Chapter 10
Translating Gene Therapy for Pain from Animal Studies to the Clinic

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Abstract The use of gene transfer techniques, designed to effect the continuous release of analgesic peptides, offers the possibility to treat what may otherwise be intractable pain. In this chapter, we review the biology underlying this approach, the results of preclinical experiments in animal models, the human trials that have been completed, and prospects for the near-term future.

Keywords Gene therapy • Inflammatory pain • Neuropathic pain • HSV • Enkephalin • GABA

Introduction

The first animal experimental studies suggesting that gene transfer might be used to reduce pain-related behaviors were published just over 15 years ago. In 1998, Michel Pohl and coworkers reported that the rat proenkephalin A (pEnkA) gene could be delivered to sensory neurons of the rat dorsal root ganglion using a herpes simplex virus (HSV)-based vector, and when expressed by a fusion promoter, consisting of the region upstream from the HSV LAT core promoter

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and elements of Moloney murine leukemia virus long terminal repeat (LTR), resulted in a substantial increase in enkephalin concentration in the dorsal horn of spinal cord [1]. This was followed soon thereafter by a report from Steve Wilson, David Yeomans, and Joe Glorioso using a related HSV-based vector to express human preproenkephalin (PENK) under the control of the human cytomegalovirus immediate early promoter that demonstrated an antihyperalgesic effect after sensitization of sensory afferents by topical application of capsaicin or dimethyl sulfoxide indicating altered responsiveness of both C and A∂ fibers to stimuli which would normally produce hyperalgesia [2]. The authors speculated that the vector employed "or a similar recombinant herpes virus may be useful for treatment of chronic pain in humans" because, they noted, "hyperalgesia, which may be important in establishing and maintaining neuropathic and other chronic pain states, was selectively blocked by infection with this proenkephalin-encoding virus." They went on to point out that "advantages of this type of gene therapy would include precise anatomical targeting of the specific nociceptors transmitting pain impulses, the lack of systemic opioid adverse effects, and a long (weeks to months) duration of action."

About the same time, Mike Iadarola and colleagues reported that an adenovirus-based vector encoding a secretable form of the endogenous opioid β-endorphin injected intrathecally effectively transduced meningeal cells of the pia mater and that β-endorphin was released into the CSF from transduced cells, while having no effect on withdrawal from a thermal stimulus in normal animals, this treatment substantially attenuated the exaggerated withdrawal response after injection of carrageenan into the paw [3]. The authors suggested that "the simplicity of this meningeal–paracrine gene therapy approach, rapidity of expression, ease of application, and apparent lack of side effects open the possibility of a more general clinical utilization…the basis for a novel therapy for pain control."

These three studies set the stage for the principal approaches that have been considered for translating gene transfer to a useful therapy for chronic pain in patients. Readers of this book should require no convincing that chronic pain is an important clinical problem that produces a substantial adverse impact on quality of life for a large number of people, resulting not only in major medical costs but producing a general adverse economic impact on society. The focus of this review will be on the challenge inherent in moving a novel therapy for pain from preclinical animal studies, through human clinical trials, and ultimately into widespread use in patients. The extensive literature reporting the effects of different animal models and gene transfer vectors in experimental studies of pain has been reviewed recently [4], and the reader is referred to that review for a comprehensive overview. After the first initial studies 15 years ago, subsequent work has largely focused on examining the effect of different gene products in different animal models of chronic pain that are considered (more or less) to mimic human conditions.

Animal Studies of Gene Transfer for Pain

DRG Transduction by Skin Inoculation

One key feature of the HSV-based approach is that DRG neurons can be transduced noninvasively by skin inoculation (Fig. 10.1). As the first-order neuron in the path of nociceptive neurotransmission from the periphery to the brain, DRG represent an important target either for strategies that would directly silence these neurons or for expression of substances that can be released to modulate nociceptive neurotransmission at the synapse in the dorsal horn between primary afferent and projection neurons. HSV-based vectors expressing preproenkephalin have been demonstrated to reduce pain-related behaviors in the delayed phase of the formalin test [5], a polyarthritis model of inflammatory pain [6], the osteolytic sarcoma model of cancer pain [7], the spinal nerve ligation model of neuropathic pain [8], pertussis toxin-induced thermal hyperalgesia [9], the infraorbital nerve ligation model of trigeminal pain [10], pain related to inflammation of the pancreas [11, 12], a rodent model of monoarthritis [13], a diabetic model of neuropathic pain [14], and a model of interstitial cystitis/painful bladder syndrome [15]. The effect of the enkephalin-expressing vector in reducing hyperalgesia has been confirmed in a primate model [16].

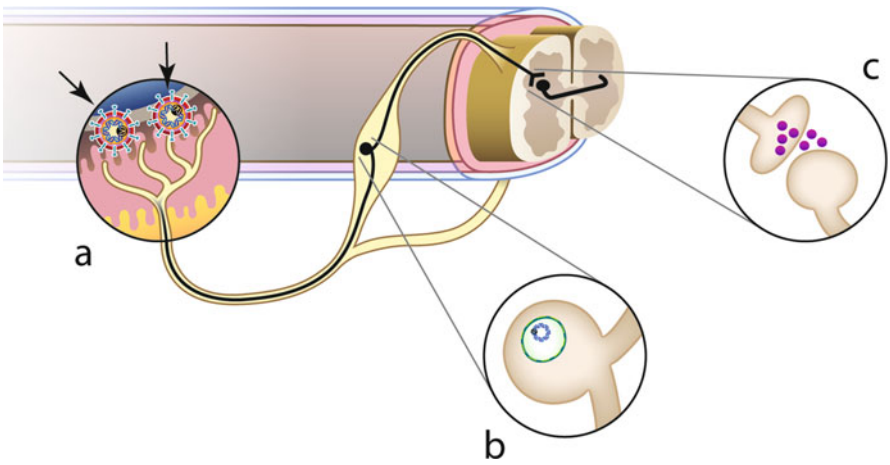

Fig. 10.1 (**a**) Non-replicating HSV vectors are injected into the skin (*arrows*, **a**) and taken up by nerve terminals. (**b**) The vector genome is retrogradely transported to the cell body in the DRG where the DNA establishes a persistent state as an intranuclear, episomal element. (**c**) Peptides coded by the vector genome are synthesized and transported to the nerve terminal where they may be released from the nerve terminal to act on second-order neurons in the pain pathway

An HSV-based vector expressing glutamic acid decarboxylase (GAD) to effect the release of the inhibitory neurotransmitter γ aminobutyric acid (GABA) reduces pain-related behaviors in rodent models of neuropathic pain resulting from spinal nerve ligation [17] or from painful diabetic neuropathy [18] and reduces pain-related behaviors [19] and detrusor overactivity [20] in a model of spinal cord injury. Unlike the preproenkephalin gene product that contains a leader sequence to direct transport into vesicles for appropriate processing and release, transgene-mediated GAD remains cytoplasmic and the resulting GABA is released from the transduced nerve terminals not by vesicular release, but rather through the GABA transporter (GAT1) that responds to the high concentration of intracytoplasmic GABA by functioning in reverse, to release GABA into the extracellular space [21].

Other genes that have been transferred by HSV vectors to produce excreted peptides to modify pain-related behaviors include glial derived neurotrophic factor (GDNF) in the SNL model of neuropathic pain [22]; interleukin 4 (IL-4) in the SNL model of neuropathic pain [23] and a bladder pain model [24]; a soluble fragment of the p55 tumor necrosis factor α receptor in the spinal nerve ligation model of neuropathic pain [25], an HIV nucleoside reverse transcriptase inhibitor neuropathy model [26] and HIV gp120 neuropathic pain model [27], and a bladder pain model [28]; IL-10 in the formalin model of inflammatory pain [29] and spinal cord injury pain [30]; and endomorphin 2 in neuropathic pain and in the CFA-induced model of inflammatory pain [31, 32].

One would anticipate that HSV-mediated gene transfer would be most effective when the transgene product is released because of the opportunity for paracrine effects to produce desired therapeutic results in nearby cells that had not been transduced by the vector. Nonetheless, HSV-mediated gene transfer of interfering RNAs to knock down expression of a pronociceptive gene product in peripheral neurons has also proven to be effective, as demonstrated by the effects of knockdown of the voltage-gated sodium channel NaV1.7 in inflammatory hyperalgesia [33] and in painful diabetic neuropathy [34]. More complicated approaches such as expression of a mutated glycine receptor have also been reported [35].

Gene Transfer by Intrathecal or Intraneural Delivery

Intrathecal injection of adenovirus has been used to deliver IL-2 in a model of neuropathic pain [36], the glial glutamate transporter (GLUT1) in inflammatory and neuropathic pain [37], the CBD peptide as a calcium channel inhibitor in neuropathic pain [38], a NaV1.3 shRNA in neuropathic pain [39], and endomorphin-2 in neuropathic pain [40]. Others have used adeno-associated virus (AAV) to deliver BDNF [41], IL-10 [42], or β-endorphin or IL-10 [43] in models of neuropathic and inflammatory pain, and lentivirus to deliver an interfering RNA [44]. Similar results have been reported using plasmid or plasmid combined with polymer-based delivery of anti-inflammatory cytokines in models of neuropathic pain [45–48]. Importantly, in at least one of the reports [43] intrathecal injection of an AAV serotype 8-derived vector was found to result in substantial infection of DRG neurons.

An alternative to skin inoculation with herpes vectors or intrathecal inoculation with other gene transfer vectors is direct injection into the DRG or nerve to transduce DRG neurons [49, 50] or into the trigeminal ganglion (TG) to transduce TG neurons [51]. In rodents, different serotypes of AAV produce infection of different classes of neurons (large diameter, producing myelinated fibers, vs. small diameter, producing unmyelinated fibers for instance). A related, less invasive alternative is direct intraneural injection to infect DRG neurons presumably by retrograde axonal transport, which can be used to express inhibitory neurotransmitters [52], inhibitory neurotransmitter receptors [53], or light-sensitive ion channels that can be activated by illumination through the skin [54].

Gene Delivery by Intraparenchymal Injection

Direct intraparenchymal injection of gene transfer vectors is an effective means to express gene products within the central nervous system, and injections into nuclei in the brain [55], brainstem, and spinal cord [56] have all been used in animal models to explore the role of specific nuclei for gene products in the phenomenon of pain perception. Of the three routes of gene delivery (skin inoculation, intrathecal or intraneural inoculation, and intraparenchymal injection), the latter in our view is the least likely to be translated to clinical application in the foreseeable future. Alternately, vectors can be injected directly into the end organ at the site causing pain, as has been demonstrated in a model of pancreatitis using an HSV-based vector [11, 12], and in models of arthritis using HSV [13] and lentivirus-based vectors [57].

Translation to Clinical Trial

General Considerations

The first question that needs to be addressed as one considers moving from preclinical animal models to the clinic investigations is to determine the appropriate pain patient population for an innovative gene therapy trial. While severe pain is disabling and often resistant to treatment, pain itself is not a fatal condition. Thus, even though a patient is suffering from pain that does not respond to standard of care therapy, the patient may not be appropriate for a treatment that involves permanent gene transfer, even if that treatment were proven to be effective in relieving pain. At a practical level, it is necessary first to identify an appropriate population for initial clinical testing of the gene therapy platform, typically in a Phase 1 trial. However, moving forward, a viable therapy will also require a large enough population of potential patients to make the treatment commercially viable. Thus, it is necessary to consider not only an initial safety study and subsequent proof-of-concept studies in

patients, but to also have a clear corporate development plan and target product profile (TPP) that will ultimately guide the therapy through clinical trials and into the marketplace.

The second question that needs to be considered is: what type of pain is the gene therapy designed to treat? What has not been discussed above is that in both humans and in animal models, there are significant and important molecular, biochemical, and electrophysiologic differences between inflammatory (nociceptive) pain, neuropathic pain, cancer pain, and other pain syndromes. Whether these known, and likely many unknown, differences are reflected in each individual patient is also an important factor to consider. The formalin test for instance is very accurate in predicting the morphine equivalent of novel opiate compounds in the treatment of inflammatory pain, but has not been as useful in predicting the response of patients to compounds acting through other mechanisms for the treatment of pain. There is also a deficiency in evidence to demonstrate that drugs that reduce pain-related behaviors in models of neuropathic pain in rodents are specifically effective in neuropathic pain in humans. But given the current state of the art, it would seem both reasonable and prudent to have preclinical evidence appropriate for the type of pain being treated prior to moving into clinical trials in patients.

Other issues to consider, such as whether the approach is appropriate for pain that is localized to one or more parts of the body, or might be applied to pain that affects patients more diffusely will depend on a combination of the rationale underlying the gene therapy approach, the preclinical data, and the clinical indication. And finally, there are practical issues that will determine whether gene therapy for pain is a viable treatment. For what indication(s) will the FDA approve the treatment? How many patients fit into that category? How heterozygous is the patient population in the chosen indication? Can the vector be produced at a cost that will make the treatment competitive with other available options? Using the developed TPP, what indication should first be tested clinically for the therapy? Ultimately, can the treatment be provided in a manner that would allow widespread use in the community, or would it be restricted to specialized medical centers? In the following sections, we describe the path we have taken to clinical trials of HSV-based vectors for the treatment of pain.

Preproenkephalin for Inflammatory Pain

The first HSV vector that we utilized in a clinical trial is a replication-defective HSV-based vector expressing human preproenkephalin (PENK). In preclinical animal studies, we initially demonstrated that an HSV vector rendered replication defective by the deletion of the gene coding for immediate early gene product ICP4 and expressing human preproenkephalin gene under control of the human cytomegalovirus immediate early promoter (HCMV IEp) reduced pain-related behaviors in the delayed phase of the formalin test of inflammatory pain [5], in the spinal nerve ligation model of neuropathic pain [8], and in the osteolytic sarcoma model of cancer pain [7].

In contrast to testing of novel small molecules where the Phase 1 dose-finding and safety trials are conducted in normal volunteers, Phase 1 gene therapy trials are typically carried out in patients. On theoretical grounds we were confident that delivery of the platform vector into the skin would be safe, because the number of vector particles injected into the skin would be orders of magnitude lower than the number of wild-type viral particles present in a typical cold sore. The safety of HSV vectors is also supported by extensive prior studies of replication competent/attenuated oncolytic HSV-based vectors that had been injected directly into the tumor of cancer patients and have advanced to Phase 3 clinical trials, or delivered peripherally as potential vaccines aimed at generating protective anti-HSV immunity (all of which have thus far failed in clinical trials). Even with this observed safety of replicating HSV vectors, our platform would be the first time a replication-defective HSV vector would be injected into the skin specifically to express potentially therapeutic genes directly in the targeted DRG. Therefore, we chose to carry out the Phase 1 safety dose-finding study in patients with intractable pain from terminal cancer.

The first step in moving forward toward a clinical trial was a public presentation to the Recombinant DNA Advisory Committee of the NIH which took place in June 2002. For this first clinical trial we proposed to employ a vector that utilized the HCMV IEp to drive transgene expression. The HCMV IEp is a transient promoter that drives expression of a transgene for a period of weeks/months in animals before the endogenous HSV latency mechanisms repress expression in vivo. We reasoned that this duration of expression should be sufficient to detect a clinical effect, and for this first Phase 1 trial represented an additional safety feature in that if adverse effects related to enkephalin expression were to occur those effects could be blocked by administration of naloxone until transgene expression would be naturally silenced.

Characterization of the Vector

1. Construction of the preproenkephalin-expressing HSV vector (NP2, now referred to as PGN-202) and complementing cell line. The NET (NEuronal Therapeutics) vector platform was constructed from an HSV genome engineered to be deleted for both the essential immediate early (IE) HSV genes ICP4 and ICP27 as well as for the UL55 coding region (Fig. 10.2). In addition truncations of the promoters for IE genes ICP22 and ICP47 removed their IE promoter nature, thereby constraining expression of these genes to complementing cells. The PENK transgene was inserted into the deleted essential ICP4 locus. Using this platform, two independent illegitimate recombination events would be required to generate a replication competent vector during manufacturing (which we have never detected). Further, the placement of the transgene into an essential gene locus mitigates the possibility of generating a replicating vector capable of expressing the transgene. The manufacturing cell line was generated by stably adding the HSV genes ICP4 and ICP27 individually into the genome of ATCC Vero (African

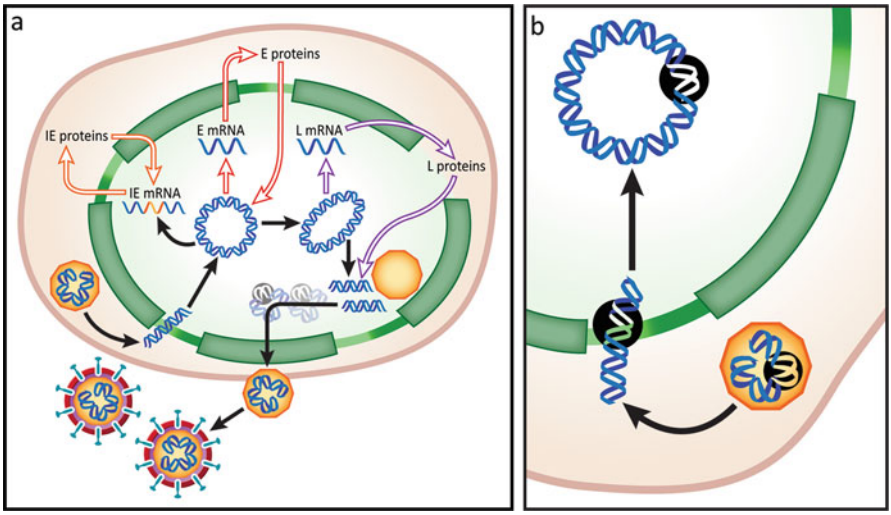

Fig. 10.2 (**a**) Wild-type HSV replication is characterized by a rigidly ordered temporal cascade that begins with the expression of immediate early (IE) genes, some of which are essential for the subsequent expression of early (E) and late (L) genes leading to the production of new virus particles. (**b**) Deletion of just one essential IE gene (indicated by *black circle*) renders the recombinant incapable of replication in any but the specific complementing cell line engineered to express the missing gene (or genes) from the cellular genome. The vector used for human trials fails to express 4 of the 5 IE genes

green monkey kidney) cells, and the resulting cell line was tested for the ability to complement replication-defective HSV production. The replication-defective HSV backbone was engineered in coordination with the complementing cell line in order to eliminate all overlaps between sequences flanking deleted essential IE genes in the vector and the IE gene sequences in the cell line in order to avoid recombination events that could generate replicating virus. The HSV vector backbone and complementing cell line was discussed with and approved by the FDA for cGMP production. NP2 was constructed by recombining a targeting plasmid containing the ICP4 flanking sequences and the human PENK expression cassette with a parental HSV vector backbone containing a GFP transgene in the ICP4 locus. The resulting recombinant was purified through three rounds of single plaque isolation and amplified into a seed stock. The seed stock was put through and passed a series of assays designed to confirm PENK expression, establish titer, demonstrate sterility, and establish endotoxin levels. The seed stock was then expanded and purified to produce material for GLP toxicology and biodistribution studies.

2. Toxicology of NP2. Following a pre-IND discussion with the FDA's Center for Biologics, Evaluation and Research (CBER), Office of Cellular, Tissue and Gene Therapies (OCTGT), a toxicology study using NP2 was performed

in compliance with US FDA (21 CFR Part 58). Four groups of 80 mice (40 male, 40 female) were dosed on study day 0 with either PBS or NP2 at 1×10^3, 1×10^5, or 1×10^7 PFU/animal, and 10 mice/sex/group/time point sacrificed on days 1, 7, 28, and 91. There was no evidence of treatment-related adverse effects as judged by clinical observations, body weight, or food consumption. Clinical pathology revealed no significant changes in hematology or clinical chemistry parameters at each time point. There were no treatment-related abnormalities in organ weights and macroscopic pathology. Histopathology examination revealed no test article treatment-related microscopic changes. Based on the predefined parameters of the toxicology study, administration of the test article according to the conditions of this study was well tolerated with no significant toxicity with the highest dose confirmed as the No Observed Adverse Effect Level (NOAEL).

3. Biodistribution of NP2. A total of 2400 tissues were harvested from the in-life phase of the biodistribution study. Using vector-specific primers, a GLP-validated QPCR assay was developed and DNA extraction efficiencies of target tissues performed. All tissues from the day 1 and day 7 cohorts were examined, as well as tissues from 10 animals of each sex from the control (PBS) group. In agreement with FDA guidance, because vector was observed only at the injection site, underlying muscle, and associated DRG on day 1 and day 7 samples, only these tissues were analyzed in the day 28 and day 90 cohorts. A total of 1180 tissue samples were evaluated. Of these, only samples from the injection site, underlying muscle, and associated DRG were found to have quantifiable vector sequences. There was no quantifiable dissemination to any other tissue. The FDA accepted the GLP biodistribution study as indicating that the vector platform is acceptably limited in distribution to the site of injection and the innervating DRG.
4. Production and certification of the master cell bank (MCB) and master viral bank (MVB). Prior to MCB production, a seed stock of complementing cells passed tests for sterility, mycoplasma, and endotoxin. A cGMP MCB of >200 vials with 1.0×10^7 cells/vial was produced at our GMP contract manufacturing organization (CMO) and tested for viable cell recovery, sterility, and mycoplasma and other standard safety and identity tests at external contract research organizations (CROs). For the NP2 vector, the seed vector stock was amplified at our CMO into an MVB using MCB cells. Final identity of the NP2 MVB was confirmed, according to guidance received from the FDA by a panel of tests including whole genome sequencing and a panel of safety, identity, and strength/potency testing parameters that constitute the Certificate of Analysis (COA).
5. GMP production of NP2 vector for human trial. NP2 was produced at our CMO using a proprietary multistep manufacturing and purification process. Sufficient GMP NP2 was produced and passed a final panel of safety, identity, and potency assays as required by the FDA to proceed with human clinical trials.

Clinical Trials of the Enkephalin-Expressing Vector

We conducted a multicenter, open-label dose-escalation, Phase 1 clinical trial of NP2 (now PGN-202) in subjects with intractable focal pain caused by cancer. NP2 was injected intradermally into the dermatome(s) corresponding to the radicular distribution of pain. The primary outcome was safety. As secondary measures, efficacy of pain relief was assessed using an 11-point Likert (0–10, 0 no pain and 10 worst pain) numeric rating scale (NRS), the Short Form McGill Pain Questionnaire (SF-MPQ), and concurrent opiate usage. Ten subjects with moderate to severe intractable pain scoring at least five on the NRS pain scale despite treatment with more than 200 mg/day of morphine (or equivalent) were enrolled into the study. Treatment was well tolerated with no study agent-related serious adverse events (SAEs) observed at any point in the study. Subjects receiving the low dose (1×10^7 PFU) of NP2 reported no substantive change in pain. Subjects in the middle (1×10^8 PFU) and high (1×10^9 PFU) dose cohorts reported pain relief as assessed by NRS and SF-MPQ. In summary, treatment of intractable pain with NP2 was well tolerated. There were no placebo controls in this relatively small study, but the dose-responsive analgesic effects suggested that NP2 may be effective in reducing pain and warranted further clinical investigation [58].

Following the completion of the Phase 1 study, we carried out a randomized, double-blind, placebo-controlled, multicenter Phase 2 clinical trial to investigate the impact of NP2 in patients with intractable pain due to malignancy. The primary endpoint of this study was change in the average NRS pain score (0–10) from the pretreatment period to the posttreatment period (day 3 to day 14 post-study drug administration) between NP2 and placebo-treated subjects. In comparison to the Phase 1 study, this Phase 2 study increased the qualifying pain score from an NRS pain score (5–7), increased the number of dermatomes to be treated (2–4), and allowed concomitant chemotherapeutic treatment with a dose of 3×10^8 PFU. In addition, subjects were allowed to receive two additional open-label doses of NP2, separated by 4–10 weeks, following the blinded portion of the study. A total of 33 patients were randomized with 30 patients (15 active, 15 placebo) included in the modified intent to treat (mITT) population. The primary therapeutic endpoint of the study was not met in this small exploratory Phase 2 study. Importantly, as in the Phase 1 study, the treatment in the Phase 2 trial was well tolerated. Details of the study design, results, and follow-up data will be provided in a future publication.

Clinical Trial of HSV-GAD in Neuropathic Pain

Because there is substantial evidence that implicates reduced spinal GABAergic inhibition in neuropathic pain [59, 60], we constructed a vector expressing GAD to produce GABA in transduced cells. In the T9 hemisection model of central

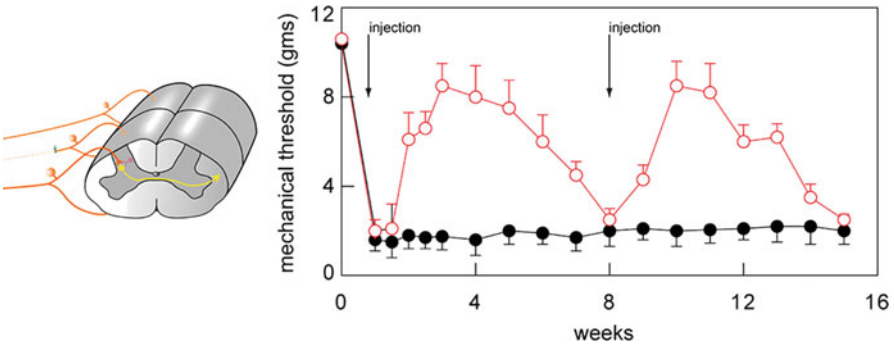

Fig. 10.3 In the selective L5 spinal nerve ligation model of neuropathic pain (*left*), subcutaneous injection of the GAD-expressing vector 1 week after nerve ligation substantially reverses mechanical allodynia. The effect persists for about 6 weeks, consistent with the time course of expression driven by the HCMV IEp from the context of the HSV vector genome, and is reestablished by reinoculation of the vector at 8 weeks

neuropathic pain resulting from spinal cord injury, subcutaneous inoculation of a GAD-expressing vector in the feet resulted in a substantial reduction in mechanical allodynia and thermal hyperalgesia in the hind limbs [19], an effect that was blocked by intrathecal injection of bicuculline or phaclofen [19]. In the SNL model of neuropathic pain, subcutaneous inoculation of the GAD-expressing vector 1 week after SNL produced a substantial antiallodynic effect that peaked about 2 weeks after inoculation and persisted for 6 weeks [17] (Fig. 10.3). The antinociceptive effect of vector-mediated GABA expression in these neuropathic pain models was substantially greater than that of vector-mediated enkephalin or endomorphin release. Like the antiallodynic effect of transgene-mediated enkephalin, the antiallodynic effect of the GAD-expressing vector waned over a time course of weeks, but was reestablished by reinoculation.

Based on the preclinical data, we went to the RAC at NIH in March 2009 with a proposal for a clinical trial of HSV GAD, and subsequently completed a pre-IND meeting with the FDA and achieved concurrence on IND enabling preclinical animal studies, on manufacturing and testing parameters, and on the clinical trial design. Similar to the PENK-expressing vector, the NET GAD-expressing vector (PGN-305) is a replication-defective HSV-1 recombinant modified as follows: (1) complete deletions of the viral ICP4, ICP27 (UL54), and UL55 genes; and (2) insertion of a human cytomegalovirus (HCMV) immediate early promoter-driven human glutamic acid decarboxylase (GAD65) expression cassette within both copies of the deleted ICP4 loci. The extent of the ICP4 deletion results in the removal of the upstream promoter sequences of the immediate early viral genes: ICP22 and ICP47. Utilizing the clinical manufacturing schema, we produced a PGN-305 seed stock, a toxicology lot, and a GMP MVB. A GLP toxicology/biodistribution study was performed at a

preclinical animal CRO. Rats were dosed by single (1×10^5 or 2×10^7 PFU per paw) or multiple (2×10^7 PFU per paw, 3 times over 2 weeks) subcutaneous injections in the plantar surface of the paw. There were no PGN-305-related changes in clinical signs, body weights, body weight changes, food consumption, clinical pathology parameters (hematology, coagulation, and clinical chemistry), and gross necropsy findings. Rats treated with repeat doses of PGN-305 did not have elevated anti-GAD65 Ab concentrations compared to vehicle controls or rats treated with one dose of PGN-305. Based on these results, the NOAEL was determined to be 2×10^7 PFU/paw, the highest dose tested.

Our proposed clinical trial includes two combined phases designed to evaluate the safety and efficacy of intradermal delivery of PGN-305 in subjects with painful diabetic neuropathy affecting the legs. The Phase 1 component is an open-label dose-escalation trial to evaluate safety of 3 escalating PGN-305 doses and determine the maximum tolerated dose (MTD). The Phase 2a component of the trial is a multicenter, randomized, double-blind, placebo-controlled trial that compares the MTD to placebo for further evaluation of safety and efficacy. Potential participants will be identified from patients seen in clinics at the participating sites having (1) Type 2 diabetes complicated by neuropathy confirmed by a score of ≥ 3 on the Michigan Neuropathy Screening Instrument (MNSI); (2) painful diabetic neuropathy with pain primarily in the legs for at least 6 months, with average daily pain score over 21 days screening period (with at least 14 days NRS assessments completed) ≥ 5 on the 0–10 NRS despite treatment with standard pain medications; and importantly, preserved nerve fibers in the skin of the lower leg ($\geq 2.5/mm^2$) determined by punch biopsy. The final inclusion and exclusion criteria will not be confirmed until just prior to filing of the IND.

For the dose-escalation safety portion of the study, PGN-305 will be delivered at doses of 0.5×10^8, 0.5×10^9, or 0.5×10^{10} PFU per leg and both legs will be dosed per subject; thus the total dose per participant will be 1×10^8, 1×10^9, or 1×10^{10} PFU cohorts. The drug will be injected intradermally in approximately 20 sites (100 μl per site) distributed over each lower leg from just above the ankle to just below the knee. For the efficacy portion of the trial, the dosing (route, volume) will be the same except that only the maximum tolerated dose of PGN-305 will be used and subjects will receive injections of PGN-305 or placebo delivered in an identical volume and number of injections.

The primary safety outcome for both the Phase 1 and Phase 2a stages will be assessed by careful evaluation of adverse events and serious adverse events. The primary efficacy variable for the phase 2a stage of the study will be changes in the average daily NRS pain score from baseline to the average daily NRS score of days 3–14 post-dosing. Secondary efficacy variables will include change in average daily NRS from days 14 to 28 post-dosing, change in the short form McGill pain questionnaire (SF-MPQ) score, change in Chronic Pain Sleep Inventory, change in the SF-12 physical component score and mental component score, and the proportion of subjects meet a 30 % reduction in the average daily numerical rating of pain. We anticipate initiating the study and enrolling patients in early 2015.

Future Directions

The first clinical trials completed with PGN-202 expressing PENK and the first clinical trial proposed for PGN-305 expressing GAD utilize the HCMV IEp to drive transgene expression because of the natural silencing of gene expression as a safety feature for early-stage trials. However, an important characteristic of our HSV vector technology includes long-term transgene expression from the episomal vector genome. Utilizing the HSV latency-associated (LAP2) promoter we have been able to demonstrate prolonged biologically active transgene expression up to 6 months after inoculation [61, 62], representing the duration of the experiments. Because LAP2 produces what appears to be lifelong expression of the latency-associated transcripts in natural infection, we doubt that 6 months is the limit of LAP2-driven transgene expression. More recently, we have shown that using the LAP2 promoter to drive a tet-on transactivator and with the transgene under the control of a minimal promoter linked to a tet-responsive element allows for expression in the DRG to be regulated under the control of oral administration of doxycycline [63]. Thus, if either or both vectors were to prove effective in the treatment of pain Phase 1/2a trials, there are options for achieving long-term or regulatable expression as we advance the HSV-based NET platform forward through clinical development toward the market.

In another line of work exploring HSV-mediated gene transfer to the DRG, we have demonstrated in a number of different preclinical models of polyneuropathy that HSV vectors expressing neurotrophic factors and delivered to the DRG by skin inoculation are effective in preventing nerve degeneration caused by drug intoxication, chemotherapeutic agents, or diabetes [62, 64–66]. In a separate project, we are proceeding forward toward a clinical trial to determine whether treatment with a vector expressing neurotrophin-3 can prevent the development of chemotherapy-induced neuropathy in patients receiving high dose chemotherapy for the treatment of cancer.

Concluding Thoughts

It is not uncommon in the current climate, particularly since NIH grant review has placed ever greater emphasis on “significance” and the implications of research proposals to have implications for treating human disease, to see basic science discoveries described in terms of their potential to be translated into novel treatments for disease. But what we have found in our experience is that the path from preclinical animal studies to the development of a human treatment is neither simple nor straightforward. Our group has been working with HSV-based vectors since 1989 and presented the proposal for our first clinical trial to the RAC in 2002. We have completed two clinical trials, dosed more than 40 subjects with some of the subjects receiving up to three doses, and to date no study agent serious adverse

events have been observed. Trials of novel treatments designed to reduce pain have the advantage that the primary readout (the patient's perception of their pain) is immediate and continuous, thus allowing for relatively short studies. The disadvantage of studying treatment for pain is the substantial placebo effect that is not limited to gene therapy trials but has frustrated the larger clinical pain research community [67, 68]. We anticipate that within the next 3–5 years we should be able to determine whether the approach of HSV-mediated gene transfer using the NET platform to treat indications such as pain or the prevention of neuropathy will introduce a viable alternative for the treatment of these conditions.

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