

Executive summary:

Neurodegenerative disorders like Alzheimer's and Parkinson's disease are affecting substantial cohorts of patients, with incidences dramatically increasing in the elderly societies of Western Europe, as ageing is the major risk factor for these otherwise idiopathic disorders. No curative therapies are currently available despite decades of intense research, and even symptomatic treatments are effective only temporally.

Gene therapy is an approach to restore function of diseases organs, e.g. the brain, by means of transferring genetic information specifically into the affected cells and tissues. This genetic information may encode proteins which can restore or protect functionality, e.g. neurotrophic factors which support neurons to function properly and to survive critical situations, or enzymes which help neurons to synthesize essential neurotransmitters needed to maintain critical communication pathways. In its current setting gene therapy is an irreversible process, as the transferred genetic information cannot be withdrawn in case of unforeseen problems, as it is possible with pharmaceutical or physical therapies. This issue can be regarded as a two edged sword: on the one hand gene therapy ensures that a potentially curative molecule can act in the diseased cells and tissues for a long time, but on the other hand this irreversibility may eventually turn into severe problems in case that unwanted side-effects occur.

The major tools for gene therapy are engineered viruses, as it can be regarded the "raison d'être" of viruses to efficiently transport genetic information into their target cells. During the last two decades these recombinant viruses ("viral vectors") have been developed into safe and efficient tools for both, basic research and for some therapeutic applications. However, substantial technical and ethical hurdles still need to be overcome in order to let gene therapy fulfil its promises in larger cohorts of patients.

The NEUGENE consortium was founded to overcome currently existing limitations of gene transfer vectors for safe and efficient gene therapy of major neurodegenerative disorders. The consortium used Parkinson's disease as a model, but the advanced tools developed are thought to be of paramount importance for development of gene therapeutic strategies for other brain diseases as well. Major objectives were:

- 1) the targeting of viral vectors to specific cell types of the brain, in order to be able to restrict expression of therapeutic molecules to either neurons or glia (which actively supports neuronal maintenance and thus may be a novel target for forthcoming therapies), or even to specific subtypes of neurons,
- 2) the regulation of transgene expression, i.e. the implementation of "switches" which can turn-on or -off the therapeutic molecule
- 3) the safety of the advanced viral vectors
- 4) and finally the efficacy of the advanced tools in standardized rodent models of Parkinson's disease.

Most (but not all) of our attempts have resulted in successful development of viral vectors with characteristics superior over previously existing material. Even though the NEUGENE consortium was mainly dedicated to basic research and not to the direct clinical application of advanced viral vector tools, the substantial success in pre-clinical evaluation has already allowed to enter the first recombinant viruses aiming to restore neurotransmitter synthesis into a clinical development program for treatment of Parkinson's patients.

Summary description of the project context and the main objectives.

According to the “Consensus document on European brain research” (*Olesen et al, J Neurol Neurosurg Psychiatry 77, 2006*) the socio-economic burden of diseases affecting the human brain is estimated to constitute 35% of all EU disease burden. Demographic changes in ageing societies of the EU will increase this rate considerably and this will represent a crucial challenge to forthcoming generations. Fortunately, enormous progress has been made during the last decade in understanding mechanisms and principles of both normal brain function and CNS disorders. This increase in knowledge makes new treatments for nervous system diseases realistic. Unfortunately, current pharmacological treatment strategies for neurodegenerative diseases are at best partially effective. Typically, they offer only some symptomatic relief. Generally, they are not able to affect disease progression. Furthermore, pharmacotherapies are limited by restrictions set by the blood-brain-barrier and by their relatively untargeted mode of action. As a consequence, they often provoke side effects, especially following long-term treatment. Thus, the targeted delivery of protective, or even curative genes to specific populations of disease-affected brain cells is an exciting future alternative. This approach may offer significant relief or even cure to presently untreatable or only symptomatically treatable brain diseases. Several proteins and regulatory RNAs involved in normal brain function and disease progression have been identified or will be identified in the near future. Targeting these molecules directly rather than developing drugs that modulate them may drastically reduce cost and time efforts in developing medications as compared to classical pharmacology. Ultimately, targeted and tightly controlled delivery of genetic medicines may open up venues for treatment strategies taking into account specific personal requirements of individual patients.

The objectives of the NEUGENE consortium are thus to develop and to validate the tools necessary for safe, efficient, durable, targeted and regulated expression of therapeutic molecules within the central nervous system (CNS).

The work plan was based on 4 experimental WPs reflecting the most important issues to be addressed in order to develop advanced gene therapy tools for the central nervous system. The additional administrative WP covered project management and dissemination of results:

WP 1: **Targeting** of transgene expression to distinct populations of brain cells

WP 2: **Regulation** of transgene expression in brain cells

WP 3: **Safety / Immunology** of novel vectors and regulated systems

WP 4: **Functional verification** of tools developed in WPs 1 – 3

WP 5: **Management and Dissemination**

In order to ensure **safety and longevity** of vectors, the consortium focused on both LV and AAV vectors, which have already demonstrated the ability to mediate long-lasting and stable transgene expression in CNS. These vectors were significantly improved through work described in the respective WPs, resulting in gene therapy vectors applicable to CNS disorders as defined by the call.

WP 1: Targeting, aimed to target gene transfer vectors and transgene expression to specific cell types. This will allow the expression of therapeutic proteins or regulatory RNAs to be restricted to the neuronal or glial cell populations which are most affected by

a given disease. Alternatively, targeting expression to a supporting cell type to serve as a source of the therapeutic molecules may be an approach for some CNS diseases. Design of targeted vectors made use of the different tropisms of various capsids/coats of AAV and LV vectors and also of transcriptional control elements such as promoters and miRNA binding sites. We anticipate that cell type specific and highly efficient transgene expression will open up novel treatment strategies for CNS disorders, with a significantly reduced risk of unwanted side effects from expression in non target cell types.

WP 2: Regulation, aimed to implement tightly regulated transgene expression systems, which will allow the regulation of therapeutic transgene expression according to the needs of individual patients. Different principles will be evaluated, based on established or emerging protein domain based systems, but also on novel strategies such as RNA aptamer based regulation. Both episomal and integrating vector systems were considered. Furthermore, based on WP1, targeted regulation of transgene expression was evaluated in different populations of brain cells. Regulation of expression of potential therapeutic transgenes with relevance to the animal model of disease used in WP 4 were also evaluated, to accelerate the translation of “basic science” into pre-clinical functional studies. As such the regulated systems were evaluated for tightly controlled expression of secreted neurotrophic factors such as GDNF, intracellular neurotransmitter synthesizing enzymes and for RNA-interference based approaches. Overall we expect to develop tightly regulated transgene expression systems which would be of clinical relevance for a wide variety of CNS diseases. Due to outstanding relevance and multiplicity of experimental approaches, this WP was headed by two participants.

WP 3: Safety, aimed to critically evaluate the safety of novel gene therapy tools in terms of potential side-effects. Special attention was given to monitoring CNS immune reactions to the various viral vector systems under evaluation and also the components of the regulated expression systems. The impact of both pre-immunization and pre-existing brain lesions on vector safety was evaluated. We expect to be able to optimise gene transfer tools which are not only effective and tightly controlled but also have a proven safety profile. Understanding a possible immune response will also allow the use of immunomodulatory regimes.

WP 4: Functional verification, aimed to demonstrate proof-of-principle that the advanced gene therapy tools developed by this program can be used in a therapeutic setting to treat a major neurodegenerative disease. After choosing the most suitable vector systems from WPs 1-3, an animal model of Parkinson’s disease was used to functionally verify and to compare the efficacy of several possible treatment regimes for PD. We were able to demonstrate that the novel vector tools developed by this program will be suitable for use in forthcoming clinical trials for the treatment of PD and other major brain diseases.

WP 5: Management and Dissemination, ensured efficient administrative and scientific project management, including supervision of the project progress, contract management, preparation of reports, organization of meetings, set-up and maintenance of a project website. Scientific management focused on identification of developments which passed the defined milestones and selection of tools best suited to fulfil the stated goals.

Work packages 1 – 3 covered the main technical developments and ran in parallel, but were highly interactive. For example, regulated systems were targeted to either neuronal or glial cell types in the brain, to evaluate whether expression kinetics may vary significantly in different types of brain cells. Along with these investigations we elucidated whether different types of vectors, transducing different populations of neurons or glial cells provoked immunological consequences depending on the transduced cell type and on vector dosages and whether these consequences depended on vector capsids or components of regulated systems. Whenever possible, potential therapeutic transgenes for PD, in addition to reporter genes, were evaluated when developing the tools described in WPs 1-3, in order to allow for timely translation of technical advances into applicable therapeutic strategies in the animal model used in WP4. For example, vectors for evaluation of regulated systems were constructed to express GDNF or TH/GCH1 transgenes, thus were suitable to restore neurotrophin levels or neurotransmitter levels.

Results obtained from WPs 1 – 3 determined the strategy for WP 4, which functionally verified the most suitable vector tools in a relevant animal model of PD. Here we performed a side-by-side comparison of different treatment regimes for PD by making use of the most advanced vector systems we have developed. We evaluated whether i) neurotrophin expression is more effective from glial cells as compared to neuronal expression, ii) short-term or intermittent neurotrophin expression is equally or better suited to reverse degeneration compared to constitutive expression, iii) regulated expression of dopamine synthesizing enzymes can reverse PD phenotype in a late stage model of the disease.

Main S & T results/foregrounds.

The Neugene consortium developed a multitude of novel viral vector constructs, several of which were successfully evaluated in a pre-clinical animal model of Parkinson's disease. A detailed description of all achieved S&T results is given below along the respective deliverables as specified in the Technical Annex of the grant application and as reported in the 1st and 2nd Periodic Reports.

Several new techniques could be implemented in the partner's laboratories, such as 2Photon live brain imaging to follow regulated and cell-type specific transgene expression in living animals, novel behavioural tests to monitor animal performance under lesion and / or therapeutic conditions, advanced reporter systems for selection of functional drug/aptamer pairs, or online *in vivo* microdialysis of dopamine and metabolites.

During the first project period the consortium assembled a tremendous number of prototype vector tools. As deduced from presentations at the NEUGENE project meetings and the more detailed reports (see below) almost 100 different vector genomes were constructed, propagated into recombinant viruses and functionally evaluated.

During the 2nd project period functional evaluations of novel vector tools were performed in a standardized animal model of PD, and optimized delivery methods were developed. The majority of proposed approaches proved to be successful, and the most suitable therapeutic concept available today is entering into clinical development already.

Main results achieved are:

Targeting of AAV and LV vectors

Functionality of the brain by no means depends on neurons only, but glial cells and especially astrocytes serve essential roles in maintenance of the CNS. Thus, making brain astrocytes available for CNS gene transfer strategies was one major focus of NEUGENE, which has been fully achieved. Using different strategies for AAV and LV vectors the consortium was successful in developing astrocytes-specific vectors, which are already used for functional studies including gene therapy in animal models. For example, Mokola pseudotyping was used to shift the tropism of lentiviral vectors toward astrocytes in association with a detargeting strategy based on the incorporation of target sequences for the neuron-specific miR124 to effectively eliminate residual expression in neurons post-transcriptionally. To achieve astrocyte-specific RNAi-mediated gene knock-down three methodologies had to be combined, tissue-specific promoters, tetracycline expression system and miR-embedded siRNA expression and the efficacy of the approach was demonstrated *in vivo*.

Transcriptional control was also used to direct transgene expression of mosaic AAV-1/2 and AAV-5 serotypes exclusively to astrocytes. These vector prototypes now offer novel venues for both gene therapy and studies of basic brain functions, and astrocyte-specific regulated vectors have proven excellent functionality in pre-clinical animal models. Moreover, we optimized gene transfer into the main target cell population affected by Parkinson's disease, nigral dopaminergic neurons. Retrograde transduction of

dopaminergic neurons in the substantia nigra after viral injections to the striatum proved most efficient for AAV6, followed by AAV9 and AAV8, and the combination of AAV serotype 6 with AAV9 opened up the possibility for repeated delivery of the same or sequential delivery of two therapeutic transgenes to the Substantia nigra. Evaluation of several neuronal subtype-specific promoters allowed for targeting of transgene expression to neuronal subtypes or specific disease-affected neurons by LV vectors: these vectors provide safe tools, that are more selective than others available, for the administration of therapeutic molecules in the CNS. Our studies did also prove that non-integrating LV can be generated with different pseudotypes and promoters and that these vectors show the same transduction properties as integrating LV. Thus, superior tools which lack the risk of insertional mutagenesis are now available for CNS gene therapy approaches.

Regulation of transgene expression

We follow two different strategies for development of systems capable of controlling transgene expression levels in the brain: regulatory protein-based and RNA aptamer-based. The RNA-aptamer-based strategy is completely novel and represents a typical high risk / high gain project. Considerable progress has been made in assembling the assay system necessary for identification of suitable regulatory RNA aptamers, and the principle to generate specific aptamers was proven. However, the generated libraries were not sufficiently complex to allow for further in vivo testing yet. This work is ongoing, but for the time being we have not met this objective.

In contrast, two different regulatory protein-based strategies have been tested successfully in vivo. Both regulated systems under development towards clinical applicability have demonstrated excellent manageability. They are based on two different principles: i) fusion of destabilizing domains (DD) to proteins involved in DOPA synthesis (TH and GCH1), expression levels of which are regulated by binding of the ligand trimethoprim (TMP) to the DD. This vector system aims at restoring DOPA in striatal neurons in late PD patients. ii) Expression of the neurotrophin GDNF in either neurons or glial cells, where the expression level is regulated by binding of the clinically approved drug mifepristone (Mfp) to the regulatory pSwitch protein, which controls activity of the minimal promoter driving GDNF expression. Both systems needed a large body of work (and indeed more work than expected) for fine tuning of their respective components to achieve optimal results in pre-clinical animal models. As outlined below, both systems have been functionally tested in relevant pre-clinical animal models of PD.

Safety / Immunology

Interference of the human immune system with viral gene transfer vectors is only poorly understood, as exemplified by recent failures in visceral gene therapy trials. However, especially for AAV based gene therapies its understanding is crucial, since most humans become naturally immunized against AAV-2 during childhood. In order to mimic the human situation as closely as possible, we developed an immunization protocol for rats making use of the antigen which humans encounter, i.e. the wild-type AAV-2 virus. We found that in response to single subcutaneous immunization with wt AAV-2 rats developed neutralizing antibodies against AAV-2 vectors. Furthermore, preimmunization with wt AAV-2 significantly decreased transgene expression in the

brain following intracerebral administration of AAV-2 vector encoding EGFP (75 % reduction in EGFP positive cells in preimmunized versus immune-naïve animals). These results clearly indicate that preexisting immunity to AAV-2 may compromise the efficiency of AAV-2-based vector systems even in immune privileged organs such as brain. Additional induction of a lesion which causes up-regulation of inflammatory markers did not further reduce transduction efficacy, indicating that it was only the specific anti-capsid directed immune reaction precluding successful transgene expression.

Furthermore, we investigated immune issues related to EIAV lentiviral vectors, to which humans are not naturally immuno-competent. Our results demonstrated that CNS application of these vectors is not substantially hindered by peripheral immunity, arguing for a superior safety profile.

During the course of our studies, novel safety aspects arose and were investigated: recently introduced serotypes of AAV (like AAV-6, -8, -9) demonstrate superior transduction efficacy as compared to e.g. the prototype AAV-2, but evidently these vectors also are processed quite differently within transduced cells. To address potential safety issues associated with the use of AAV vectors, we explored virus persistence following injection in the nigrostriatal system. It was shown that AAV vector genomes are detectable extra-nuclearly for long time in CNS neurons and that intact AAV particles persist within the nucleus without disassembly, which might impose an additional safety issue. However, our studies also demonstrated that these particles are unlikely to spread to undesired location upon release from degenerating neurons, suggesting that the novel serotypes of AAV are not *per se* introducing uncontrollable safety risks.

Functional validation

The major goal of the NEUGENE consortium was to evaluate the developed advanced genetic treatment options in a pre-clinical animal of PD. The major results obtained during these studies are:

- An AAV vector was generated to constitutively express optimized levels of DOPA synthesizing TH/GCH1, which successfully restored motor performance in a model of late PD, without induction of any dyskinesia. This tool is in a clinical development program already.
- An AAV vector was generated to constitutively express GDNF in astrocytes, thereby preventing off-target delivery of the neurotrophin without any restriction in therapeutic efficacy. This tool offers novel delivery options for neurotrophic factor-based gene therapy.
- An AAV vector system was generated to express GDNF in a tightly regulated manner, and short-term intermitted induction of GDNF expression provided substantial improvements in motor performance and neuroprotection in a model of early PD. This tool is currently developed further towards clinical applicability.
- A non-integrating LV vector expressing GDNF was generated and provided the same level of neuroprotection as conventional integrating LV vectors. These tools will provide added safety for forthcoming therapeutic strategies using LV vectors.
- Neuronal subpopulation-specific and glia-specific AAV and LV vectors were generated, which are currently exploited to generate advanced animal models of neurodegenerative diseases.

Detailed description of important scientific and technological results:

Note: This section contains partly unpublished results and thus must be treated confidentially! Despite the fact that the runtime of the NEUGENE consortium was prolonged by 6 months, some *in vivo* studies are not yet published due to long observation times necessary to prove long-lasting therapeutic effects. The expected date to have these data published is Jan-2013, but we ask the Commission to verify this fact by communication with the coordinator of NEUGENE before making results publicly available.

"Construction of glial-specific AAV serotypes, *in vitro* and *in vivo* evaluation of vector tropism"

We have evaluated several different serotypes of AAV for their potential to restrict transgene expression exclusively to astrocytes. The hybrid serotype 1/2 was initially chosen as a promising candidate. In combination with a 2.2 kbp sized human GFAP promoter this vector allowed for highly preferential transgene expression in striatal astrocytes *in vivo*. However, the capsid of AAV-1/2 demonstrated instabilities after long-term storage, and the size of the promoter occupied almost half of the capacity of the viral genome, making it not feasible for a regulated vector system. Thus, we tested shorter versions of the GFAP promoter and finally found that a 0.7 kbp sized fragment (called gfab1d) was sufficient to fully restrict transgene expression to astrocytes when incorporated into a serotype-5 AAV vector.

Functional verification of this astrocyte-specific transgene expression was achieved in the MPTP model of Parkinson's disease: astrocyte-specific expression of the neurotrophic factor GDNF protected nigral neurons, their striatal projections and dopamine content against MPTP mediated neurotoxicity as efficient as neuronal expression of GDNF, but with substantially increased safety. The enhanced safety profile of this novel tool for gene therapy for PD was mainly due to the fact that delivery of GDNF to remote sites as seen after neuronal GDNF expression was completely avoided by astrocytic GDNF expression.

" Immunological study on LV vectors"

The goal of this study was to investigate the immunological response of EIAV-based lentiviral vectors and whether the development of an immunological response prevents transgene expression. This study has been completed and therefore met its goals.

In the study adult rats were treated with an EIAV-LacZ vector using different delivery approaches and antibody responses against the vector components were tested in serum samples taken from the study animals. Histology was performed on the animals post mortem and gene expression evaluated.

Animals were treated as shown in the table below:

Group	Test Article	Route of Administration	Dose Level (TU/animal)	Number of animals
1	EIAV-lacZ	i.v.; i.v.; striatal (bilateral)	2.73 x 10 ⁶ /per i.v. injection	3
2	EIAV-lacZ	striatal (left); striatal (right)		3
3	EIAV-lacZ	striatal (bilateral)		3
4	EIAV-lacZ	striatal (bilateral); i.v.; i.v.	1.71 x 10 ⁶ /per striatal injection	3
5	EIAV-lacZ	Striatal (left); i.v.; i.v.; striatal (right)		3

Summary of Results

Antibody responses against the VSV envelope glycoprotein (VSV-G) were observed in most animals that received repeated i.v. administration of vector (see below for details). Virtually no antibodies against any other component of the EIAV vector were detected.

In group 1, two out of three animals that were treated i.v. with EIAV-LacZ prior to intra-striatal administration developed antibody responses to the VSV-G envelope protein. Both of these animals demonstrated lower levels of transduction in the striatum (by histology) than the single animal that did not develop an immune response, however, positive cells were still observed indicating that the presence of an immune response against VSV-G did not prevent EIAV vector transduction.

In groups 2 and 3, no animals developed antibody responses to the vector, indicating that an intrastriatal injection is less likely to induce an immune response. In both groups, LacZ-positive cells were observed in the striata of all animals, indicating gene transfer.

In group 4, all animals developed antibodies against VSV-G after i.v. injection (but not after the initial intrastriatal administration), however gene transfer was observed in all animals, indicating that induction of an immune response by i.v. administration after initial striatal transduction did not compromise gene expression.

In group 5, all animals developed antibodies against VSV-G following i.v. delivery but in two out of three animals this did not compromise transduction of the right striatum when vector was subsequently delivered.

"Construction and production of LV vectors with the 5'-upstream sequences of neuron-specific genes"

A strong neuronal tropism was observed when VSV-G pseudotyped lentiviral vectors express transgenes under the control of the ubiquitous cytomegalovirus (CMV) or phosphoglycerate kinase 1 promoters (PGK). However, to explore pathophysiological conditions and in particular the selective vulnerability of neurons and contribution of

various cell-types in neurodegenerative diseases, restricting transgene expression to specific sub-populations of neurons is required. Most core promoters available have been identified and characterized on a gene-by-gene basis and a limited number of promoters with regulatory domains spanning a restricted area of the genome suitable in size for a cloning in LV have been characterized. In addition, most tissue-specific promoters have weak transcriptional activities relative to viral (CMV) promoters. In the present study, we have therefore integrated seven pan-neuronal and cell-type specific neuronal promoters in constitutive or tetracycline-regulated LV containing the WPRE element and assessed the tropism and transcriptional activity in rodent CNS. The pan-neuronal promoter NSE and five cell-type specific neuronal promoters (GAD67, Dlx5/6, GluR1, Drd1a, Tac1) were cloned in the SIN-W-AcGFPnuc LV. As control a LV with the housekeeping PGK promoter was used (SIN-W-PGK-AcGFPnuc). All promoters showed a preserved specificity for neuronal expression in the striatum. Confocal analysis and cell counts revealed that more than 95% of transduced cells co-localized with NeuN, whereas only scarce AcGFPnuc-positive cells were double-stained for S100b (Delzor et al. in revision). However, there was substantial diversity of transcriptional activity and cell-type specificity of expression. The promoters with the highest activity were those of the 67 kDa glutamic acid decarboxylase (GAD67), homeobox Dlx5/6, glutamate receptor 1 (GluR1), and preprotachykinin 1 (Tac1) genes. Neuron-specific enolase (NSE) and dopaminergic receptor 1 (Drd1a) promoters showed weak activity, but the integration of an amplification system into the LV overcame this limitation. In the striatum, the expression profiles of Tac1 and Drd1a were not limited to the striatonigral pathway. Finally, regulation of the Dlx5/6 promoter was observed in a disease condition whereas Tac1 activity was unaffected. These vectors provide safe tools, that are more selective than others available, for the administration of therapeutic molecules in the CNS. Nevertheless, additional characterization of regulatory elements in neuronal promoters is still required.

" In vitro analysis of different cell-type specific LV vectors"

We used Mokola pseudotyping to shift the tropism of lentiviral vectors toward astrocytes and a detargeting strategy with miRNA to eliminate residual expression in neuronal cells. In primary cultures, we showed that incorporating target sequences for the neuron-specific miR124 effectively abolished transgene expression in neurons post-transcriptionally. Targeted expression of the LacZ reporter gene in astrocytes was achieved in the hippocampus, striatum, and cerebellum of the adult mouse in vivo. Doubleimmunofluorescence staining confirmed that the miR124T insertion restricted the LacZ transgene expression almost exclusively to astrocytes (89% of S100b-positive cells and 6% of NeuN-positive cells in striatum). So we have demonstrated that insertion of miR124T into a Mokola-pseudotyped vector led to restricted transgene expression in astrocytes. As a proof-of-principle, this new lentiviral vector was used to either overexpress or downregulate (RNA interference) the glial glutamate transporter GLAST into striatal astrocytes in vivo (Colin A, Faideau M, Dufour N, Auregan G, Hassig R, Andrieu T, Brouillet E, Hantraye P, Bonvento G, Déglon N. Engineered lentiviral vector targeting astrocytes in vivo. *Glia*. 2009 Apr 15;57(6):667-79). However, species differences were observed with this system, with an astrocytic tropism in mice and only a partly shift in the tropism in rats. To solve this issue, we assessed the efficacy of a new miR124 target with a perfect complementary for the rat miR-124 sequence. The number of neurons expressing the LacZ transgene was

dramatically decreased in rat cells with a strong colocalization in astrocytes and a very weak neuronal transgene expression (Escartin et al. unpublished data). In the study by Colin et collaborators, we have also shown that we can downregulate a glial gene (glutamate transporter GLAST; not expressed in neurons) into striatal astrocytes in vivo. However, this first generation vector is not suitable for an astrocyte-specific downregulation of an ubiquitous gene. Indeed, due to the maturation/processing of siRNA, the miRNA target sequence is cleaved during the synthesis of the siRNA. In order to solve this issue and be able to selectively silence mutant Htt in astrocytes, we have combined three methodologies, tissue-specific promoters, tetracycline expression system and miR-embedded siRNA expression and demonstrated the efficacy of the approach in vivo (Delzor et al. in Revision).

"In vivo evaluation of AAV serotypes in both SNpc and striatum"

The main goal of D1.6 and D1.7 was the characterization of novel AAV serotypes as vehicles for future therapeutic gene delivery to the nigrostriatal system. The capacity of certain AAV serotypes such as AAV2 to transduce neurons in the Substantia Nigra and in other brain regions has previously been investigated in various animal models, prompting the use of this vector for gene therapy in PD. However, many facets related to the use of AAV vectors remain unexplored, such as the ability of novel serotypes to infect nigral neurons from distal nerve terminals. Thoroughly addressing these questions first in rodent models is needed to expand the arsenal of tools available for therapeutic gene transfer, and possibly restore motor function and oppose neurodegeneration. A need for serotype alternatives to AAV2 and AAV5 also arises from the fact that wild-type AAV2 and AAV5 are widespread in the human population and efficient gene transfer can thus be impaired by pre-existing immunity. In our analysis we focused on AAV serotypes 6, 8 and 9, all of which had been reported to efficiently transduce neurons. AAV6 was selected because of its potential for efficient retrograde axonal transport, and AAV serotypes 8 and 9 were chosen for their reduced risk of eliciting an immune response, since neither one of them binds heparin, and heparin binding has been postulated as a prerequisite for dendritic cell infection and development of persisting immunity.

Experiment 1: Comparison of the efficiency of dopaminergic neuron transduction, cellular tropism and the inflammatory response after injection of AAV serotypes 6, 8 or 9 to the Substantia Nigra.

Result: AAV serotypes 6, 8 and 9 transduce dopaminergic neurons of the Substantia Nigra with equal efficiency when directly injected to the Substantia Nigra. AAV serotypes 6 and 9 exhibit exclusively neuronal transduction in the Substantia Nigra (TH, NeuN co-label), whereas AAV8 transduces oligodendrocytes (Olig-2 positive cells) in addition to neurons. AAV serotypes 6, 8 and 9 do not cause any inflammatory response in the Substantia Nigra when administered at a titer of 1.9×10^{10} vgs, as assessed by the absence of cellular infiltrate (Cresyl violet stain), and the absence of microglial (Iba-1 label) and astrocytic activation (GFAP label).

Experiment 2: Evaluation of the capacity of AAV serotypes 6, 8 and 9 to retrogradely transduce dopaminergic neurons in the Substantia Nigra after injections to the Striatum. Result: AAV serotype 6 (368 ± 54 (SEM) cells) can more efficiently retrogradely transduce nigral dopaminergic neurons than AAV9 (259 ± 28 cells) or AAV8 (98 ± 15 cells). As observed for the Substantia Nigra, AAV6 and AAV9 display pure neuronal

transduction profiles in the Striatum, AAV8 also transduces oligodendrocytes in addition to neurons. No EGFP expressing cells with astrocytic morphology could be detected. Spread of the heparin binding serotype AAV6 was confined to ca. 1 mm around the needle tract. Within this region, fluorescence intensity is significantly higher for AAV6 compared to AAV8 or 9. These two non-heparin binding AAV serotypes, by contrast, spread throughout the entire injected striatum. As a consequence of these different spread characteristics, EGFP-filled axons of medial spinal neurons could be detected throughout the entire pars reticulata of the Substantia Nigra after striatal AAV8 or AAV9 injections, whereas only a distinct portion of the reticulata contained EGFP-filled axons after AAV6 CMV EGFP delivery to the Striatum.

Experiment 3: Evaluation of repeated dopaminergic neuron transduction in the Substantia Nigra: delivery of AAV serotypes 6, 8 and 9 to the Substantia Nigra, followed by striatal injection of AAV6, 4 weeks later

Result: The combination of AAV9 with AAV6 was most efficient for sequential transgene delivery (73 ± 8 cells (SEM)) followed by AAV8 with AAV6 ($47 \text{ cells} \pm 11.2 \text{ cells}$), and turned out to be twice as efficient as repeated delivery with AAV6 (36 ± 5 cells). We thus identified the combination of AAV6 with AAV9 as suitable for repeated administration of the same therapeutic gene or sequential delivery of different therapeutic genes to the Substantia Nigra.

In the frame of this research, we found a significant persistence of viral genomes in the striatum of animals that had been injected in the Substantia Nigra with AAV vectors. This aspect was further investigated and these data are described in the significant results section of Workpackage 3 "Safety/Immunology".

The development of various types of AAV vectors targeting the nigrostriatal system may have future implications in the development of gene therapy for PD. Nevertheless, these vectors have already found applications in the lab for the development of genetic animal models based on alpha-synuclein overexpression and testing of targets for neuroprotection, such as PGC-1alpha (see EPFL publications related to these aspects). This research is relevant for mid-term therapeutic outcomes that aim at slowing down disease progression, and may therefore greatly improve the long-term efficacy of symptomatic treatments including the currently proposed gene therapy applications.

"Efficiency of nigral and striatum expression of non-integrating LV vectors"

The main goal from RHUL's participation in NEUGENE was the *in vivo* assessment of transduction with stereotactically-delivered integration-deficient lentiviral vectors (IDLVs) in the context of Parkinson disease. We used Sprague-Dawley rats for all experiments. The results were always compared with identical lentiviral vectors of standard integrating configuration. PCR-based techniques were used in our studies to monitor vector integration in control and Parkinsonian rat striatum.

We compared the efficiency of nigral and striatal expression of both integrating and non-integrating LV vectors. Three different promoters (CMV, hGFAP and hSYN) were used, each with an *eGFP* transgene. Groups of animals ($n = 3$ per group) were stereotactically injected with either integrating or non-integrating lentiviral vectors to the striatum or substantia nigra pars compacta. Three weeks post-injection, the animals

were perfused and nigral and striatal sections were taken on a vibratome for immunohistological analysis.

For all promoters tested, there were no significant differences in expression between integrating and non-integrating lentivectors. Using the CMV promoter, a mixture of neurons and astrocytes expressed *eGFP*, the hGFAP promoter gave predominantly glial expression and the hSYN promoter conveyed neuronal specificity to *eGFP* expression. hSYN mediated particularly extensive expression. In agreement with previously published studies, transduction of nigral tyrosine hydroxylase (TH)-positive neurons was relatively low, whilst striatal expression remained high.

To demonstrate lack of integration of non-integrating lentiviral vectors *in vivo* we sham-treated or lesioned animals by injection of 6-hydroxydopamine (6-OHDA) in the median forebrain bundle. 2.5 weeks later animals received a unilateral injection of non-integrating or integrating lentivector into the striatum. One week after vector administration the injected striata were harvested for analysis of vector genomic integration by linear amplification-mediated PCR (LAM-PCR), performed in collaboration with the laboratory of Christof von Kalle/Manfred Schmidt (our partners in the PERSIST consortium). The conclusions of this experiment are as follows:

- LAM-PCR analyses of vector integration in the intact and lesioned striata demonstrated only residual levels in non-integrating vector samples.
- Integration analyses of standard integrating vector showed high-level integration and similar patterns in intact and lesioned striata. Integration levels within genes were near random and significantly reduced compared to proliferating tissues, as well as biased against integration in promoter regions. Regarding integration frequencies in repetitive genomic elements, the lesioned striata showed increased integration frequencies in LINES and satellite DNA, but reduced within LTR elements. Integration in SINEs was not affected.

"Efficiency of non-integrating LV vectors with different pseudotypes"

We have compared the efficiency of nigral and striatal expression of both integrating and non-integrating lentiviral vectors with different pseudotypes. Three different pseudotypes (VSV-G, Ross River and Rabies protein G) were used, each with an *eGFP* transgene. Three different promoters were also investigated (CMV, hGFAP and hSYN). Groups of animals ($n = 3$ per group) were stereotactically injected with either integrating or non-integrating lentiviral vectors to the striatum or substantia nigra pars compacta. Three weeks post-injection, the animals were perfused and nigral and striatal sections were taken on a vibratome for immunohistological analysis.

For all pseudotypes tested, there were no significant differences in expression between integrating and non-integrating lentivectors. Striatal injections of Rabies G vectors with CMV promoter resulted in limited nigral transduction, via retrograde transport. Ross River envelope pseudotype depended on promoter for cell-type specificity, expressing in astrocytes if driven by hGFAP and in neurons if under hSYN promoter. Similarly, VSV-G envelope gave either neuronal (CMV or hSYN promoters) or astrocytic expression

(hGFAP promoter). We conclude that the choice of envelope is less determinant for specificity of expression than that of promoter, regardless of integration proficiency.

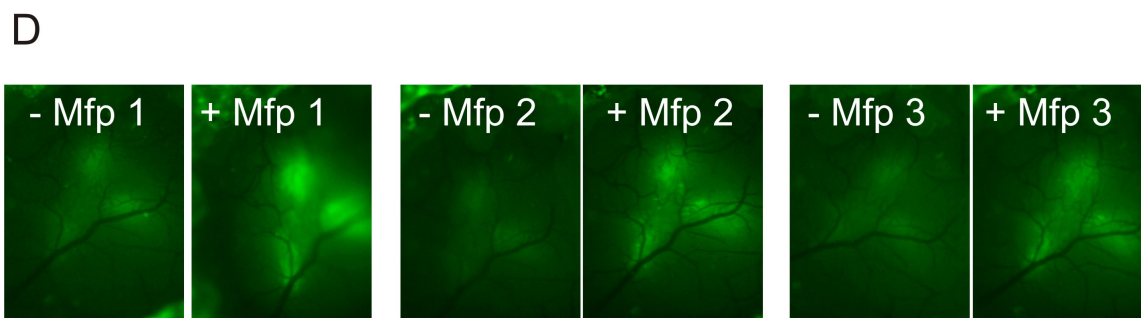
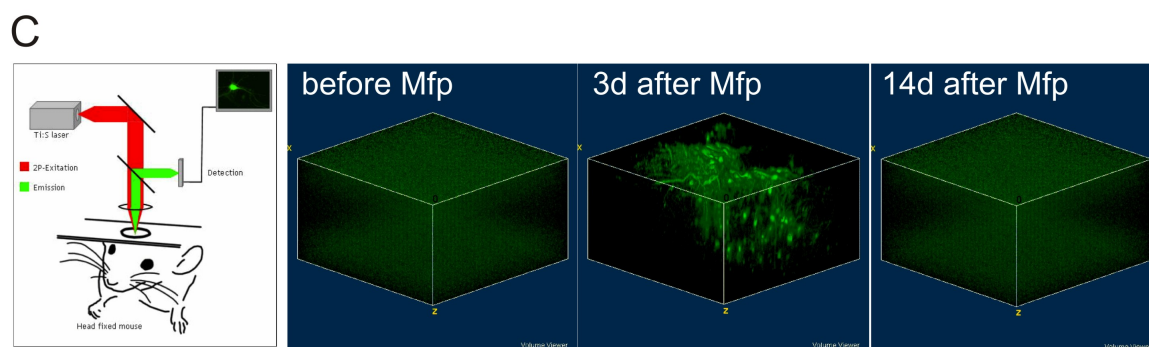
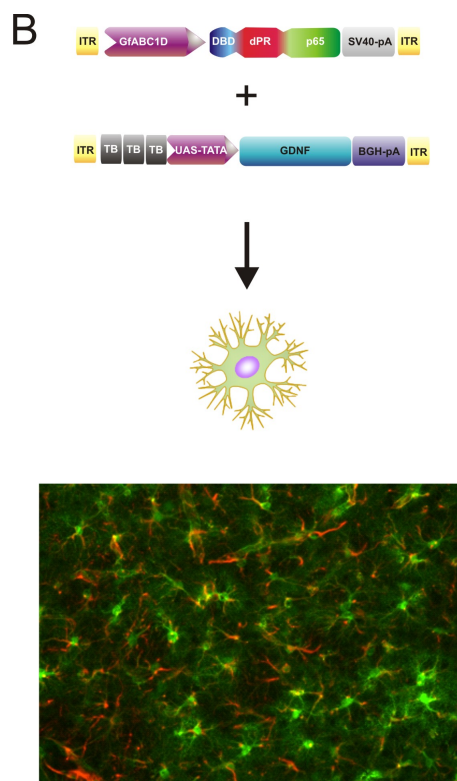
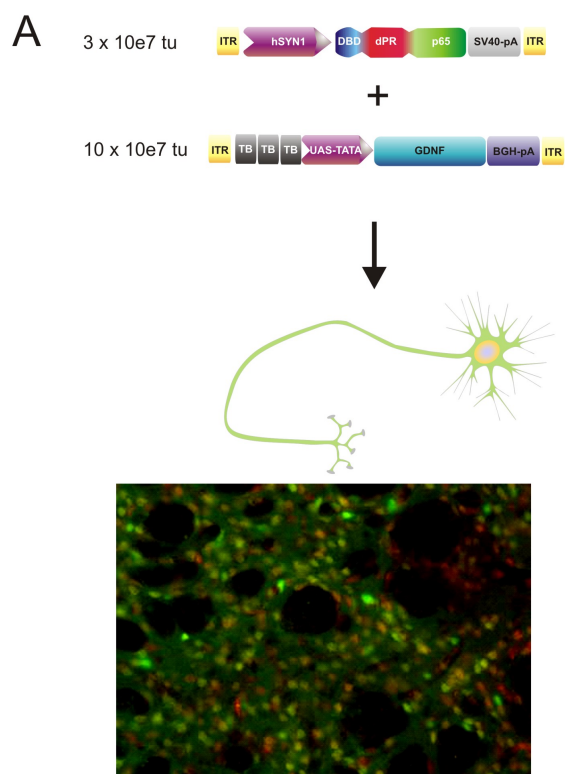
“In vivo validation and analysis of the cell-specificity and level of expression from regulated vectors in normal and lesioned brain”

Mfp-regulated vectors for GDNF expression:

As outlined in more detail in the report for D2.4 the mifepristone (MfP)-regulated GDNF expressing vector system had to be constructed in a two-vector design, in order to allow for negligible background expression of this potent neurotrophin. This configuration (see Fig 1) allowed making use of astrocyte-specific or neuron-specific promoters to drive the pSwitch protein, and by this means we could successfully target transgene expression (EGFP as a fluorescent reporter, or GDNF) to either astrocytes or neurons in an almost mutually exclusive manner.

Application of Mfp at moderate dosages (5 – 20 µg/kg, i.p.) resulted in robust induction of transgene expression in both neurons and astrocytes. For GDNF, levels of induction were 7-10-fold over baseline, as compared to 70-fold over baseline when constitutively expressing vectors were used.

Fig. 1 (next page): Examples for in vivo assessment neuron-specific and astrocyte-specific Mfp-regulated vectors. Vector genomes for neuron-specific (A) and astrocyte-specific (B) expression of transgenes in rat striatum, overlays of EGFP/NeuN or EGFP/GFAP are shown in the striatal sections. In vivo 2-photon microscopy was used to demonstrate that AAV-5 mediated regulated EGFP expression in mouse cortex was background-free and tightly regulated (C), while wide-field fluorescence imaging of AAV-6 mediated Mfp-regulated expression in mouse cortex demonstrated some background in the non-induced state (-MfP), but also demonstrated repeated responsiveness of the regulated system over 3 cycles (D, the same brain area imaged over a period of 3 months with 3 rounds of Mfp application).



While the main emphasis for the Mfp-regulated vector system was on astrocytic targeting and GDNF expression (see also D4.4), a comparative study demonstrating appropriate targeting and regulation is currently being finalized and prepared for publication. No significant differences in background expression, level of induction and necessary Mfp dosage were found in astrocytes versus neurons. (Maddalena A, et al., “A mifepristone-regulated AAV vector system for targeted transgene expression in the CNS”), however, the serotype of AAV vectors used turned out to be very important: AAV-5 allowed for background free expression, while AAV-6 delivered too many genomes per cell and demonstrated considerable background expression (Fig 1 D).

TMP-regulated vectors for TH/GCH1 expression:

Regulated vectors were driven by CBA promoters and intrastriatal injections resulted in transduction of predominantly neurons. Animals were injected with CBA-DD-TH and CBA-GCH1 vectors and received either diluted TMP emulsion (0.5, 1 or 2 mg/ml) or water, and were followed 37 weeks in different behavior tests. A subset of animals were not included in the behavioral assessment but were sacrificed for histology after 6 weeks of TMP administration (0.5 mg/ml). TMP administration resulted in TH positive cells in the striatum, whereas the animal receiving water had limited TH expression. This is an ongoing study and the remaining animals will be killed during May 2012.

“Long-term stability of gene expression from selected non-integrating LV”

Following encouraging results of *eGFP* expression from non-integrating lentivectors at three weeks post-stereotactic injection, we performed two separate sets of experiments:

- A 6-week follow up with non-integrating and integrating *eGFP* lentivectors with various promoters (hSYN, hGFAP, CMV) and pseudotypes (VSV, Rabies, Ross River) in the striatum and substantia nigra. Groups of animals ($n = 3$ per group) were stereotactically injected with either integrating or non-integrating lentiviral vectors to the striatum or substantia nigra pars compacta. Six weeks post-injection, the animals were perfused and nigral and striatal sections were taken on a vibratome for immunohistological analysis.
- An 18-week follow up of *eGFP* expression with a selected four-vector set (integrating and non-integrating, VSV pseudotype with hSYN and hGFAP promoters) following 6-hydroxydopamine lesion. Animals were followed for 18 weeks post-vector injection (16 weeks post-lesion).

The 6-week *eGFP* expression patterns were similar to those described in deliverables D1.8 and D1.9 above for the 3-week follow up. There were no major differences between IDLVs and integrating vectors for *eGFP* expression. Vectors expressed *eGFP* efficiently in striatum and to a lesser extent in the substantia nigra. Promoter and to a lesser extent pseudotype alterations were able to target sub-populations of cells within the rodent basal ganglia. Using the CMV promoter, a mixture of neurons and astrocytes expressed *eGFP*, the hGFAP promoter gave predominantly glial expression and the hSYN promoter conveyed particularly efficient neuronal specificity. VSV-G envelope gave either neuronal (CMV or hSYN promoters) or astrocytic expression (hGFAP promoter). Ross River envelope in conjunction with hGFAP promoter conferred glial specificity, while

with hSYN promoter mediated neuronal expression. Striatal injection of Rabies-CMV vectors resulted in limited nigral transduction, via retrograde transport.

Efficient, very long-term *eGFP* expression from VSV vectors (18 weeks) was not prevented by 6-OHDA lesion two weeks post-vector injection, regardless of vector integration proficiency (Fig. 2). Sets of coronal sections demonstrated *eGFP* expression throughout the vector-injected striatum. Promoter-driven cell-type specificity was demonstrated for both hSYN (neurons) and hGFAP promoters (astrocytes). Quantitative imaging showed that hSYN-driven non-integrating and integrating vectors had high and similar *eGFP* expression levels. hGFAP-mediated expression was somewhat lower, and about 2-fold higher from integrating compared to non-integrating vectors (Fig. 2).

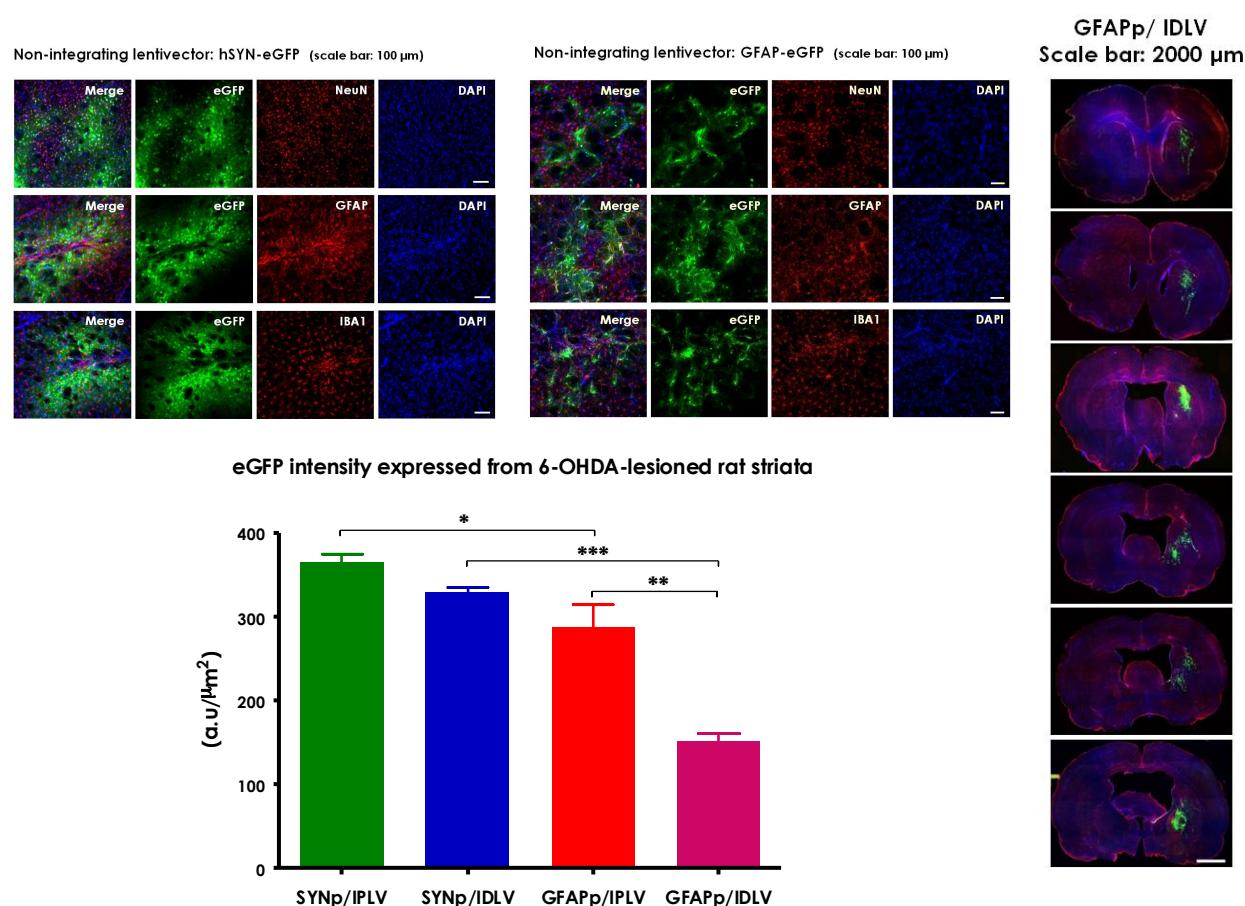


Fig. 2. Effective long-term *eGFP* expression from non-integrating lentivectors in the 6-OHDA lesioned rat. Integrating and non-integrating *eGFP* vectors were injected in the right striatum, and two weeks later animals were lesioned at the same location by 6-OHDA injection. Samples were processed 18 weeks post-vector injection (16 weeks post-lesion). Micrographs illustrate *eGFP* expression from non-integrating lentivectors driven by hSYN (top left panel) and hGFAP (top middle panel), showing effective expression and cell-type specificity (NeuN neurons with hSYN promoter and GFAP astrocytes with hGFAP promoter). The right panel shows a set of coronal sections demonstrating effective *eGFP* expression throughout striatum following non-integrating hGFAP-*eGFP* vector injection. Fluorescence levels in the striatum were quantitated for all vector groups (bottom panel). IPLV: integration-proficient lentivector; IDLV: integration-deficient lentivector.

" In vivo characterization of regulated vectors: Mfp-regulated GDNF expression"

In vitro and in vivo studies clearly demonstrated that the Mfp-regulated system required a two-vector design in order to allow for an off-state which is indeed characterized by negligible GDNF expression. As GDNF is a highly potent neurotrophin, it was considered to be absolutely critical to achieve this "zero-expression" off state, and this goal was achievable only by construction of expression cassettes, which were tailored to express low levels of the Mfp-regulated pSwitch protein. Furthermore, it was necessary to adjust the amount of viruses expressing pSwitch and the responsive GDNF expression cassette to 1 : 3 to achieve both background-free and readily inducible GDNF expression in the striatum of rats. In addition, only AAV vectors which are characterized by only moderate transduction efficacy per cell (AAV-5) were suited to provide background-free expression, while vectors which demonstrate very high transduction efficacy per cell (like AAV-6) accumulated too many vector genomes per neuron or astrocyte to allow for appropriate regulation.

The advantage of the two-vector approach is the easy shift between promoters driving pSwitch expression in either neurons or astrocytes and thereby targeting the GDNF expression also to either one cell type. Using moderate dosages of the inducer mifepristone (Mfp, 10 – 20 µg/kg) we could achieve induction of GDNF expression in both cell types to almost the same extent. Induced GDNF levels reached 7 – 10 fold baseline levels of GDNF in both normal and partially 6-OHDA lesioned rat striatum, which after a single application of Mfp lasted for about 2 – 3 weeks. These levels were considerable lower than those achieved through constitutive GDNF expression (reaching about 50 – 70 fold the baseline level of GDNF), but were sufficient to provide at substantial motor performance recovery in 6-OHDA lesioned rats (see D4.4)

Using EGFP as a reporter gene we exploited both in vivo live imaging and immunohistochemistry to demonstrate that the Mfp-regulated vector system is repeatedly responsive. To this end mice and rats were injected with an optimal dosage of AAV-vectors expressing pSwitch or EGFP into either cortex (for live imaging) or striatum (for immunohistochemistry). Our results demonstrate that repeated cycles of induction can be accomplished (Fig 1 D) making the system suitable for both a single short-term expression or a repeated, intermitted short-term expression of therapeutic transgenes.

" In vivo characterization of regulated vectors: TMP-regulated TH/GCH1 expression"

In vitro characterization of a regulated DOPA producing construct

Destabilizing domains (DD) is a post-translational regulation concept where an instable protein is continuously expressed but readily degraded in the cell due to specific point mutations. These attributes can be transferred to other proteins by creating fusion proteins. The DD, and any protein coupled to it, are rescued in presence of a ligand. The DD version described here utilize a mutant version of dihydrofolate reductase (DHFR) in combination with its high affinity ligand trimethoprim (TMP).

Initial *in vitro* attempts focused on how to regulate DOPA production with the DD concept in terms of placement, i.e. on the N- or C-terminal side of the protein, and to what transgene the DD, i.e. TH or GCH1. HEK cells were transfected with different plasmid combinations of regulated or constitutive active TH and GCH1. Plasmid combinations were compared with or without TMP and with mock-transfected cells or cells transfected plasmids expressing both TH and GCH1 constitutively. Four hours after transfection the culture medium with lipofectamine was changed to either fresh medium or medium with $1\text{E-}5$ M TMP. Twelve hours later a sample of the medium was taken for DOPA quantification with HPLC. In short, coupling the DD to the N-terminal side of the TH gene in combination with constitutively expressing GCH1 gave the most favorable results in terms of low basal DOPA levels and high levels in presence of TMP. This was also true for GCH1, although combining it with a constitutively expressing TH plasmid resulted in high basal DOPA levels. Coupling the DD to the N-terminal side of the protein was also the best choice when regulating both TH and GCH1.

Regulation of DD-TH with TMP in the complete 6-OHDA lesion model

The vector combination where DD was coupled to the N-terminal side of TH combined constitutively expression of GCH1 (DD-TH+GCH1) was tested *in vivo* in the complete 6-OHDA lesion model. The DD-TH+GCH1 group was divided to either receive TMP or water. The regulated vector groups were compared with a group receiving vectors with constitutive expression of both TH and GCH1 (TH+GCH1) and lesion and intact controls. All animals were pre-scored in corridor and stepping test and followed 37 weeks after AAV-treatment. When all groups reached stable performance in the behavior tests at 15 weeks post-AAV, oral administration of TMP was initiated. TMP emulsion was diluted in drinking water with a starting concentration of 0.5 mg/ml, which resulted in a daily average dose of 22.8 ± 2.8 mg TMP/kg. At this time point animals in the TH+GCH1 group performed similar in behavior tests as intact controls. Animals in the DD-TH+GCH1 group showed minor improvement with TMP administration compared the same group receiving only water. The motor performance in the DD-TH+GCH1 group receiving TMP was only slightly increased when the TMP dose in the drinking water was increased to 1 mg/ml and 2 mg/ml (average daily dose 40.9 ± 6.2 and 87.1 ± 14.6 mg TMP/kg, respectively).

"Analysis of the effect of pre-immunization with WT-AAV-2 and brain lesion on transduction efficacy "

Twenty Wistar female rats were immunized with 1×10^9 tu of wild type (wt) AAV-2 in Complete Freund's Adjuvant (CFA) (pre-immunized group). Another 20 rats were immunized with CFA only (immune-naïve group). Three month later, 10 pre-immunized and 10 immune-naïve rats received intrastriatal injection of 6-OHDA ($10\mu\text{g}$, single injection) whereas remaining 10 animals from pre-immunized and immune-naïve groups received vehicle only. One month after the 6-OHDA lesion, all animals were administered with 2×10^8 tu of AAV2-GDNF-EGFP in the lesioned area. Rats were sacrificed 4 weeks after vector injection. Brain tissue and blood were collected at the sacrifice. Blood was also collected two weeks after the vector injection. Transduction efficiency was estimated by quantifying EGFP+ cells in the brain sections using stereology. Number of EGFP+ cells were compared between 4 groups of animals

described above. Two-way ANOVA revealed significant effect of immunization ($F(1,30) = 80.1$; $p < 0.0001$) but no effect of lesion ($F(1,30) = 2.01$; $p = 0.17$) or interaction between immunization and lesion ($F(1,30) = 0.53$; $p = 0.47$).

Brain sections were also stained with the markers of activated microglia (OX-6). In agreement with previous results, there was a significant effect of lesion on the number of activated microglia cell (two-way ANOVA, $F(1,28) = 8.44$; $p = 0.007$). We also observed an effect of immunization on microglia activation ($F(1,30) = 13.5$; $p = 0.001$) but no interaction between immunization and lesion.

Effects of wt AAV-2 immunization and 6-OHDA lesion on the number of transduced EGFP⁺ cells and activated OX6⁺ microglia

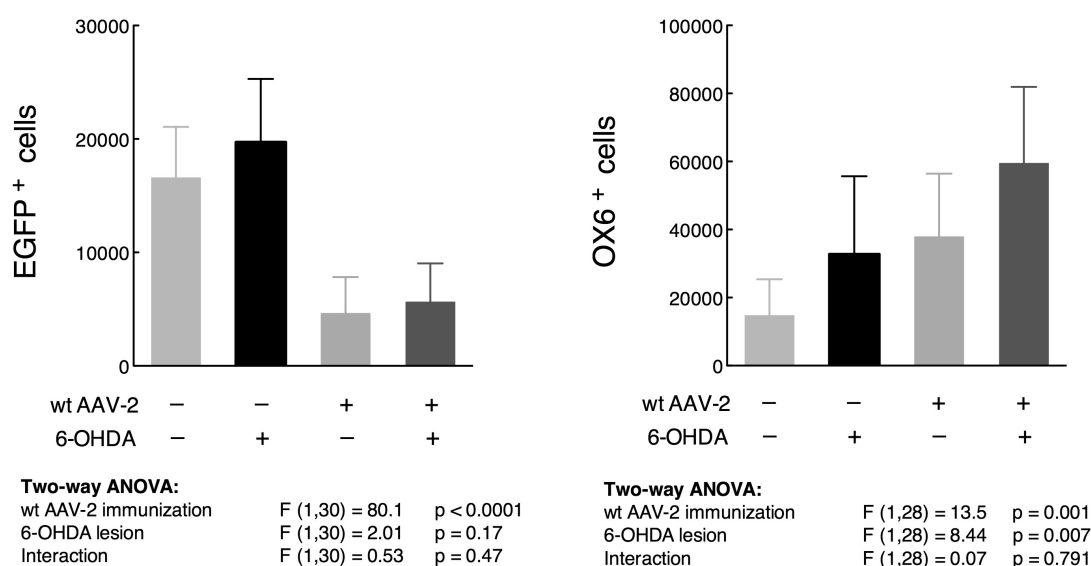


Figure 3: Pre-immunization but not the additional striatal 6-OHDA lesion impair transduction efficacy of AAV vectors

“Evaluation of immune responses to components of inducible vector systems”

At this time point, no adverse effects or immunological reactions have been observed with the destabilizing domain regulated vectors or with oral administration of TMP. This needs to be carefully reviewed in post-mortem analysis of vector-treated animals. The same holds true for the Mfp-regulated vectors for GDNF expression.

“Analysis of the persistence of genome-containing viral elements in the nigrostriatal system following intranigral injection of AAV6, AAV8 and AAV9 vectors”

Four weeks after injection of 1.9×10^{10} genome copies (gc) of AAV6, 8 and 9 to the Substantia Nigra, we could detect by real-time PCR between 0.5 and 1.4% of total amount of injected gc within the Substantia Nigra, and approximately 0.1% within the striatum. Among the remaining viral genome copies, the majority persisted as single-

stranded viral DNA, estimated between 74% and 81% of the gc detectable in the Substantia Nigra, and to an even higher degree in the striatum. The persistence of single-stranded viral DNA was likewise confirmed in the Substantia Nigra by *in situ* hybridization. Finally, intact assembled AAV6 capsids could be detected by immunohistochemistry within the neuronal cell bodies in the Substantia Nigra using a conformation-sensitive antibody. Intact viral AAV6 capsid appeared enriched within the nuclei of nigral neurons. Altogether, these data indicate that there is vector persistence in the nigrostriatal system following nigral injection of viral particles. In particular, the presence of vector genomes in the striatum, a brain structure distal to the site of injection, prompts further study addressing the possible spread of infection that may result from the potential release of particles retaining infectivity.

“Potential spread of persisting intact AAV particles in the course of induced neurodegeneration.”

We designed an experiment to determine if the viral particles persisting in the nigrostriatal system could be released in the course of degeneration and lead to a spread of infection. Animals injected in the Substantia Nigra with EGFP-expressing AAV6, 8 or 9 vectors received four weeks later 6-hydroxydopamine (6-OHDA) injections to the ipsilateral medial forebrain bundle. Six weeks after toxin injection, 6-OHDA had induced near total nigrostriatal degeneration. However, we did not observe any indication of AAV infection spread, as concluded from the lack of novel neuronal transduction in the striatum (no increased density of EGFP-expressing striatal cells). Interestingly, we measured a 69% reduction of the amount of viral genome copies present in the striatum following 6-OHDA-induced degeneration of the nigral dopaminergic neurons, in line with viral genome persistence in dopaminergic axon terminals and elimination of these viral genomes upon nigral neuron degeneration. Therefore, we conclude that persisting AAV particles were either no more infectious, or became cleared in the process of neurodegeneration. The fact that no undesired spread of transduction was observed following degeneration of infected neurons is an important finding with regard to the safety of AAV as a gene delivery tool for PD.

" Optimal combination of the TH/GCH1 vector for maximum L-DOPA production"

The optimal ratio of expression between the TH and GCH1 genes in a two-vector system was 5:1, confirming the high stability of the GCH1 enzyme relative to the need for high levels of TH for highest L-DOPA production *in vivo* (Björklund et al, *J Neurochem*, 2009). We generated a single vector construct where both TH and GCH1 was expressed from a single AAV vector (Cederfjäll et al, *Mol Ther*, 2012). To obtain the favourable ratio between the two transgenes a WPRE sequence was positioned downstream the TH gene. Treatment with TH-GCH1 single vector in the complete 6-OHDA lesioned animal resulted in complete recovery in both drug-induced and drug-naïve rotation tests associated with a widespread neuronal transduction in striatum, cortex and globus pallidus. However, the transduction was associated with a marked loss of NeuN positive cells in globus pallidus. Post mortem striatal brain tissue online microdialysis revealed increased levels of both dopamine and DOPA in vector-treated animals.

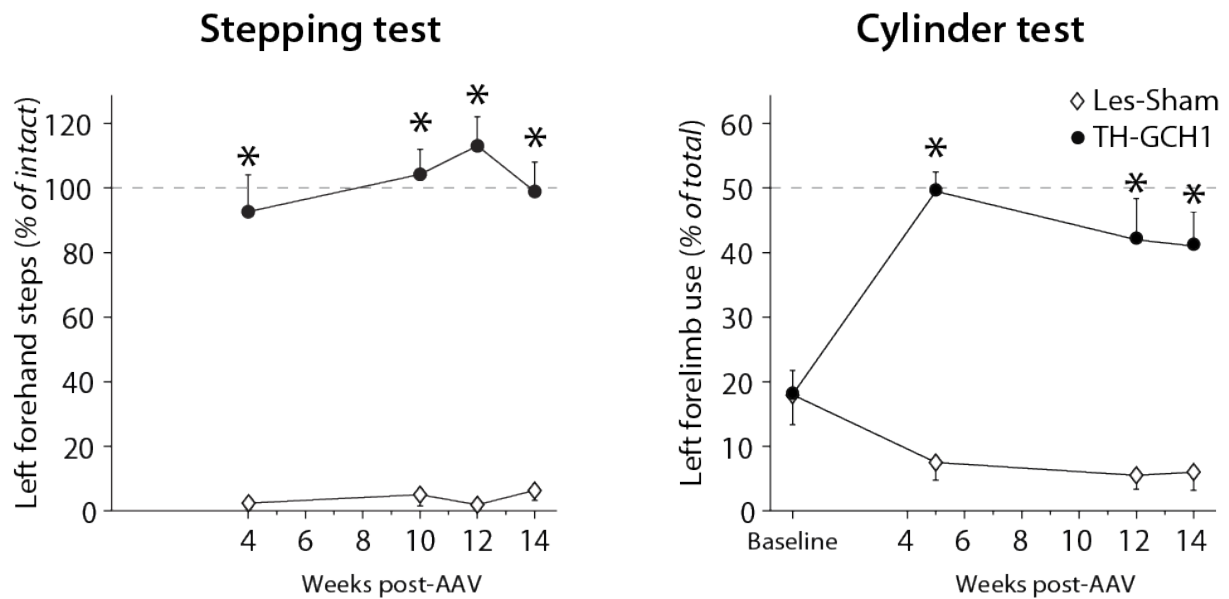


Fig. 4: Complete and sustained recovery of rats from 6-OHDA lesion by DOPA restoration therapy. Animals were injected with the optimized AAV-TH/GCH1 vector and monitored over long time by drug-independent behavioural tests. TH-GCH1 expressing rats showed full motor recovery to control values (stippled lines).

“Therapeutic efficacy of regulated expression of the TH/GCH1 in the rat 6-OHDA model”

Complete 6-OHDA lesioned animals with regulated expression of TH and continuous expression of GCH1 (DD-TH+GCH1) showed incomplete functional recovery with TMP administration (0.5 mg/ml) compared with the same group receiving only water or lesion controls. The motor performance in the DD-TH+GCH1 group was only slightly increased when the TMP dose was increased to 1 mg/ml and 2 mg/ml.

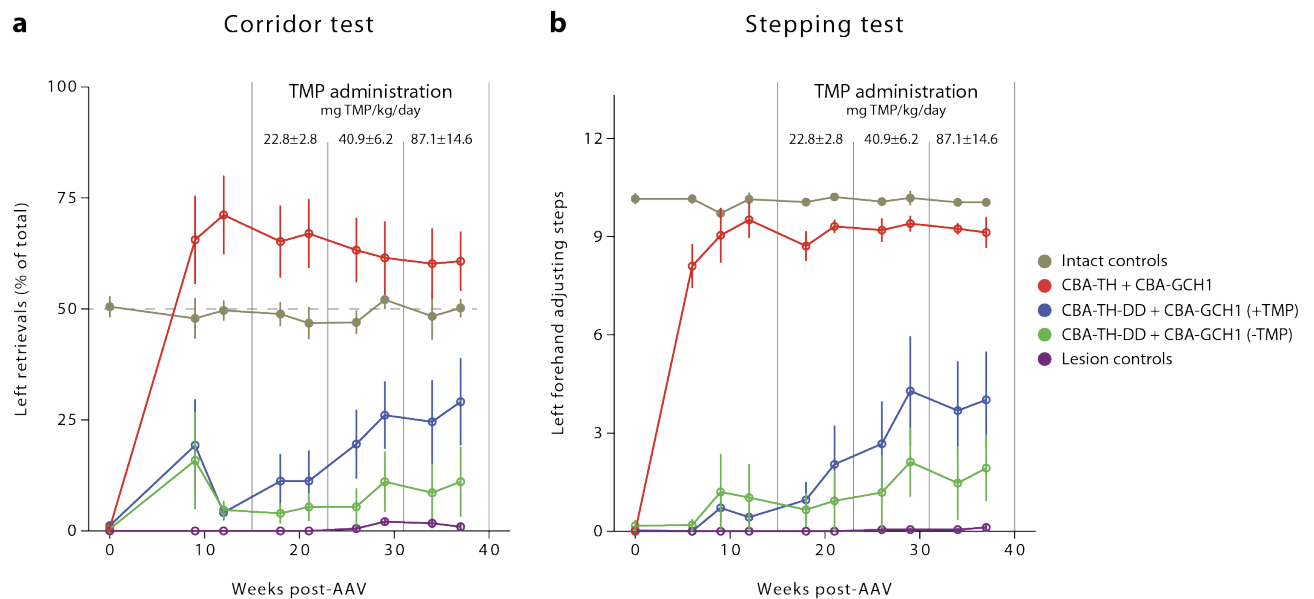


Fig 5: Significant but as yet incomplete recovery of rats from 6-OHDA lesion by TMP-regulated DOPA restoration. As compared to the constitutive TH/GCH1 expression (red line), TMP-induced TH/GCH1 expression (blue line) provided substantial, but less, therapeutic benefit during the time course evaluated at time of this report.

“Long-term efficacy of integrative vs non-integrative LV vectors for therapeutic transgene delivery in the striatal 6-OHDA model”

Following encouraging results of *eGFP* expression described in D1.8, D1.9 and D1.10, a therapeutic neuroprotection study was undertaken to establish the effectiveness of *hGDNF* expression from a non-integrating lentivector to alleviate the symptoms of 6-hydroxydopamine-mediated Parkinsonism in a rat model. *hGDNF* or *eGFP* were expressed from integrating or non-integrating lentivectors pseudotyped with VSV envelope and driven by hGFAP promoter. Animals were injected with vector in the right striatum, and two weeks later they were lesioned by injection of 6-OHDA at the same location. Animals were followed for 18 weeks post-vector injection (16 weeks post-lesion), during which they were tested monthly for amphetamine-induced rotational behaviour.

Non-integrating lentivectors expressed *hGDNF* efficiently in the lesioned striatum, about-50-fold above background levels at the contralateral side according to an ELISA assay (Fig. 6). This compared to about 250-fold overexpression mediated by the equivalent integrating vector. Non-integrating vectors significantly enhanced survival of tyrosine hydroxylase (TH)-positive cells in the lesioned substantia nigra, from 50 to 62%, while integrating vectors performed significantly better at 70%. However, despite the difference in *hGDNF* expression level and TH-positive cell survival, both non-integrating and integrating *hGDNF* vectors prevented to a similar extent rotational behaviour in response to amphetamine treatment (Fig. 6).

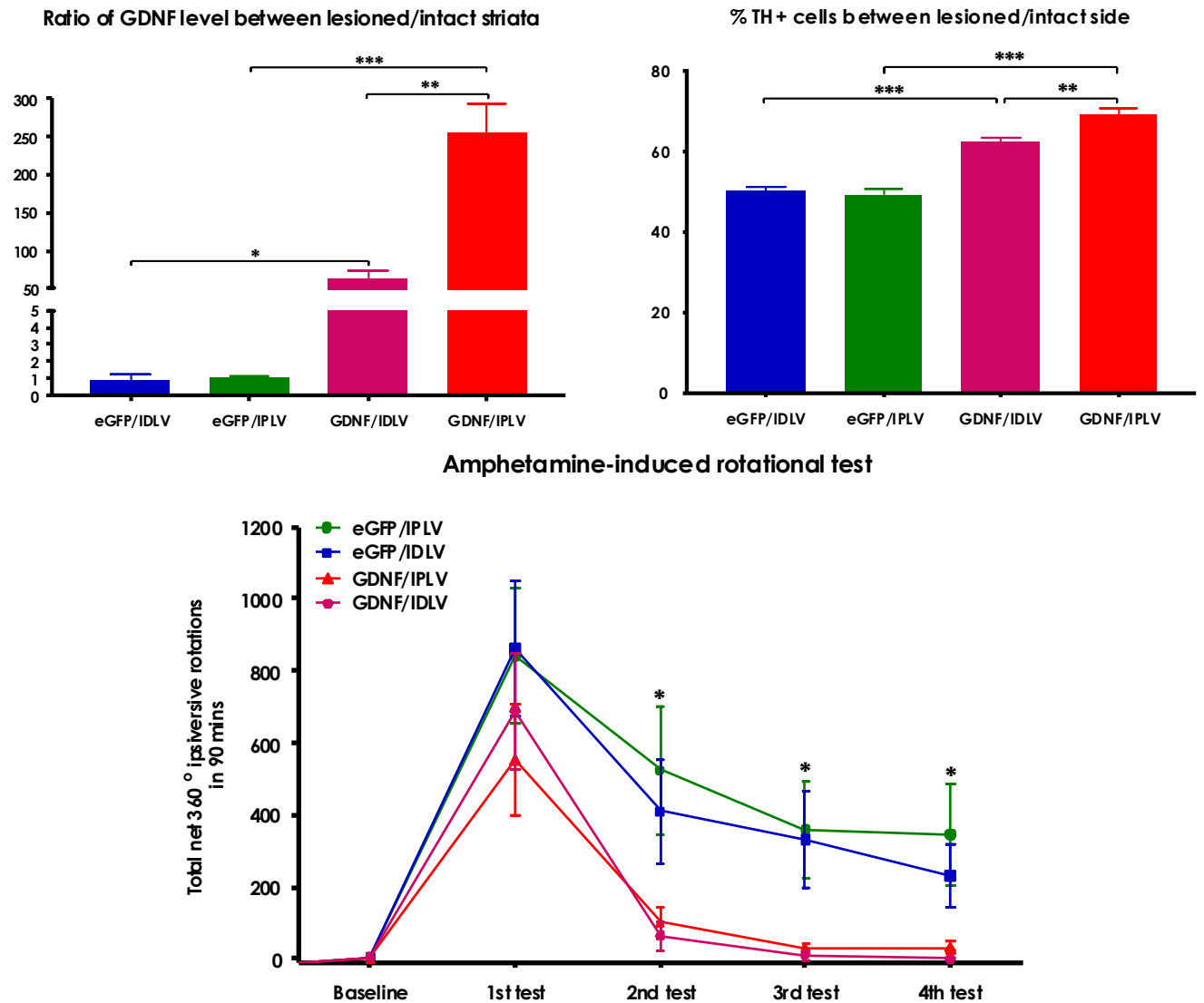


Fig. 6: Effective long-term hGDNF expression from non-integrating lentivectors protects against Parkinsonian symptoms in the 6-OHDA rat model. Integrating and non-integrating eGFP or hGDNF vectors were injected in the right striatum, and two weeks later animals were lesioned by 6-OHDA injection at the same location. Samples were processed 18 weeks post-vector injection (16 weeks post-lesion). Top left panel: ratio of GDNF levels between lesioned, vector-injected and contralateral striata. Top right panel: percentage of TH-positive cells remaining in the lesioned, vector-injected compared to contralateral striata. Bottom panel: ipsiversive rotations in response to amphetamine treatment in the various vector-treated groups, using monthly tests starting three weeks post-lesion. IPLV: integration-proficient lentiviral vector; IDLV: integration-deficient lentiviral vector.

“Therapeutic benefits of constitutive vs. short-term / intermitted expression of GDNF in the striatal 6-OHDA model”

A major goal in the attempts to develop regulated transgene expression vectors was the demonstration that a single short-term or at least intermitted short-term expression of GDNF would provide significant benefit in a relevant rodent model of PD. A partial striatal 6-OHDA lesion was implemented to generate a slowly and progressive degeneration of the nigro-striatal projection, with clearly evident motor pathology in drug induced and spontaneous behavioural tests already early after lesion, and a delayed degeneration of nigral dopaminergic cell bodies.

The astrocyte-specific Mfp-regulated GDNF-expressing vector system was tested against an astrocyte-specific but constitutively GDNF expressing vector in two different paradigms: in a **neuroprotection** setting, where a short-term GDNF expression was induced *before* the application of 6-OHDA, and in a **neurorestoration** setting where vector injection and induction of short-term GDNF expression was performed several weeks *after* the lesion.

Beforehand we proved that constitutive GDNF expression in astrocytes was fully neuroprotective in the MPTP model and almost completely neurorestorative in the 6-OHDA model (Drinkut et al, Mol Ther. 2012), and that Mfp-induced GDNF expression lasted for no more than 2 weeks after induction.

a) Neuroprotection through a short-term expression of GDNF:

Mfp-regulated GDNF-expression vectors were applied unilaterally into the striatum of rats and two weeks later GDNF expression was induced by Mfp (20 µg/kg, i.p. on three consecutive days). One week after induction of GDNF expression the partial, striatal 6-OHDA lesion was executed, and animals were monitored for 4 weeks behaviourally (apomorphine-induced rotations, cylinder test) and biochemically (determination of dopamine and metabolites). While control groups which had received a control vector (EGFP) or which were not induced with Mfp, developed strong motor impairment and a 50%-loss of striatal dopamine, no motor symptoms developed in the Mfp-induced GDNF expressing animals, and DA loss was limited to less than 10%. The group of animals which was injected with the constitutively GDNF-expressing vector was not significantly better, indicating that a short-term GDNF expression can completely *prevent* motor pathology in the 6-OHDA model.

b) Neurorestoration through short-term or intermitted GDNF expression:

In a more clinically relevant paradigm we then tested efficacy of short-term GDNF expression if the regulated vectors were applied several weeks after induction of nigro-striatal degeneration. Regulated GDNF-expressing vectors or constitutively GDNF expressing vectors were injected into the lesioned striata at 2 weeks after partial striatal 6-OHDA lesion, when robust drug-induced (apomorphine-induced rotations) or spontaneous (cylinder test) impairment of motor control was documented. Three weeks after vector injection (i.e. 5 weeks after 6-OHDA lesion) the inducer Mfp was applied (20 µg/kg, i.p., on 3 consecutive days), and animals were monitored behaviourally and biochemically for 3 further months.

These studies demonstrated that:

- a single induction of GDNF expression was sufficient to significantly improve motor performance in drug-induced and spontaneous test, and to partially restore striatal

dopamine levels. However, at two months after GDNF induction motor symptoms worsened, indicating that a single short-term induction of GDNF may not be sufficient to restore motor performance in the long run (Fig. 7 C).

- a second group of animals received a second induction of GDNF expression at 6 weeks after the first induction. In these animals the improvement in motor performance lasted through the time of analysis, indicating that short-term intermittent GDNF expression may substantially restore motor performance in a long-lasting manner (Fig 7 D).

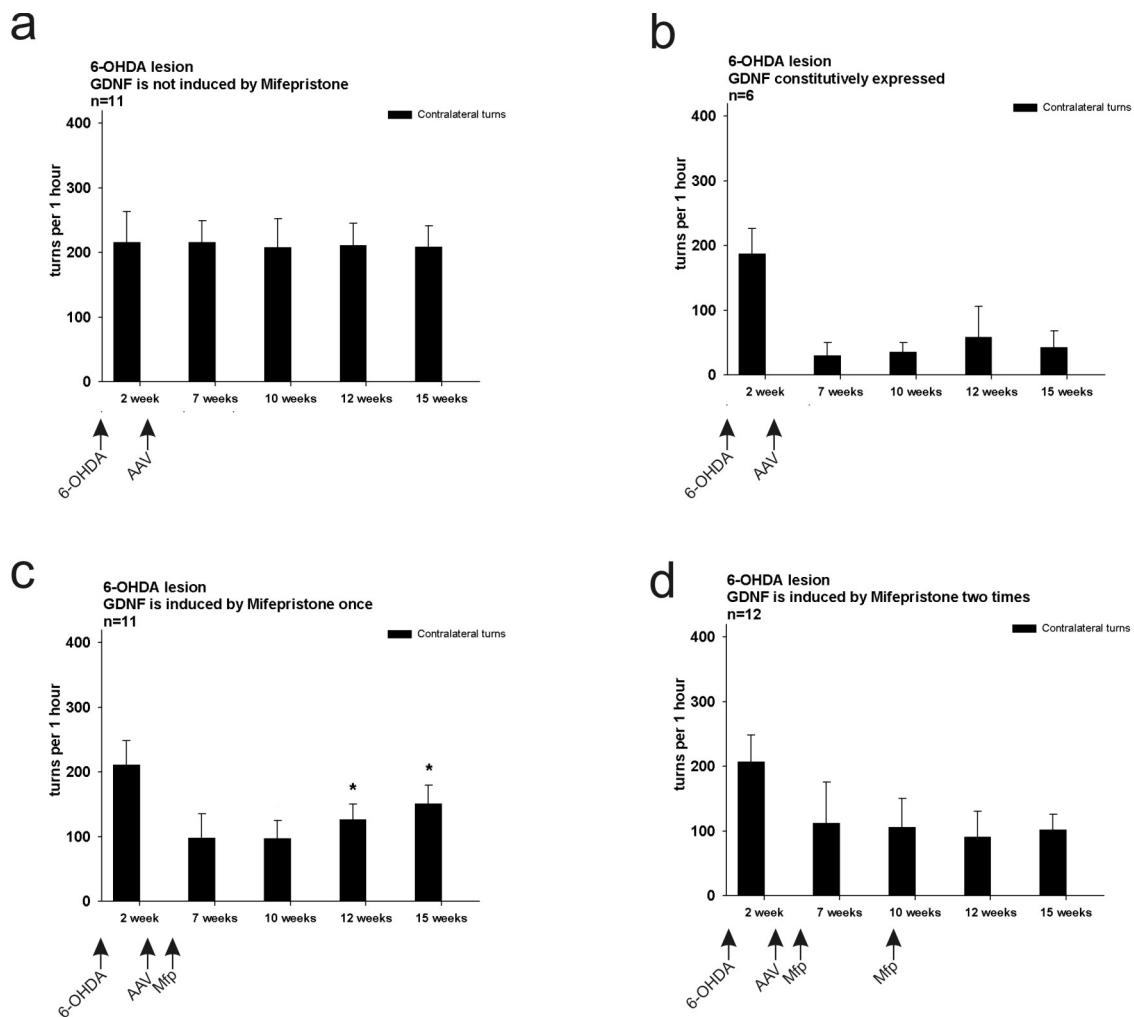


Fig. 7 Apomorphine-induced rotation behaviour in rats after partial, striatal 6-OHDA lesion. Animals injected with the regulated GDNF expressing vectors but not induced by Mfp showed stable rotation behaviour (> 200 / h) over 4 months (a). Animals injected with a constitutively GDNF expressing vector showed stable reduction of rotations to about 40 per hour (b). Animals injected with the regulated vector and induced only once showed reduction of rotation behaviour for up to 10 weeks after lesion, but then rotations significantly increased (c). Animals injected with the regulated vectors and induced twice showed stable reduction of rotation behaviour over 4 months (d). It should be noted that the constitutively expressing vector could start to express GDNF already at 2 weeks after lesion, while the regulated vectors were induced to express for the first time at 5 weeks after lesion.

These studies demonstrated that in principle an intermitted expression of GDNF is sufficient to achieve significant restoration of motor performance. In order to include all necessary control groups and to be able to study several aspects of the inducing drug Mfp we needed over 300 rats to complete the study. While all behavioural and biochemical studies are now completed, the stereological assessment of numbers of nigral dopaminergic neurons is still on-going and expected to be completed in June/July, at that time we also plan to submit the respective manuscript.

“Role of target cell specificities (neuronal vs glial) in the development of functional therapeutic benefits in the striatal 6-OHDA model”

Studies on the development of regulated GDNF-expressing vectors focussed on astroglial expression, as our data obtained through the runtime of the Neugene consortium have clearly shown that restricting neurotrophin expression to astrocytes provides safety benefits without any restrictions in efficacy (Drinkut et al, Mol Ther., 2012). Thus, at least for constitutive GDNF expression it was shown that therapeutic efficacy is the same for astrocytic and neuronal GDNF expression.

To substantiate these findings for the regulated GDNF expression systems we have developed neuron-specific regulated vectors, which are tested in the neurorestorative paradigm described for D4.4. A cohort of another almost 100 rats has been behaviourally assessed, and tissue samples have been collected for biochemical analysis of dopamine and GDNF levels, and for immunohistochemistry / stereology to quantify striatal dopaminergic projections and nigral cell bodies. These studies are on-going and will be completed within 2-3 months.

Thus, while it was shown in principle that constitutive astrocytic and neuronal GDNF expression demonstrated the same therapeutic efficacy, this remains to be proven for the regulated vector systems.

Summary of significant results:

- Development and in vivo validation of a LV allowing both the overexpression and silencing of target genes in astrocytes
- Development and in vivo validation of LV with neuronal regulatory element providing new tools to explore the selective vulnerability of neurons and contribution of various cell-types in neurodegenerative diseases
- Protocols for the production of high quality vector batches of AAV6, 8 and 9 was established.
- AAV6, 8 and 9 can infect nigral dopamine neurons with equal high efficiency
- AAV6 has a superior efficacy for retrograde transduction of nigral neurons from striatal axonal projections
- Striatal injection of AAV6 can be used to iterative neuronal transduction following nigral injection of AAV8 and 9 vectors

- There were no major differences between non-integrating and integrating lentivectors for *eGFP* expression. Vectors expressed *eGFP* efficiently in striatum and to a lesser extent in the substantia nigra. Promoter and to a lesser extent pseudotype alterations were able to target sub-populations of cells within the rodent basal ganglia. Follow up reached 18 weeks post-vector injection in the 6-OHDA-lesioned striatum.
- Linear-amplification mediated (LAM)-PCR analyses demonstrated lack of significant genomic integration of non-integrating lentivectors in the intact and lesioned striata, underscoring their much improved bio-safety
- Ongoing *in vivo* assessment indicates that incomplete recovery can be achieved in animals treated with CBA-DD-TH and CBA-GCH1 with oral administration of TMP.
- Neuron- and astrocyte-specific regulation of transgene expression was achieved, best results obtained with AAV-5 vectors.
- Regulation of *in vitro* DOPA production can be achieved by coupling destabilizing domains to TH and/or GCH1 by TMP administration. Ongoing *in vivo* assessment indicates that partial functional recovery can be achieved in animals treated with DD-TH+GCH1 with oral administration of TMP.
- Cell-type specific, tightly regulated transgene expression was achieved using a two-vector design for the Mfp-regulated vectors.
- Immunization with wt AAV-2 had a significant effect on the transduction efficiency of the intracerebrally applied AAV2 vector. 6-OHDA lesion however did not affect the number of transduced cells. At the same time both immunization with wt AAV-2 and 6-OHDA lesion increased number of activated microglia.
- Until now, no adverse effects or immunological consequences were observed in rodent brains due to expression of proteins coupled to destabilizing domains, expression of the pSwitch protein or administration of the inducing drugs TMP or MfP.
- AAV particles and vector genomes were shown to persist extra-nuclearly in the nigrostriatal system for several weeks to months after injection.
- Following toxin-induced degeneration of the nigrostriatal system, no spread of infection was observed in the striatum despite the persisting presence of viral genomes in axon terminals.
- Non-integrating lentiviral vectors:
Our results confirm the efficiency and usefulness of non-integrating lentiviral vectors for the treatment of Parkinson disease in a pre-clinical model. Non-integrating vectors expressed *hGDNF* in the striatum 50-fold above background, significantly enhanced survival of TH-positive cells in the lesioned substantia nigra and essentially abolished amphetamine-induced rotational behaviour. Non-integrating lentivectors contribute to enhanced bio-safety at two major levels:

mediating moderate rather than extremely high *hGDNF* expression, and avoiding insertional mutagenesis. These results are of considerable relevance for the use of safer lentiviral vectors in the treatment of Parkinson and other CNS disorders.

- **TH/GCH1 expression for generation of DOPA**
A single vector system to express TH/GCH1 at optimal levels was constructed and successfully restored motor performance in the 6-OHDA model. Although further fine-tuning of expression levels may be necessary, this vector is estimated to enter clinical applicability soon.
- **Mfp-regulated GDNF expression**
Astrocyte-specific GDNF expression demonstrated the same therapeutic efficacy as neuronal GDNF expression in several model systems of PD (neuroprotection in MPTP lesion, neurorestoration in 6-OHDA lesion). Regulated astrocyte-specific GDNF expression provided significant restoration of motor performance and striatal dopamine levels after a single short-term induction, but this effect was transient in long-term studies. Regulated astrocyte-specific GDNF expression provided long-term improvement of motor restoration through an intermitted induction profile. Taken together, these results open new and potentially safer and more efficient venues for neurotrophic factor and dopamine-restoration based gene therapy of PD.

Description of the potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results.

The majority of projects conducted by the NEUGENE consortium were of a basic research nature, and thus direct economical or wider societal impacts were not intended. Clearly, it will be the scientific community (neuroscientists, gene therapists etc.) who will have the major benefit from the work presented so far and from further work resulting from current projects.

Optimized AAV vectors to constitutively express DOPA restoring TH/GCH1 proteins in striatal neurons have entered clinical development programs at ULUND. The respective regulated versions are expected to reach clinical applicability within 2-3 years. The regulated GDNF expression vector system is currently being further optimized to fit into a single vector design together with a leading European gene therapy SME (uniQure, formerly AMT) in cooperation with UMG-GOE. The same company is interested in RNAi-based strategies developed by CEA for further pre-clinical research. Thus, tools developed through NEUGENE are very close to clinical applications already. Further benefit is delivered by tools developed for advanced basic research on genetic therapies for CNS disorders, i.e. optimized and safe non-integrating LV vectors, LV and AAV vectors targeted to specific neuronal or glial sub-populations, protocols for re-delivery of AAV vectors into the CNS etc. In addition, optimized vector tools are currently used to generate advanced animal models of neurodegenerative disorders, targeting key components like the synuclein and huntingtin genes. In conclusion, we anticipate that tools developed by the NEUGENE consortium will substantially promote applicability of gene medicines for the brain, thereby offering treatment opportunities for large numbers of patients.

Much of the work progress of the NEUGENE consortium is freely available to the scientific community. Our results have been presented at 19 conference contributions and in 14 peer-reviewed publications, mostly in high-ranked neuroscience or gene therapy journals. The number of high ranked publications will further increase in the next months, as several manuscripts are in revision, submitted, or in preparation. Actually, several highly interesting studies resulting from work in the WP4 (Functional validation) are just about to be prepared for publication.

Dissemination activities

Results of the studies performed by the NEUGENE consortium were presented to the public at several scientific meetings and through publications.

Conference communications and peer-reviewed publications, acknowledging support by NEUGENE are listed below:

Meeting contributions:

Francophone Society of Cell and Gene Therapy (SFTCG) meeting (June 13 – 15, 2010), Paris, France. Pre-clinical studies of neurodegenerative diseases. *Déglon et al.*

FENS-IBRO Summer School on translational research in basal ganglia disorders (September 20 – 24th, 2010), Mallorca, Spain. Genetic models of neurodegenerative diseases. *Déglon et al.*

Society for Neuroscience Meeting (November 13 – 17, 2010), San Diego, USA. Tissue-specific targeting of lentiviral vectors using neuronal or astrocytic promoters and various pseudotyping. *Delzor, et al.*

16th Annual meeting of the German Society of Neurogenetics : « Functional Genomics of Parkinson's disease », Tuebingen, Germany. Viral gene delivery to nigral neurons: animal models of Parkinson's disease in prelude to neuroprotective therapies. *Schneider B.L.*

Swiss Society for Neuroscience, Zürich, Switzerland. The potential of viral tools: from optogenetics to gene therapy - Symposium « Viral Strategies in Brain Research », *Schneider B.L.*

12th International Neuroscience Winter Conference, Soelden, Austria Adeno-associated vectors to model and treat Parkinson's disease. *Schneider B.L.*

World Parkinson Congress, Glasgow, UK Alpha-synuclein animal models. *Schneider B.L.*

World Parkinson Congress, Glasgow, UK Effective delivery of the neuroprotectant to the brain. *Schneider B.L.*

40th Annual Meeting of the Neuroscience Society 2010, San Diego, USA The therapeutic potential of PGC-1 α in Parkinson's disease: promise and pitfalls. *Ciron C., Bensadoun J.C., Canto-Alvarez C., Mlynarik V., Schneider B.L., Aebischer P.*

40th Annual Meeting of the Neuroscience Society 2010, San Diego, USA Adeno-associated mifepristone-regulated viral vector system for controllable GDNF expression in the central nervous system. *Tereshchenko Y., Maddalena A., Bähr M., Kügler S.*

7th British Society for Gene Therapy Meeting, 29-31 March 2010, Egham, UK. Transcriptional targeting of integration-proficient and integration-deficient lentiviral vectors for the treatment of Parkinson's disease. *Broadstock, M and Yáñez-Muñoz RJ*

Federation of European Neurosciences (FENS), 2010, Amsterdam, Netherlands. Dose-response study of continuous DOPA delivery using AAV gene therapy reveals threshold and kinetics for restoration of motor function in a rodent model of Parkinson's disease. *Cederfjäll E, Björklund T and Kirik D.*

Monitoring Molecules in Neuroscience, 2010, Brussels, Belgium. Dose-response study of continuous DOPA delivery using AAV gene therapy reveals threshold and kinetics for restoration of motor function in a rodent model of Parkinson's disease. *Cederfjäll E, Björklund T and Kirik D.*

Monitoring Molecules in Neuroscience, 2010, Brussels, Belgium. Regulation of AAV-mediated continuous DOPA delivery using destabilizing domains. *Björklund T.*

Collaborative British Society for Gene Therapy/European Society for Gene and Cell Therapy Congress, 27th-31st Oct 2011, Brighton, UK. Therapeutic strategies with integration-deficient lentiviral vectors. *Yáñez-Muñoz, R.J*

Clinical Gene Transfer: state of the art/e-CHIPS European Congress on Human iPS Stem Cell Reprogramming Conferences, 7th-9th Apr 2011, Paris, France. Vector Integration Patterns of Integration-Deficient and Integration-Competent Lentiviral Vectors in a Preclinical Model of Parkinson Disease. *Schliesser, M., Broadstock, M., Bartholomae, C., Arens, A., Cartier, N., Aubourg, P., von Kalle, C., Yáñez-Muñoz, R.J. and Schmidt, M.*

41th Annual Meeting of the Neuroscience Society 2011, Washington DC, USA Knock-down of PGC-1 α expression increases vulnerability to human α -synuclein in nigral dopaminergic neurons. *Ciron C., Kelly D.P., Padrun V., Aebischer P., Schneider B.L.*

41th Annual Meeting of the Neuroscience Society 2011, Washington DC, USA Adeno-associated mifepristone-regulated viral vector system for controllable GDNF expression in astrocytes provides therapeutic effects in the 6-OHDA Parkinson disease model. *Tereshchenko Y, Bähr M., Kügler S.*

CMPB symposium "A bridge between microscopy and neuroscience", Goettingen, Germany Gene delivery to the adult central nervous system to explore genetic determinants of aging and neurodegeneration. *Schneider B.L.*

Published papers:

[Engineered lentiviral vector targeting astrocytes in vivo.](#)

Colin A, Faideau M, Dufour N, Auregan G, Hassig R, Andrieu T, Brouillet E, Hantraye P, Bonvento G, Déglon N.
Glia. 2009 Apr 15;57(6):667-79.

[Optimization of continuous in vivo DOPA production and studies on ectopic DA synthesis using rAAV5 vectors in Parkinsonian rats.](#)

Björklund T, Hall H, Breyse N, Sonesson C, Carlsson T, Mandel RJ, Carta M, Kirik D.
J Neurochem. 2009 Oct;111(2):355-67

[Dose optimization for long-term rAAV-mediated RNA interference in the nigrostriatal projection neurons.](#)

Ulusoy A, Sahin G, Björklund T, Aebischer P, Kirik D.

Mol Ther. 2009 Sep;17(9):1574-84

[In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects.](#)

Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auresan G, Dufour N, Guilleminier M, Brouillet E, Hantraye P, Déglon N, Ferrante RJ, Bonvento G.
Hum Mol Genet. 2010 Aug 1;19(15):3053-67.

[Optimized adeno-associated viral vector-mediated striatal DOPA delivery restores sensorimotor function and prevents dyskinesias in a model of advanced Parkinson's disease.](#)

Björklund T, Carlsson T, Cederfjäll EA, Carta M, Kirik D.
Brain. 2010 Feb;133(Pt 2):496-511.

[Gene therapy for dopamine replacement.](#)

Björklund T, Cederfjäll EA, Kirik D.
Prog Brain Res. 2010;184:221-35.

[Glutathione depletion and overproduction both initiate degeneration of nigral dopaminergic neurons.](#)

Garrido M, Tereshchenko Y, Zhevtsova Z, Taschenberger G, Bähr M, Kügler S.
Acta Neuropathol. 2011 Apr;121(4):475-85.

[Efficient gene therapy for Parkinson's disease using astrocytes as hosts for localized neurotrophic factor delivery.](#)

Drinkut A, Tereshchenko Y, Schulz JB, Bähr M, Kügler S.
Mol Ther. 2012 Mar;20(3):534-43.

[Aggregation of \$\alpha\$ Synuclein promotes progressive in vivo neurotoxicity in adult rat dopaminergic neurons.](#)

Taschenberger G, Garrido M, Tereshchenko Y, Bähr M, Zweckstetter M, Kügler S.
Acta Neuropathol. 2011 Dec 14. [Epub ahead of print]

[Key factors determining the efficacy of gene therapy for continuous DOPA delivery in the Parkinsonian brain.](#)

Cederfjäll E, Sahin G, Kirik D.
Neurobiol Dis. 2011 Oct 25. [Epub ahead of print]

[Nigrostriatal overabundance of \$\alpha\$ -synuclein leads to decreased vesicle density and deficits in dopamine release that correlate with reduced motor activity.](#)

Gaugler MN, Genc O, Bobela W, Mohanna S, Ardah MT, El-Agnaf OM, Cantoni M, Bensadoun JC, Schneggenburger R, Knott GW, Aebischer P, Schneider BL.
Acta Neuropathol. 2012 Feb 24. [Epub ahead of print]

[Sustained expression of PGC-1 \$\alpha\$ in the rat nigrostriatal system selectively impairs dopaminergic function.](#)

Ciron C, Lengacher S, Dusanochet J, Aebischer P, Schneider BL.
Hum Mol Genet. 2012 Jan 19. [Epub ahead of print]

[Design of a Single AAV Vector for Coexpression of TH and GCH1 to Establish Continuous DOPA Synthesis in a Rat Model of Parkinson's Disease.](#)

Cederfjäll E, Sahin G, Kirik D, Björklund T.

Mol Ther. 2012 Jan 31. doi: 10.1038/mt.2012.1. [Epub ahead of print]

[Challenges for gene therapy of CNS disorders and implications for Parkinson's disease therapies.](#)

Broadstock M. and Yáñez-Muñoz R.J.

Hum Gene Ther (2012) 23, 340-343. doi: 10.1089/hum.2012.2507

Submitted manuscripts:

Direct and retrograde transduction of nigral neurons with AAV6, 8 and 9 and intraneuronal persistence of viral particles

K. Löw, Aebischer P., Schneider B.L.

Gene Therapy, submitted

Restricted transgene expression in the brain with cell-type specific neuronal promoters

Delzor, A., Dufour, N., Petit, F., Guillermier, M., Houitte, D., Auregan G., Brouillet, E.,

Hantraye, P., and Déglon, N. in revision

Several manuscripts describing the results of the functional validations in animal models are currently under preparation. Thus, we expect that further major scientific contributions will be published within the next few months.

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