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Summary and general discussion. Gene therapy and peripheral nerve repair: a perspective

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SUMMARY

The mammalian peripheral nervous system (PNS) consists of primary sensory neurons in the dorsal root ganglia, motor neurons in the ventral horn of the spinal cord and their axonal projections to the muscles and skin (Figure 1). Most peripheral nerves contain axons of sensory and motor neurons and patients that sustain an injury experience loss of sensory and motor function. In patients, regeneration of injured peripheral axons does occur but is almost never complete. This is due to the low velocity of axon growth, the deterioration of pro-regenerative Schwann cells in the distal nerve stump following longer periods of denervation, and the misrouting of regrowing axons. As reviewed in Chapter 1, nerve regeneration is studied in well-defined rodent models of nerve injury. A widely used model is transection of the sciatic nerve of the rat followed by end-to-end repair of the nerve stumps or implantation of an autograft or artificial nerve guide to bridge the gap between the stumps. In this model axons reinnervate the end organs within weeks to months. In humans surgical repair of peripheral nerves has reached its optimal refinement. Recovery of function as a result of surgical repair has significantly improved but remains unsatisfactory. Novel adjuvant therapeutic strategies to promote axon regeneration in the injured peripheral nerve are needed. One of these strategies is gene therapy. The overall goal of the work presented in this thesis is to enhance peripheral nerve regeneration by stimulating axonal growth, reducing misdirection and prolonging the pro-regenerative environment through the use of gene therapy.

In Chapter 2, we investigated whether the functional outcome of a standard procedure in nerve surgery (autografting) could be improved through the overexpression of neurotrophic factors in nerve autografts by using gene therapy. We performed a large in-vivo comparison of six neurotrophic factors overexpressed in a rat model of nerve autografting. We assessed early (2) and late (20 weeks) effects of each neurotrophic factor by performing a histological, electrophysiological and functional analysis. Three of the six neurotrophic factors investigated (BDNF, GDNF and NGF) showed early stimulatory effects on axon growth but persistent expression revealed selective but excessive sensory (NGF) or motor axon (BDNF, GDNF) growth and uncontrolled cell proliferation. This resulted in trapping of regenerating axons in the autograft which interfered with recovery of function but also led to the discovery of the most relevant and more interestingly modality specific candidates for further studies.

In Chapter 3 we investigated whether it was possible to selectively guide regenerating motor axons by using gene therapy. We selectively overexpressed a neurotrophic factor (GDNF) in a nerve injury and bifurcation model and observed its effects on axon routing. We demonstrated that it is technically feasible to increase the expression of a neurotrophic factor in a specific branch of a surgically repaired peripheral nerve. We assessed axon routing at 4 weeks by performing retrograde tracing and histology. Although the variability was high and the results did not reach statistical significance, retrograde
tracing revealed that there were on average more motor neurons from which axons had regenerated towards the peroneal branch that overexpressed GDNF than in the group with the control vector. In some animals the normal peroneal to tibial motor neuron distribution was even reversed (from 1:2 to 2:1). Taken altogether this proof of principle study indicates that viral vector mediated neurotrophic factor expression may be used to direct regenerating axons.

The studies in Chapter 2 and 3 indicate that in the context of PNS gene therapy the creation of a safe regulatable gene therapy vector is essential. This is important for two reasons. First, persistent expression of growth factors leads to local trapping of axons. Second, uncontrolled expression has been shown to cause local and systemic side effects, including hyperalgesia (NGF) or systemic weight loss and anorexia (BDNF and CNTF). In Chapter 4 we developed a viral vector system that allows regulated expression of a neurotrophic factor in the peripheral nerve. It was based on the prototypical system for regulating gene expression which involves a transactivator (TA) that binds to a promoter in the presence of doxycycline. However, the TA is a bacterial protein and is therefore a permanent immunological target. Clinical use of analogous systems using alternative
TAs is precluded for the same reason. Viruses have evolved several strategies to escape immune surveillance (Zaldumbide and Hoeben, 2008). We took advantage of the long Gly-Ala repeat (GAr) domain of Epstein Barr virus to generate an immunologically inert version of the TA. This idea was based on the observation that Schwann cells which express a foreign protein (e.g. GFP) are cleared from the nerve by an immune response. This does not happen when these proteins are fused to GAr. We fused GAr to TA and showed that GAr-TA retains its sensitivity to dox. We used this system successfully to turn GDNF expression “on” and “off” in the rat sciatic nerve. The GAr-TA system is several fold less “leaky” compared to the TA protein and displays strongly reduced immunogenicity in a bioassay for antigenic peptide generation.

Almost all gene therapy studies targeting the PNS have been based on lentiviral vectors (LV). Unfortunately, the random insertion of genetic material following transduction with LV can potentially lead to the activation or inactivation of genes that control cell proliferation leading to uncontrolled proliferation. Although this risk seems limited an alternative, adeno-associated viral vectors (AAVs), is gaining increased acceptance as the preferred clinical gene delivery platform. In Chapter 5 we investigated whether AAVs were capable of transducing the rat and human PNS. We compared the transduction efficiencies of nine AAV vector serotypes for cultured primary rat and human SCs as well as their ability to genetically modify rat and human nerve segments. We found that AAV1 is the preferred serotype to transduce cultured rat SCs, whereas AAV2 and AAV6 are the best serotypes to target human SCs. We found a toxic effect of AAV6 in rat but not in human SCs. In rat nerve segments AAV1, AAV5, AAV7 and AAV9 transduced equally well, whereas in human nerve segments AAV2 outperformed all other serotypes. LV had superior efficacy throughout the study. Taken together, the key findings of this study were that 1) we could successfully transduce the rat and human PNS derived Schwann cells and nerve segments with AAV vectors, 2) Schwann cells derived from rat and human nerve require different serotypes, highlighting the necessity for comparative pre-clinical studies, and 3) AAV2 seems to be a promising vector for the genetic modification of human peripheral nerve autografts. The latter finding is important because AAV2 is the best characterized serotype available and has been used in several clinical trials involving hundreds of human patients.

Discussion

Our results in perspective and the path to the clinic

As a result of the findings described above further research is crucial before clinical translation can be considered. In this section I will discuss three key areas of future research. In the first part I will discuss further research that is required to create a regulatable vector with improved doxycycline sensitivity. Then I will give my view on the developments required to allow clinically safe transgene delivery to Schwann cells. Finally, beyond the
research performed in this thesis I will elaborate on the goal to identify novel targets to enhance regeneration.

Creating a safe and clinically applicable regulatable vector
The criteria for safe regulated vector-based therapeutic gene expression are that 1) it can be induced by a small molecule that is safe, 2) it can be turned off effectively by withdrawal of the inducer whereas “leaky” expression should be minimal and preferably undetectable, and 3) the transactivator protein (TA) that is employed should be non-immunogenic and well-tolerated. The GAr-TA based system presented in Chapter 4 requires doxycycline levels that are 40-fold higher than clinically acceptable. Fusion of GAr to rtTA results in a three times larger protein (248 versus 678 amino acids) with significantly reduced sensitivity to doxycycline. Doxycycline an analogue of tetracycline is a well-tolerated antibiotic drug that has been administered orally and intravenously in humans for over 30 years. At high levels however, renal toxicity and dose-dependent photosensitivity might occur (Stieger et al., 2009).

Two main strategies could potentially create a more efficient transactivator: 1) reduction of the size of the GAr tail and 2) further optimization of GAr-TA through viral evolution. Understanding of the GAr sequence has permitted to identify some of the essential structures permitting immune evasion. Indeed, GAr sequences as short as 24 and even 7 amino acids have shown conserved immune evasive properties (Zhang and Coffino, 2004; Hammer et al., 2008). Das and colleagues have performed several elegant studies were viral evolution was used to optimize rtTA leading to variants that were 7-fold more active and 100-fold more dox-sensitive than the original Tet-on system (Das et al., 2004; Zhou et al., 2006). To achieve this they incorporated the Tet-on components into the human immunodeficiency virus (HIV)-1 genome such that viral replication was controlled by this regulatory circuit. During replication of this HIV-rtTA in human T cells, genetic diversity is continuously generated due to the error-prone reverse transcription process, followed by selection of faster replicating variants. Thus, generation of genetic diversity and selection of improved more doxycycline sensitive variants were combined in this viral evolution approach. As the fusion of GAr to rtTA results in a novel much larger protein with modified pharmacokinetics repeating this assay could lead to interesting and more efficient variants. It is however likely that viral evolution would remove the GAr sequence as it would not have an evolutionary disadvantage in their in-vitro assay. A possible solution to this problem would be to add an extra selection based on the immune evasive capacity of the transactivator. Cytotoxic T cells targeting rtTA could be used to remove strains that have lost their immune evasive capacity.

A second concern of an antibiotic driven regulatory system is the potential of creating antibiotic-resistant bacteria. Chronic systemic exposure to doxycycline might lead to evolutionary selection of resistant bacterial strains. The use of 4-epidoxycycline, a hepatic metabolite of dox lacking antibiotic activity might be a possible solution to this
problem. Finally, additional evidence of the non-immunogenic nature of GAr-rtTA is required. In our in-vivo experiment we were unable to show its immune evasive advantage. This was due to a lack of immune response against the classical rtTA in the rat sciatic nerve. Previously our group has shown an immune response directed against the foreign protein GFP expressed in Schwann cells in a peripheral nerve (Hendriks et al., 2007). Several studies in non-human primates have shown an active immunological response against rtTA (Favre et al., 2002; Latta-Mahieu et al., 2002) and an experiment in which the GAr-rtTA is tested in non-human primates might be unavoidable.

Apart from ligand (i.e. dox) regulated promoters, promoters induced by physiological stimuli associated with neural injury may emerge as tools to restrict transgene expression to the post-lesion period (Jazwa et al., 2013). The glia fibrillary acidic protein (GFAP)-promoter is an example of an injury induced promoter that has been used in transgenic mice to turn on gene expression in a diseased peripheral nerve (Keller et al., 2009). However, GFAP continues to be expressed in non-myelinating Schwann cells in an intact nerve which would result in some level of persistent transgene expression after nerve regeneration has been completed.

Enhancing clinically safe transgene delivery to Schwann cells

In Chapter 5, we have shown that AAV vectors were capable of transducing rat and human cultured nerve segments. A next step would be to investigate the efficacy of in-vivo AAV-mediated therapeutic gene targeting in our rodent models of peripheral nerve injury. The culture results showed that lentiviral vectors remained superior to the best AAV vectors. Thus, further research should focus on (1) identifying newly engineered AAV vectors with an improved tropism for Schwann cells (Kotterman and Schaffer, 2014), and on (2) developing lentiviral vectors with an improved safety profile, e.g. non-integrating lentiviral vectors (Yanez-Munoz et al., 2006; Cesana et al., 2014).

New AAV vectors are being developed continuously. In our comparison we worked with AAV 1 to 9. Currently 11 mainstream serotypes are available but many other novel AAV-vector capsids are being developed. Some of these variants may exhibit enhanced tropism towards SCs (Kotterman and Schaffer, 2014). One of the strategies might be to study and understand AAV evolution leading to common ancestors with a broader transduction profile (Zinn et al., 2015). Further understanding the binding of AAV receptors on Schwann cells in both their demyelinated state versus their myelinated state could also lead to the development of more selective vectors.

Lentiviral vectors are derived from the human immunodeficiency virus type 1 (HIV-1) and as such have the ability to integrate efficiently into quiescent or non-dividing cells. Their capacity to integrate their genetic material into target cells allows long term expression but can potentially cause insertional mutagenesis. Insertional mutagenesis occurs when viral DNA integrates in regulatory regions disrupting genes involved in growth
regulation and genetic stability potentially causing uncontrolled proliferation. For the PNS it is questionable whether long term expression is required or even wanted. As we have shown in this thesis temporary expression of a selected transgene, if of adequate duration and level, could rather be an advantage than a limitation. In the past decades non-integrating lentiviral vectors (NILV) have been developed. These vectors conserve the transduction efficiency of the classical vectors but do not integrate their genetic material. In the classical lentiviral vector, integration into the target cell’s genome is mediated by the viral protein called integrase. Integrase binds to both endings of the viral cDNA called the long terminal repeats (LTR) which prepares and allows its integration into the cell genome. The two main strategies that have been developed are the modification of the 5’ or 3’ LTR or the integrase protein greatly reducing the capacity to integrate. Unfortunatey NILV have been shown to have a significantly reduced level of transgene expression compared to the classical vectors mainly because episomal transgene expression is naturally inhibited (Philippe et al., 2006; Apolonia et al., 2007; Kantor et al., 2011). Furthermore these vectors are still not completely integration free. Both these problems are being currently assessed and several modifications have already assessed these issues demonstrating the potential utility of NILV in human gene therapy (Bayer et al., 2008; Pelascini et al., 2013). Finally, an alternative to viral vector-directed gene delivery could be the direct in vivo electroporation of expression plasmids in Schwann cells (Aspalter et al., 2009; Pereira Lopes et al., 2013). Plasmid-mediated gene transfer is a straightforward procedure, however, the strong electrical currents required for the electroporation, the relatively low transduction rate, and short-lived expression of the therapeutic gene indicate that in vivo plasmid-based gene transfer will in my opinion have limited utility.

Identifying novel targets
A key area of future research will be the gathering of further fundamental biological know-how on the cellular and molecular properties of Schwann cells in a regenerating nerve. A nerve injury induces major, tightly coordinated changes in gene expression in Schwann cells in the distal nerve. Together with the typical alignment of Schwann cells in pathways for growing axons, this creates a unique environment for successful regeneration. The signals that transform stable Schwann cells into the specialized repair cells in an injured nerve are not clearly understood and it is not known why Schwann cells gradually lose their pro-regenerative properties after longer times of denervation (Gordon et al 2011). Moreover, growing evidence indicates the existence of Schwann cells with distinct phenotypes preferentially supporting either motor or sensory neuron regeneration (Wright et al., 2014) which is relevant to direct growing axons to their correct target cells. To develop new strategies to stimulate axon regeneration, an analysis of the mechanisms that underlie the pro-regenerative properties of Schwann cells is needed. Conditional knock-out of the gene for the transcription factor c-Jun in Schwann cells has a negative impact on axon regeneration and results in simultaneous down-regulation of multiple pro-regenerative proteins in Schwann cells in an injured nerve (Arthur-Farraj et al., 2012). Neurotrophic factor expression in Schwann cells can
be enhanced by overexpression of c-Jun (Huang et al., 2015). C-Jun appears to be one, of perhaps a small set, of central transcriptional “master switches” which, in a cooperative manner, control the pro-regenerative phenotype of Schwann cells (Hung et al., 2015). If, in future experiments, the key regulatory complex of transcription factors is identified, these genes would be prime targets for Schwann cell gene therapy. “Transcriptional reprogramming” of Schwann cells is fundamentally different from PNS-gene therapy with a vector encoding a single neurotrophic factor because this would result in an elaborate repertoire of molecular changes (Huang et al., 2015), which would be particularly beneficial during the intermediate and later phases of the regeneration process when the ability of Schwann cells to support axonal outgrowth deteriorates (Gordon et al., 2011). The identification of the transcriptional “master switches” and studies on their combinatorial role in determining the repair-properties of Schwann cells may also shed new light on the occurrence of specific “motor” and “sensory” specific Schwann cells (Wright et al., 2014).

Path to a clinical study
The preclinical issues discussed above require several more years of systematic research in rodents. A clinical study to promote PNS regeneration by gene therapy is therefore currently hypothetical. However, the rapidly growing clinical experience with gene therapy for other neurological diseases and the steady advances in preclinical PNS-gene therapy support the conception of a framework for a future clinical study. The development of a PNS-gene therapy study will benefit particularly from experience with gene therapy for pain and neuromuscular diseases. In these disorders the sensory (pain) and motor neurons and muscle cells (neuromuscular disorders) are the primary target cells (Pleticha et al. 2014a, Cheever et al. 2015). Gene therapy for traumatic nerve injury has to include methods for safe gene transfer to the nerve Schwann cells as well. The following three topics need careful consideration in the context of preclinical-to-clinical translation of PNS-gene therapy and will be discussed below: 1. the route and mode of delivery of the vector, 2. the efficacy and safety of the vector, and 3. the choice of the patient population.

Route and mode of delivery of the vector
Pleticha and colleagues presented a roadmap for the preclinical evaluation of AAV-based genetic modification of dorsal root ganglia for clinical trials on pain (Pleticha et al., 2014a). This roadmap covers the essential preclinical steps needed to realize safe AAV-mediated targeting of primary sensory neurons in human patients. The human dorsal root ganglion (DRG) is approximately 50 times larger than the rat DRG (Shen et al., 2006). The rat motor neuron pool that supplies the nerves that innervate the forepaw (equivalent to the brachial plexus in humans) spreads over 0.5 cm of cervical cord, whereas the motor neuron pool innervating the brachial plexus in humans spans at least 10 cm of the spinal cord. The longest rat peripheral nerve, the sciatic nerve, is approximately 12 cm long while the nerves that innervate the human arm measure 80 to 100 cm. Therefore, translating gene therapy to the PNS of humans poses specific challenges with
respect to the route and mode of delivery of the vector because of the diverging anatomical dimensions of the rodent and human PNS.

To target primary sensory and motor neurons two routes of delivery have successfully been used: direct intraganglionic or intraspinal injection and intrathecal (IT) delivery. In the rat a single intraganglionic injection of an AAV vector results in efficient transduction of sensory neurons with very little if any spread of the vector to other locations (Mason et al., 2010). In contrast, intrathecal delivery results in transduction of sensory and spinal motor neurons and other non-neuronal cell types (Snyder et al., 2011). In humans, lumbar puncture is a relatively safe and standard technique to approach the cerebrospinal fluid and it would be feasible to deliver a vector to human DRGs and spinal motor neurons via this route. AAV vectors were delivered to the cat, the pig and to non-human primates using a lumbar puncture technique (Bucher et al., 2013; Gray et al., 2013; Pleticha et al., 2013; Samaranch et al., 2013; Dirren et al., 2014; Passini et al., 2014). If expression of a transgene in areas outside the DRG is not desirable, direct injection would be a requirement. Convection enhanced delivery (CED) relies on enhanced extracellular transport of a solution infused in tissue over an extended period of time [typically ranging from 20 minutes to 2 hours, (Krauze et al., 2005a; Krauze et al., 2005b)] and results in equal tissue distribution of the infusate. Minimally invasive intra-ganglionic gene transfer by CT-guided percutaneous injection and CED of AAV1 in lumbar DRGs of the pig resulted in 33% transduction of DRG neurons (Pleticha et al., 2014c).

Gene transfer to the injury-repair site of a human peripheral nerve will require a method to deliver a vector to a sural nerve graft inserted to connect the proximal and distal stump or to the nerve distal to the repair site. In rats, when relying on diffusion of the viral vector during a single manually guided 1 to 2 ul injection, the vector spreads in a nerve graft or in a nerve stump distal to a repair site over several millimeters (Tannemaat et al., 2008; Hoyng et al., 2014). Four injections placed at 5 to 8 mm distances from each other resulted in the transduction of a 4 to 5 cm long segment of rat sciatic nerve (Eggers et al., 2013). This injection technique results in rather unequal transduction of Schwann cells, with “hot spots” containing many transduced cells, and areas with no or very little transduced cells. CED carries macromolecules (such as Gadolinium-labeled Albumine for direct monitoring of the infusion process) over a distance of 1 cm in a rat nerve (Pleticha et al., 2014b) and over distances of 2.7 to 3.5 cm in a nerve of a non-human primate (Ratliff and Oldfield, 2001; Chen et al., 2011). Importantly, and in contrast to manual injection of small volumes of vector solution, CED resulted in an equal distribution of the infusate over the nerve. Future studies have to test whether CED of a viral vector to an injured nerve of a larger animal is a feasible option. Taken together, gene therapy for traumatic nerve injuries will benefit significantly from the encouraging observations in larger animals which show that the neuroanatomical dimensions do not preclude efficient gene delivery to the human PNS.
Safety and efficacy of the vector
Rigorous toxicity, and serological and cellular immune assessments have been performed for AAV1, AAV2, AAV5 AAV8 and AAVrh10. These serotypes have been used in clinical trials for LPLD [AAV-1; (Scott, 2015)], Canavan, PD and AD [AAV-2; (Leone et al., 2000; Kaplitt et al., 2007; Richardson et al., 2011; Leone et al., 2012; Bartus et al., 2013; Rafii et al., 2014)], liver mediated diseases [AAV5; (Grosios and Pañeda, 2013)], San Fillipo B [(AAV5, AAVrh10; (Tardieu et al., 2014), http://www.uniqure.com/news/182/182/ Clinical-trial-launched-to-treat-Sanfilippo-B-syndrome-using-gene-therapy.html] and Haemophilia B [AAV-5, AAV-8; (Nathwani et al., 2014)]. Although most humans have natural occurring neutralizing antibodies against AAV and treatment with AAV usually results in enhanced levels of these antibodies this occurred without detectable pathological effects (Salmon et al., 2014). Screening of patients following application of an AAV-1 vector to skeletal muscle resulted in seropositivity for AAV1 (Ferreira et al., 2014; Salmon et al., 2014). Antibodies which develop after the administration of AAV1 would not interfere with the therapeutic effect as the AAV vector has already delivered its therapeutic cargo. However, preexisting antibodies may interfere significantly with the transduction process as has been shown in some studies (Samaranch et al., 2013), whereas neutralizing antibodies had no effect on gene delivery with AAV after intraparenchymal or intrathecal injection in other studies (Gray et al., 2013).

Transduction differences between different serotypes in rat, larger animals and human complicates the choice of the vector for preclinical-to-clinical translation. The use of primary human tissue, either biopsy material or autopsy tissue, may prove to be critical in determining the optimal serotype for human patients. In our hands, cultured human peripheral nerve segments, obtained as left-over tissue from the operation theatre after nerve repair surgery, were transducible by lentiviral vectors (Tannemaat et al., 2007), whereas AAV-serotype testing showed that AAV2 was superior to eight other common serotypes investigated (Hoyng et al., 2015) (Figure 2). To date, AAV2 has been used in several clinical trials and, together with AAV1, is one of the best characterized serotypes. AAV2 outperforms other serotypes in human nerve segments and is therefore currently the leading vector for a clinical study that aims at enhancing the therapeutic potential of Schwann cells in a human peripheral nerve.

The choice of the patient population
Animal models will provide information about the efficacy and safety of the delivery technique, the vector and the transgene. However, the predictive value of animal studies is limited and eventually a study on a small number of human subjects with a PNS-lesion will be a necessary step in the translation process. An early gene therapy study for Alzheimer’s disease enrolled eight patients (Tuszynski et al., 2005). This study was too small to demonstrate efficacy, but showed that the gene therapy procedure was feasible and well-tolerated. The transgene was NGF, a growth factor relevant in the context of PNS-gene therapy. NGF expression was detectable in post-mortem brain tissue of a subject
that died of causes unrelated to the gene delivery procedure. This shows that a small clinical study can be highly informative and may form the basis of a larger randomized gene therapy trial (Cheever et al., 2015).

Nerve injury is a heterogeneous condition, ranging from brachial plexus injuries to distal injuries of the digital nerves that innervate the hand. Established guidelines on the design of clinical trials for the evaluation of novel treatments for nerve injury do not (yet) exist. Previous trials to test experimental treatments to promote nerve regeneration involved patients that sustained very different types of injuries. A recent successful clinical trial on the beneficial effect of electrical stimulation was performed on patients with complete transection injury of the digital nerve (Wong et al., 2015). An advantage of this study population is its relative homogeneity. Although a clinically meaningful degree of regeneration occurs spontaneously in these patients, enhanced sensory reinnervation was detectable following a short period of per-operative electrical stimulation. A follow-up trial with electrical stimulation as adjuvant treatment to surgical repair in patients with a severe brachial plexus injury, a severe lesion that causes serious dysfunction of the arm with prospects of only limited functional recovery of biceps function, is

Figure 2. AAV2-mediated transduction of a human sural nerve segment. Surplus human nerve material was obtained from the operation room and anonymized as stated in the code of conduct for responsible use of human tissue and medical research (Federa, 2011). Nine AAV serotypes were compared for their transduction efficiency by injecting $1.85 \times 10^{10}$ gc/cm nerve and culturing the nerve segments for 14 days. After 14 days nerve segments were immersion fixed with paraformaldehyde and 20 mm sections were prepared. The upper panel shows a section through a human sural nerve stained for GFP. The middle panel shows the same image stained for the nuclear stain Hoechst and the lower panel shows the merged images of the two panels. AAV2 transduced numerous cells that display the typical longitudinal shape of Schwann cells and this serotype was superior to all other serotypes tested. More details on this study can be found in Hoyng et al. (2015).
currently underway (www.clinicaltrials.gov). Thus, although electrical stimulation is a straight-forward procedure shown to be effective and safe in animals (Al-Majed et al., 2000; Brushtart et al., 2005; Gordon et al., 2008; Gordon et al., 2010; Haastert-Talini et al., 2011), tolerability and efficacy were first studied in a patient population that sustained a lesion with relatively moderate medical consequences before translating the procedure to lesions associated with long-lasting disability. A similarly cautious and phased translational path for PNS-gene therapy is mandatory.

Gene therapy for neurotrophic factors was well-tolerated in Alzheimer’s (NGF; (Rafi et al., 2014)) and Parkinson’s disease (Neurturin (Bartus et al., 2013)) patients. In contrast to neurotrophic factor gene therapy in the brain, neurotrophic factor gene delivery to an injured peripheral nerve is not without risk, as it may induce uncontrolled growth of axons, hypersensitivity and unwanted changes in Schwann cells (Mason et al., 2011). As discussed above, animal studies must first provide robust experimental evidence showing that control over the dose and the timing of viral vector-derived neurotrophic factor expression is effective, before gene therapy in a small group of patients with a nerve lesion can be undertaken. A gene therapy study in patients with a digital nerve injury, as performed for electrical stimulation, may reveal potential unwanted effects, and monitoring benefit is possible with the current battery of sensory tests (Wong et al., 2015). The vector, preferably an immune-inert regulatable AAV vector encoding NGF (a growth factor with stimulatory effects on sensory fibers) would be delivered to the denervated digital nerve by CED or by multiple injections along the 6 to 10 cm long digital nerve immediately following end-to-end repair. While this pilot study could be important in demonstrating safety and tolerability of PNS gene therapy, the therapeutic benefit of gene therapy for digital nerve injury patients is probably limited as this nerve displays a significant degree of spontaneous regeneration. Patients with a brachial plexus injury, a lesion which has a permanent negative impact, are a target group where gene therapeutic intervention could develop into a genuine adjuvant regenerative treatment strategy to further promote repair after neurosurgical intervention.

CONCLUSION:

Injury to the peripheral nervous systems can lead, in particular following proximal lesions, to extreme disabilities. The current gold standard, microsurgical repair, has reached optimal refinement. Unfortunately the functional outcome following surgery remains highly unsatisfactory and novel therapeutic strategies are needed. The delivery of therapeutic genes to the injured nerve through gene therapy might be a valuable addition to the surgeon’s armamentarium to promote nerve repair. Clinical phase I/II studies have demonstrated the safety of gene therapy for a variety of central nervous system disorders, including Canavan’s, Parkinson’s (PD) and Alzheimer’s disease (AD), retinal diseases and pain (REFs) and the first AAV-based therapeutic, a vector encoding...
lipoprotein lipase, is now marketed in Europe under the name Glybera. We have shown in this thesis that applying gene therapy to the peripheral nervous system could be a powerful tool to enhance regeneration. Our main findings were 1) the identification of modality specific pro-regenerative neurotrophic factors, 2) the creation of a potentially non immunogenic regulatable vector and 3) identifying a clinically applicable vector to deliver our therapeutic genes. Future research will build on these findings to further advance gene therapy towards a clinical strategy that can help to promote repair of the injured peripheral nerve in patients.
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