirus vectors in nonhuman primate brain.***
12:30-12 :40 **Nicole Déglon,** Lausanne (CH) ***Presentation of the Swiss Transmed gene therapy network.***
Lunch 12:45-14:00
Session 5: ***Gene therapy clinical trials setup and monitoring*** Chairmen: **Hueseyin Firat** and **Otto Merten**
14:00-14:30 **Ana Filipa Rodrigues**, Oeiras (PT**)** ***Impact of the cell source on the production of clinical-grade vectors: cGMP considerations and cell line engineering perspectives*.**
14:30-15:00 **Saliha Moussaoui**, Huningue (F) ***Peripheral biomarkers of dementia of Alzheimer and Parkinson types.***
15:00 – 15:30 **Marie-Lise Gougeon ,** Paris (F) ***Immune response after rAAV2/5-hNAGLU injection in the brain of children with Sanfilippo type B syndrome.***
15:30-16:45. **Round table:** ***Final conclusions and future directions of brain gene therapy: expectations and limitation issues***
Krystof Bankiewicz, Hueseyin Firat, Nicole Déglon, Marie-Lise Gougeon and **Marc Levivier.**
Moderator: **Pavlina Konstantinova.**

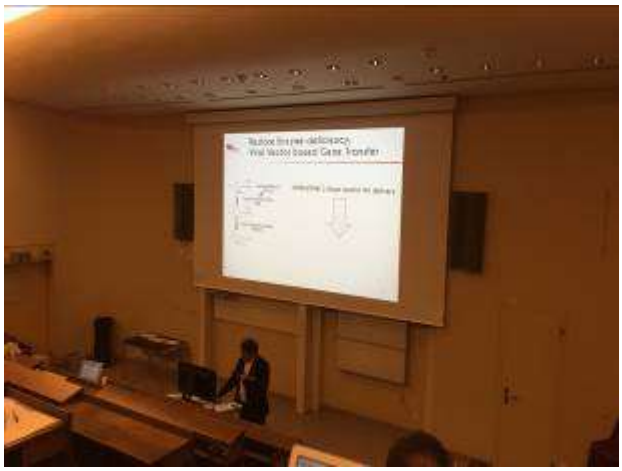
Introduction

Glial cell line-derived neurotrophic factor (GDNF) and Neurturin (NRTN) were shown to protect nigro-striatal dopaminergic neurons and reduce motor symptoms when applied terminally in toxin-induced Parkinson's disease models. However clinical trials based on intraputaminal GDNF protein administration or adeno-associated virus (AAV)-mediated NRTN gene delivery have been disappointing.

During the workshop, several factors that could have limited the clinical benefits were discussed.

On-going gene therapy clinical trials for Parkinson's disease (PD)

Prof. Krys Bankiewicz and Prof. Stéphane Palfi described 2 pioneer clinical trials for PD: GDNF delivered by a AAV vector and the 3 enzymes of the dopamine biosynthesis delivered by a lentiviral vector. Both discussed the issue of the disease state at the time of enrolment: only very advanced patients could be involved. This fact seems to have a negative impact on the outcome of the trial because it is highly probable that at this advanced state of diseases the gene therapy treatment could not alleviate the already existing damage. Furthermore Prof. Krys Bankiewicz indicated that all growth factor based gene therapy trials failed mainly because the present animal models do not really reflect the natural disease (PD) in humans. Open questions are also: vector delivery, patient selection, and axonal transport. Prof. Stéphane Palfi reported on the optimisation of the lentiviral vector construct which will be evaluated in a new clinical trial in France and the UK.



Dr Pavlina Konstantinova, discussed the role of the industry and showed the involvement of uniQure in another clinical trial for a genetic disease affecting the central nervous system using AAV5 vectors: San Filippo. In this clinical trial the neurocognitive development is improved, however, it is evident that the treatment has to be done as soon as possible for avoiding advanced damage of the brain tissue. Another project of uniQure is the development of an AAV5 based gene therapy of Huntington's Disease, which is based on a gene silencing approach. For the moment there are no data available.

Prof. Marie-Lise Gougeon showed the first data of the immunological analysis in the San Filippo clinical trial. These data are crucial for the establishment of the safety of AAV5 vectors for brain diseases.

Dr Ana-Filippa Rodrigues (IBET) presented issues related to the development of gene therapy products: in particular, the importance of the producer cell line for the quality of viral preparations.

Dr Saliha Moussaoui discussed the importance of the assessment of disease diagnosis and progression using biomarkers, in particular for Alzheimer disease.

Mechanism of action of neurotrophic factors (NF)

Prof. Mart Saarma summarized the history of NFs and their mechanism of action via a receptor of the trk family (NGF, BDNF and neurotrophins) or of the GFR-□ family (GDNF, NRTN, Artemin and Persephin). He then described 2 new NFs isolated in his laboratory which act through a different mechanism: CDNF and MANF. These factors lack a secretory signal and are secreted in response to endoplasmic reticulum stress (induced e.g. by alpha-synuclein). In a MPTP induced PD model (in macaques) CDNF improves the overall wellbeing of the animals. CDNF is involved in the protection of neurons and regeneration of axons of remaining neurons. Furthermore, it downregulates the activation of unfolded protein stress. A phase I/II clinical trial is planned to be done in Scandinavia. Dr Mikko Airavaara further discussed the evaluation of CDNF in rodent models of PD.

Regarding GDNF, in addition to its anti-apoptotic and neurotrophic properties, it also interferes with DA homeostasis via time and dose-dependent effects such as: stimulation of DA neuron excitability, inhibition of DAT activity, tyrosine hydroxylase (TH) phosphorylation and inhibition of TH transcription. Depending on the delivery parameters, the net result of this intricate network of regulations could be either beneficial or deleterious. Prof. T. Gonzalez-Hernandez, using a tetracycline-inducible AAV vector developed in L. Tenenbaum's group, showed that enhancement of dopaminergic activity appears early and at low GDNF doses, whereas compensatory effects appear at long-term and high doses. These data are in favour of regulating the expression of GDNF in particular and NFs in general.

Dr Sebastian Kugler, Prof. Cecilia Lundberg and Dr Marie Humbert-Claude presented 3 different regulated vectors allowing to control GDNF expression at the transcriptional or post-transcriptional level in response to clinically-acceptable antibiotic treatments. They discussed data in rodent models which confirm than long-term uninterrupted GDNF treatment can be deleterious and does not provide more protection than short-term treatment. Dr Atze Das, described useful new mutants of the tetracycline-inducible system, and their respective usefulness for transient and stable transfections and in animals.

Dr Jose Luis Lanciego and Prof. Bankiewicz discussed different modalities used in the MPTP macaque model and their relevance for modelling Parkinson's disease (PD). As alternatives to toxin-induced models, local transgenesis using viral vectors to express mutated genes isolated in familial cases of PD exist. Several groups use AAV vectors to locally deliver alpha-synuclein (□-syn) cDNA in the adult substantia nigra (SN). Dr Mikko Airavaara reported difficulties in obtaining reproducible PD pathology in the rAAV-mediated alpha-synuclein model and described data obtained using direct injection of □-syn fibrils.

However, the most common mutation in PD is in the LRKK2 gene. LRKK2 is a very large protein and its cDNA cannot possibly be cloned in AAV or LV vectors. Dr Eric Kremer described another family of viral vectors, the canine adenoviral vectors (CAV) which can afford very large inserts and contrarily to human adenoviruses are not immunogenic. These could be particularly useful to modelize PD via local virally-mediated transgenesis (e.g. using LRKK2) as well as to study brain connectivity.

Other therapeutic approaches for neurological diseases with a genetic etiology.

To open a debate on the relevance of using NFs, paradigms based on the correction of mutations in other neurological diseases with a genetic etiology were discussed. These could be applied to genetic forms of PD or to correct aberrantly expressed factors (e.g. wt □-syn is toxic when overexpressed). Prof. Nicole Deglon presented data on huntingtin silencing. She discussed the importance of allele-specific silencing and presented strategies to achieve this goal. Dr Bernard Schneider presented data on SOD silencing in amyotrophic lateral sclerosis. Targeting separately neurons and astrocytes suggests that, beside neuronal degeneration, astrocytes play an important, direct or indirect, role in the pathology. Dr Nicolas Serratrice presented data from the group of Dr Eric Kremer on overexpression of the GUSB gene, which is deficient in mucopolysaccharosidosis type VII using a CAV2 vector in a dog model.

Finally, PD can also be approached by cellular replacement therapy. Dr Jocelyne Bloch presented data on auto-transplantation of neural stem cells. The advantage of these cells is that they become precursor cells when put into culture. They are useful for autologous transplantation. The use of cells from the patient himself could overcome the limitation of immune rejection.

Round tables

During the first round table, moderated by Dr Tenenbaum, Prof. Bankiewicz stated that to his opinion, the main reason for the failure of the AAV-NRTN trial was the poor coverage of the targeted structure, the putamen. He described his data on monkeys treated by the MPTP toxin which recapitulate the degeneration scheme: neurons terminals degeneration preceeding cell bodies loss. Retrograde transport of GDNF/NRTN to the dopaminergic neurons soma is thought to be necessary for NRTN/ GFR-□/Ret signaling mediating the pro-survival effect. Therefore, the feasibility of treating advanced patients with NFs is questioned by recent data showing that : i) TH-positive putaminal innervation has almost completely disappeared at 5 years post-diagnosis and ii) in patients enrolled in the AAV-NRTN trial more than 5 years post-diagnosis, NRTN was almost not transported to the SN pars compacta. However, in K. Bankiewicz pre-clinical study in MPTP monkeys, TH+ neurons could be preserved to some extent, presumably due to anterograde transport of GDNF.

Prof M Saarma insisted on the importance to deliver GDNF from the right cells (GDNF is naturally secreted by parvalbumin-positive interneurons) and at a physiological dose.

All participants agreed that enrolling patients at earlier stages will considerably increase the chances to demonstrate clinical benefits. Although, the mechanism of the disease onset (starting from the neuron terminals or from the cell soma) is still not completely elucidated. Indeed, neuroprotective approaches should take into account J. Kordower's study showing that the terminals disappeared in patients at 4 years post-diagnosis. Jose Lanciego stated that, in the MPTP macaque model, when clinical

symptoms appear, it is too late for neuroprotection. Mart Saarma confirmed that in his opinion, if innervation is lost, NFs will not work.

Harald Petry discussed the fact that whereas for genetic diseases it is easy to convince of the relevance of performing gene therapy clinical trials, it is much more complicated to defend it in cases of diseases that are not caused by a mutation.

Disease modelling is also primordial to obtain relevant pre-clinical data. Jose Lanciego commented that MPTP-induced neurodegeneration is not PD. Concerning genetic models, Mart Saarma commented that the virally-induced ?-syn models are difficult to use.

In the second round table, moderated by Dr Bas Blits (uniQure) participants summarized the discussions and concluded that:

New clinical studies in less advanced patients are warranted to evaluate the potential of AAV-mediated NF gene delivery in PD. These will be facilitated by the growing evidence that rAAV administration into the human brain is safe. Neurosurgical techniques allowing a widespread distribution of viral particles are requested to optimize clinical benefits.

Further unraveling of the mechanism of action of GDNF gene delivery in relevant animal models is still needed to optimize the clinical benefits of this new therapeutic approach. Recent developments have generated regulated viral vectors which allow to finely adjust GDNF dose and period of administration.

Poster session

Posters covered most aspects of the workshop:

- Methods for producing large-scale AAV batches: Daniel Blessing.
- Pre-clinical assessment of AAV vectors in NHP brain: Bas Blits
- Design of brain cell type specific AAV vectors: Diego Pignataro, Charlotte Jolle and Juan Gerez
- Modelling PD using AAV-mediated alpha-synuclein delivery in the rodent brain: Juan Gerez
- RNA interference for huntingtin silencing. Mergim Ramosaj.
- Pre-clinical gene therapy for MPSVII using a CAV2 vector: Nicolas Serratrice
- Regulation of NF gene delivery: Naika Prince, Luis Quintino, Marie Humbert-Claude.
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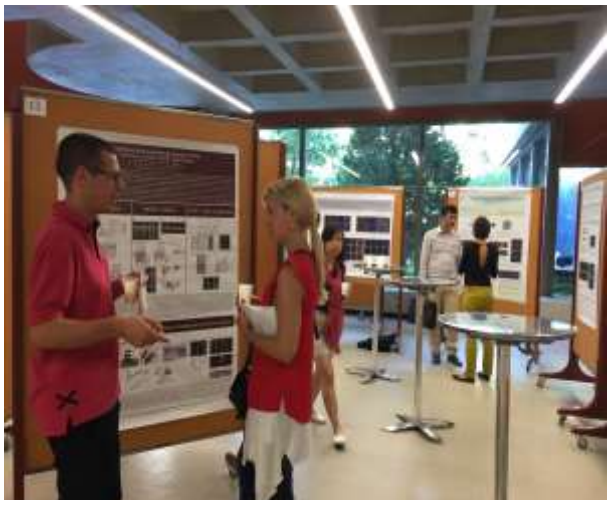

Other themes not directly related to the workshop's discussions but of general interest for developing novel therapies:

- Mechanism of Tau pathology in Alzheimer disease. Kevin Richetin.
- Role of regulation of neuroinflammation in PD: Marie Humbert-Claude.
- Telomere-related processes: Georgia Zanetti and Isabella Saggio.

Networks

This workshop was organized in the context of the last meeting of the BrainVectors project, under the auspices of the FP7 Marie Curie IAPP program.

Liliane Tenenbaum presented the structure of this network, its research goals and achievements as well as the training activities. All partners were present and discussed data generated during the project and further collaborations. The input of external experts fostered these discussions

Nicole Deglon presented another national (Swiss) network on gene therapy for neurological diseases financed by the Swiss TransMed initiative.

Social event

In the evening of the second day a social event was organized which consisted on an evening ship trip on the Lake Geneva. The ship was the entirely renovated 'La Suisse' and brought the group from Lausanne-Ouchy to Montreux and back with marvelous views on both sides of the lake side. On the way back a dinner was served. Everybody was in good mood allowing a lot of scientific but also non-scientific discussions.

Publication related to the workshop and acknowledging the Marie Curie IAPP contract n° 286071 and the Swiss foundation FNS:

Glial cell line-Derived Neurotrophic Factor gene delivery in Parkinson's disease: a delicate balance between neuroprotection, trophic effects and unwanted compensatory mechanisms. Mini-review by Tenenbaum L. and Humbert-Claude M. in**:** ***Frontiers in Neuroanatomy***, 2017, 11:29. doi: 10.3389/fnana.2017.00029 Editor: Jose-Luis Lanciego; Topic: Gene Therapy for Parkinson's disease. *(download the pdf)*