Published in final edited form as:

J Gene Med. 2018 July ; 20(7-8): e3026. doi:10.1002/jgm.3026.

Human interleukin-10 delivered intrathecally by self-complementary adeno-associated virus 8 induces xenogeneic transgene immunity without clinical neurotoxicity in swine

Mark D. Unger^{1,3}, Josef Pleticha^{1,3}, Lukas F. Heilmann¹, Laura K. Newman¹, Timothy P. Maus², and Andreas S. Beutler^{1,*}

¹Departments of Anesthesiology and Oncology, Mayo Clinic, Translational Science Track, Mayo Graduate School, Rochester, MN 55905, USA

²Department of Radiology (Section of Interventional Pain Management), Mayo Clinic, Rochester, MN 55905, USA

Abstract

Introduction—Intrathecal interleukin-10 delivered by plasmid or viral gene vectors has been proposed for clinical testing because it is effective for chronic pain in rodents, a potential therapeutic for various human diseases, and was found to be non-toxic in dogs, when the human interleukin-10 ortholog was tested. However, recent studies in swine testing porcine interleukin-10 demonstrated fatal neurotoxicity.

Aims—To deliver vector-encoded human interleukin-10 in swine, measure expression of the transgene in cerebrospinal fluid, and monitor animals for signs of neurotoxicity.

Results—Human interleukin-10 levels peaked 2 weeks after vector administration followed by a rapid decline that occurred concomitant with the emergence of anti-human interleukin-10 antibodies in the cerebrospinal fluid and serum. Animals remained neurologically healthy throughout the study period.

Conclusions—This study suggests that swine are not idiosyncratically sensitive to intrathecal interleukin-10 because, recapitulating previous reports in dogs, they suffered no clinical neurotoxicity from the human ortholog. These results strongly infer that toxicity of intrathecal interleukin-10 in large animal models was previously overlooked because of a species mismatch between transgene and host. The present study further suggests that swine were protected from interleukin-10 by a humoral immune response against the xenogeneic cytokine. Future safety studies of interleukin-10 or related therapeutics may require syngeneic large animal models.

Keywords

Interleukin-10; Intrathecal; Adeno-associated virus vector; Xenogeneic; Swine; Animal model

*To whom correspondence should be addressed: Andreas S. Beutler, M.D., Associate Professor, Departments of Anesthesiology and Oncology, Mayo Clinic, Translational Science Track, Mayo Graduate School, 200 First Street SW, Rochester, MN 55905, USA, Tel.: +1-507-774-1873; fax: +1-507-293-1058, beutler.andreas@mayo.edu.

³These authors contributed equally to this work.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

INTRODUCTION

The anti-inflammatory cytokine interleukin-10 (IL-10) is a therapeutic candidate for several diseases of the nervous system.¹ Numerous groups have delivered plasmid- and vector-encoded IL-10 to cerebrospinal fluid (CSF) in different animal species to determine safety and efficacy in models of neuropathic pain and other chronic nervous system diseases.¹⁻⁴ Outside the nervous system, IL-10 has been tested in patients with non-life threatening disorders, including rheumatoid arthritis,^{5,6} vasculitis,⁷ and psoriasis,⁸ among others.

In a recent preclinical study, intrathecal (IT) IL-10 was tested in a swine model wherein porcine IL-10 (pIL-10), encoded by self-complementary adeno-associated virus serotype 8 (scAAV8) vectors, was delivered via lumbar puncture (LP).⁹ Animals developed fatal neurotoxicity and a neuropathologic workup demonstrated sterile, lymphocyte-predominant meningitis.⁹ Fatality was positively correlated to IL-10 concentration in CSF and after an extensive analysis, no pathogen was identified to be the cause of demise.

Prior to the above study, IT IL-10 had not been associated with death, neurotoxicity, or another adverse effect based on all available preclinical data.⁹ A closely related study in dogs found no evidence of toxicity after IT administration of human IL-10 (hIL-10).¹⁰ Literature review revealed that testing of IL-10 in CSF had involved xenogeneic IL-10 in both small and large animal species or syngeneic IL-10 in small animal species only.⁹ The fatal swine study was the first to test syngeneic IT IL-10 in a large animal model.

IL-10 is not the first investigational agent associated with a variable safety profile and other cytokines have posed similar challenges to the interpretation of preclinical data.^{11,12} For example, interleukin-2 is likely to cause toxicity regardless of the host species because systemic administration of vector-encoded interleukin-2 caused comparable adverse effects in rat and humans.¹³ Activity of cytokines human interferon-gamma and human interleukin-12 demonstrated activity in non-human primates but not in other large animal species.^{12,14} These observations suggest that preclinical testing requires several host-transgene combinations to assess toxicity and activity of cytokines.^{14,15} Given the array of gene therapy products under development, the United States Food and Drug Administration can offer no single guidance applicable to the design of all preclinical safety studies.¹⁶ Instead, a product-specific approach is recommended with a focus on lessons learned and practical considerations to improve translatability of preclinical data.¹¹

Two factors could account for conflicting observations regarding safety of IT IL-10 in dogs and swine. Possibly, a unique sensitivity of swine exists, i.e., relative resistance against adverse IL-10 effects in dogs. Alternatively, the immunological compatibility between transgenic IL-10 and its host influenced the observed outcomes. In dogs the human ortholog was used,¹⁰ i.e., a xenogeneic form was tested, while in swine the porcine form was used, i.e., a syngeneic model.⁹ To improve interpretation of available large animal data regarding safety of IT IL-10, we sought to study xenogeneic IL-10 in a second large animal species. We delivered vector-encoded hIL-10 to the IT space in swine and found the treatment to be well-tolerated by all animals.

MATERIALS AND METHODS

Animals

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Mayo Clinic and in accordance with relevant national guidelines for the care and use of laboratory animals. A total of $n = 4$ domestic swine of Landrace origin were used throughout the study. All animals were examined twice daily by certified veterinary personnel and showed good appetite, growth, and remained neurologically intact during the 12-week study.

Investigational agent

The scAAV8 vector expressing hIL-10 complementary DNA was the same construct as used previously.¹⁰ Table 1 outlines the vector doses used. The vector was produced by Penn Vector Core (University of Pennsylvania, Philadelphia, PA) and assessed for purity and endotoxin concentration. Purity was measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis, determined to be greater than 90%, and endotoxin concentration was measured by the limulus amebocyte lysate assay, determined to be less than 1.0 units/ml. The expression cassette consisted of CMV promoter/enhancer and bGH polyA sequence. The vector was packaged in AAV8 capsid.

IT vector administration

Vector was administered as previously described.⁹ Briefly, procedures were performed using strict aseptic technique and under general anesthesia induced by intramuscular injection of tiletamine/zolazepam (Telazol), xylazine, and glycopyrrolate. Animals were intubated and anesthesia was maintained by 1.5–2.5% isoflurane in 50% O₂ titrated to effect. The lateral recess of the lumbar IT space was accessed under computed tomography fluoroscopic (CTF) guidance. The lumbosacral spine was visualized on a pre-procedure CTF scan using a Beekley biopsy grid (Beekley Medical, Bristol, CT). A 25 gauge, Quincke point spinal needle (Kimberly-Clark, Irving, TX) was advanced through the interlaminar space and into the subarachnoid space lateral to the spinal cord. IT position of the needle tip was verified by imaging after injection of 200 μ l Omnipaque 240 contrast media (GE Healthcare Inc., Princeton, NJ) diluted 1:3 in phosphate buffered saline to a final concentration of 80 mg/ml. Vector was administered using a programmable syringe pump (model #10050, Chemyx Inc., Stafford, TX) at a flow rate of 1 ml/min. Following infusion, the needle was left in place for 3 minutes to prevent reflux of injectate. A post-injection CTF scan was obtained to document successful delivery. Each injection was completed successfully on the first attempt.

Serial CSF and serum collection

CSF and serum was collected as previously described.⁹ For baseline expression of hIL-10 in CSF and anti-hIL-10 antibodies in CSF and serum, CSF and blood samples were obtained at the week 0 time point immediately before vector administration.

Measurement of hIL-10 concentration

A hIL-10 enzyme-linked immunosorbent assay (ELISA) kit (Pierce Biotechnology, Rockford, IL) was used to quantify the hIL-10 levels in CSF. The samples were analyzed in duplicate exactly according to the manufacturer's instructions. CSF of animals that had not received the vector served as negative controls. pIL-10 levels of up to 600 pg/ml, the upper limit of the hIL-10 standard curve range, were tested and no cross-reactivity was observed.

Detection of anti-hIL-10 antibodies

The anti-hIL-10 antibodies were detected by ELISA in the CSF and serum using the method described previously.¹⁰ Briefly, the experimental samples were serially diluted in a coating solution (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MA) and adsorbed to a polystyrene 96-well plate (Corning, Corning, NY) in duplicate. The plate was blocked with 1% bovine serum albumin and recombinant hIL-10 at 1 µg/ml (Shenandoah Biotechnology, Inc., Warwick, PA) was added to the plate, followed by biotinylated anti-hIL-10 detection antibodies at 0.3125 µg/ml (Pierce) and streptavidin-horseradish peroxidase conjugate at 1:400 (Pierce). The plate was developed by adding 3,3',5,5'-tetramethylbenzidine substrate and the stop solution (Kirkegaard). A total of 3 washes with 2 mM imidazole buffered saline and 0.02% Tween-20 were performed between steps. As a standard, anti-hIL-10 IgG (Abcam, Cambridge, MA) of known concentrations was adsorbed to the plate instead of experimental CSF or serum, resulting in a dynamic range of the assay of 12.5–0.125 µg/ml. The hIL-10 capturing capacity of the experimental samples (consisting of antibodies of all isotypes) was thereby quantified as an equivalent of the capturing capacity of respective IgG at a given concentration.

Detection of anti-AAV8 capsid antibodies

CSF and serum obtained from each animal prior to vector injection and at sacrifice was analyzed for the titer of anti-AAV8 capsid antibodies. The assay was performed as described by Martino *et al.*¹⁷ Briefly, 2.0×10^5 HeLa cells/well were planted in a 24-well cell culture plate. Serial dilutions of CSF or serum (1:4, 1:10, 1:100, 1:1,000, 1:10,000) were incubated with 8.0×10^8 genome copies of scAAV8 for 30 minutes to achieve a multiplicity of infection of 4,000. The mixture was then added to the plate in triplicate. After incubation for 24 hours, the cells were fixed with 4% phosphate-buffered saline and the highest dilution resulting in greater than or equal to 50% inhibition of transduction compared to pre-immune serum was determined by fluorescence-activated cell sorting.

Euthanasia and tissue harvest

Animals were deeply anesthetized via intramuscular tiletamine/zolazepam and xylazine and euthanized with intravenous injection of pentobarbital. A panel of peripheral organ samples was collected, including heart, kidney, liver, lung, skeletal muscle, and spleen. Select dorsal root ganglia, cerebellum, forebrain, and spinal cord segments were collected, snap-frozen on dry ice, and stored at -80°C . No gross pathology was observed upon dissection and harvest of all animal tissues.

Histology

Spinal cord, dorsal root ganglia, forebrain, and cerebellum were cryoembedded in optimal cutting temperature compound (Sakura Finetek USA Inc, Torrance, CA). Tissues were sectioned to a thickness of 20 µm using a cryostat (Leica Biosystems, Wetzlar, Germany) and stained with H&E.

Vector biodistribution by quantitative polymerase chain reaction (qPCR)

DNA from the forebrain, heart, kidney, liver, lung, skeletal muscle, and spleen was isolated by the DNAeasy Blood and Tissue Kit (Qiagen, Germantown, MA). The SYBR Green (Roche Diagnostics, Mannheim, Germany) based qPCR was used and the number of AAV vector genome copies was normalized to the number of copies of the diploid host genome. hIL-10- (forward, 5'-CTACGGCGCTGTCATCGAT-3'; reverse, 5'-TGGAGCTTATTAAAGGCATTCTTCA-3') specific primers were used for detection of the vector target sequence. Swine β-actin- (forward, 5'-CTGCGTCTGGACCTGGCTG-3'; reverse, 5'-ACGCGGCAGTGGCCATCTC-3') specific primers were used as a reference. The amplification was performed using the LightCycler 480 (Roche).

RESULTS

Clinical course and tolerability of LP

Each animal was observed daily by veterinary staff for changes in behavior, vocalizations, activity level, affect, appetite, bowel and bladder function, gait, and gross motor function. All parameters were found to be within normal limits for all animals before and after vector administration. Throughout the study, no animal displayed signs of weakness, ataxia, seizure, overt pain, or moribundity. All animals survived to the primary study endpoint of 12 weeks and were sacrificed at that time. Table 1 displays a summary of animals used.

CSF collection via LP required the induction of general anesthesia and intubation. Despite the frequency and repetition of these procedures, no animal developed signs of respiratory dysfunction, CSF leakage, or infection. As shown in Figure 1, all animal weights trended upwards throughout the study suggesting overall well-being. Gross analysis of CSF at the time of each LP revealed clear fluid without turbidity or other evidence of infection at all time points. The model allowed for frequent CSF sampling using an approach similar to bedside methods and provided a longer time series with higher resolution of IT IL-10 secretion kinetics compared to similar studies in swine and dogs.^{9,10}

Transgene expression in CSF

Baseline hIL-10 concentrations in CSF were measured immediately prior to vector administration and weekly thereafter. As shown in Figure 2, hIL-10 was not detected in CSF of any animal at baseline and was found to be expressed in CSF during the first week after vector administration. The mean peak CSF hIL-10 concentration of 3.0×10^4 pg/ml was measured during week 2. Levels decreased each week thereafter, approximating baseline levels by the sixth week and persisted there throughout the remainder of the study.

Xenogeneic immune response

A xenogeneic immune response was observed together with (inversely correlated to) the rise and fall of hIL-10 in CSF (Figure 2, inverted bars). Anti-hIL-10 antibodies were detected in CSF and sera beginning at week 2. Antibody levels peaked by week 7 in CSF and serum (mean 7.0×10^8 pg/ml and 8.0×10^9 pg/ml, respectively) and were sustained above baseline for the remainder of the study (mean 3.0×10^8 pg/ml and 6.0×10^9 pg/ml, respectively).

Neuropathologic assessment

Gross examination at the time of necropsy did not reveal evidence of pathology in any organ system. Microscopic examination performed on nervous tissues did not demonstrate evidence of meningeal cellular infiltration at the cerebellum, forebrain, spinal cord, or dorsal root ganglion (Figure 3A – 3D). There was also a complete lack of parenchymal infiltration by immune cells at all sites. These observations differed markedly from the microscopic evidence of sterile meningitis observed for moribund animals that received pIL-10 delivered under identical conditions.⁹

Vector biodistribution and immune response to scAAV8 capsid

qPCR was used to measure the presence of scAAV8 genomes in peripheral tissues. Analysis revealed minimal spread with less than 0.01 transgene copies/diploid host genome in all samples, including the liver (Figure 4). For comparison, the dog study of IT scAAV8-hIL-10 resulted in over 50-fold greater viral spread to the liver¹⁰ than detected here. It appears that the diminished systemic spread of vector genomes to peripheral organs may be due to the different means of vector delivery. Here, a computed tomography fluoroscopic (CTF)-guided LP was used to access the lumbar IT space as previously described,¹⁸ while the dog study externalized a lumbar catheter through the cisterna magna.^{10,19} The use of a lumbar catheter may have allowed vector escape from the CSF to the blood through the surgical incision in the atlantooccipital membrane. While species differences between the two studies and some difference in the time course of the experimental design may limit the direct comparability of the studies, the results may suggest that LP (without catheter implantation) may decrease the rate of vector biodistribution compared with the method of IT vector administration used in our previous study in dogs.¹⁰

As shown in Table 2, IT administration of scAAV8 resulted in a serum anti-capsid titer rise above baseline at the time of sacrifice for all animals and a CSF titer rise above baseline in 3 of 4 animals. The range of titer values recorded for the humoral response to the AAV8 capsid were greater in CSF compared to serum but no correlation between anti-AAV8 titers and hIL-10 levels was seen. These findings suggest that the therapeutic potential of repeat IT delivery may be limited if an identical AAV serotype is used in subsequent interventions, an interpretation that would be consistent with previous reports of neutralizing anti-AAV capsid antibodies in other experimental paradigms.^{10,20}

DISCUSSION

In a swine model of IT gene therapy, we found hIL-10 to be safe and not associated with the development of neurotoxicity for at least 12 weeks. This observation contrasts our recent

report where swine receiving IT pIL-10 (delivered via scAAV8 as in the present study) succumbed to fatal meningitis no later than the fourth week after administration.⁹ No pathogen responsible for demise was identified in that study and fatality was correlated to peak CSF IL-10 concentration. The same swine breed, experimentalists, and methods were involved in both studies. The variable that differed in the present study was the use of a xenogeneic IL-10 ortholog.

The present study provides evidence that swine are not uniquely sensitive to any IT IL-10; if this were the case, the present study should have revealed neurotoxicity. We therefore conclude that swine are not idiosyncratically sensitive to IT IL-10.

Instead, we conclude that the host-transgene compatibility of the transgene may be a significant factor in the development of neurotoxicity. Neither dogs (previous study¹⁰) nor swine (enclosed study) suffered toxicity from hIL-10, while both species developed anti-transgene product antibodies quickly (within 2 weeks) that neutralized hIL-10. Neurotoxicity was observed only when swine received the porcine ortholog, i.e., syngeneic IL-10, in which case toxicity unfolded without inhibition, i.e., without the self-limiting effect of serologic immunity that could not develop because the transgene was syngeneic.

Small animal studies of IT IL-10 did not identify neurotoxicity regardless of the IL-10 ortholog used, suggesting that preclinical testing of IL-10 (and potentially other biologics) should not be considered complete until syngeneic studies in large animals have been conducted. Such has been the case for other cytokines^{13–15} Furthermore, our study implies that other large animal species may also be sensitive to toxicity of IL-10 only when the syngeneic form is tested, such as canine IL-10 in dogs, a prediction that could be addressed in future studies.

We observed a transient rise in CSF IL-10 concentrations during the first 2 weeks of study followed by a decline and return to baseline which persisted for the remainder of the study. The rise and fall of IL-10 occurred concurrently with the emergence of anti-hIL-10 antibodies in CSF and serum, signifying a xenogeneic immune response against the human ortholog of IL-10. Such xenogeneic immunity seems to occur in many large animal models (and potentially not to the same degree in rodents), as previously shown by others.^{10,20} While xenogeneic immunity likely contributed to the sustained decline in CSF IL-10 levels, an additional possibility is that the CMV promoter may have been silenced. Gene expression driven by the CMV promoter has been shown to decline over time in some types of neural tissue and may be due to cellular recognition of non-eukaryotic DNA motifs within the promoter region of the vector.^{21–23} Regardless of the mechanism for falling IL-10 concentrations in CSF, swine may have been shielded from its neurotoxic effects due to endogenous production of IL-10-neutralizing antibodies.

CSF IL-10 concentrations varied considerably among animals in each dose group and the range of peak IL-10 concentrations was larger than any effect that may have been expected from doubling the vector dose (Figure 2). Similar variability in peak CSF IL-10 levels was seen previously in dogs¹⁰ and swine.⁹ Inter-individual variation in IT transgene levels unfortunately remains significant, suggesting that improvements in our understanding of IT

vector distribution or better administration techniques will need to be developed in order to achieve clinical relevance. Variability has also been seen in humans and existing literature on the pharmacokinetics of conventional IT drug delivery provides a critical context for these results. Similar to our findings for gene therapy, pharmacokinetics studies of conventional drugs by others have shown substantial variation in the measured drug concentrations in CSF. Clonidine delivered via IT push resulted in a 15-fold variation of CSF levels within 6 hours after injection. This result was observed in both large animal models and humans.^{24,25} IT administration of ropivacaine or doripenem led to a 10-fold variation in CSF levels within 3 hours after administration.^{26,27} These findings also extend to intra-CSF administration via continuous infusion through an implanted IT pump over days to weeks. IT infusion of morphine or hydromorphone at constant rates resulted in an inter-individual variation on the order of 15-fold in CSF concentrations after 1 month,^{28,29} indicating that the high variation in the CSF concentrations persists even after a steady state of the drug has been attained. Given the optimized settings of IT drug administration employed in those studies, variation in CSF drug levels appeared to be an inherent characteristic of the delivery route.

Acknowledgments

Support was provided by the National Institute of Neurological Disorders and Stroke (NINDS) under R01NS063022 and R01NS100725 (to A.S.B.). JP, TPM, and ASB designed the study. JP, TPM, LKN, and LFH performed animal experiments. JP, LFH, and MDU performed laboratory analyses. MDU, JP, TPM, and ASB analyzed data. MDU and ASB wrote the manuscript. ASB is responsible for the overall integrity of the study.

References

1. Kwilasz AJ, Grace PM, Serbedzija P, Maier SF, Watkins LR. The therapeutic potential of interleukin-10 in neuroimmune diseases. *Neuropharmacology*. 2015; 96(Part A):55–69. DOI: 10.1016/j.neuropharm.2014.10.020 [PubMed: 25446571]
2. Storek B, Reinhardt M, Wang C, et al. Sensory neuron targeting by self-complementary AAV8 via lumbar puncture for chronic pain. *Proc Natl Acad Sci*. 2008; 105(3):1055–1060. DOI: 10.1073/pnas.0708003105 [PubMed: 18215993]
3. Storek B, Harder NM, Banck MS, et al. Intrathecal long-term gene expression by self-complementary adeno-associated virus type 1 suitable for chronic pain studies in rats. *Mol Pain*. 2006; 2:4.doi: 10.1186/1744-8069-2-4 [PubMed: 16445862]
4. Milligan ED, Sloane EM, Langer SJ, et al. Controlling neuropathic pain by adeno-associated virus driven production of the anti-inflammatory cytokine, interleukin-10. *Mol Pain*. 2005; 1:9.doi: 10.1186/1744-8069-1-9 [PubMed: 15813997]
5. Semerano L, Minichiello E, Bessis N, Boissier MC. Novel Immunotherapeutic Avenues for Rheumatoid Arthritis. *Trends Mol Med*. 2016; 22(3):214–229. DOI: 10.1016/j.molmed.2016.01.005 [PubMed: 26875450]
6. Ferrari M, Onuoha SC, Pitzalis C. Going with the flow: Harnessing the power of the vasculature for targeted therapy in rheumatoid arthritis. *Drug Discov Today*. 2016; 21(1):172–179. DOI: 10.1016/j.drudis.2015.10.014 [PubMed: 26523772]
7. Lúdvíksson BR, Sneller MC, Chua KS, et al. Active Wegener's granulomatosis is associated with HLA-DR+ CD4+ T cells exhibiting an unbalanced Th1-type T cell cytokine pattern: reversal with IL-10. *J Immunol*. 1998; 160(7):3602–3609. [PubMed: 9531324]
8. Saxena A, Khosraviani S, Noel S, Mohan D, Donner T, Hamad ARA. Interleukin-10 paradox: A potent immunoregulatory cytokine that has been difficult to harness for immunotherapy. *Cytokine*. 2015; 74(1):27–34. DOI: 10.1016/j.cyto.2014.10.031 [PubMed: 25481648]
9. Unger MD, Pleticha J, Collins JE, et al. Fatal Meningitis in Swine after Intrathecal Administration of Adeno-associated Virus Expressing Syngeneic Interleukin-10. *Mol Ther*. 2017; 25(11):2526–2532. DOI: 10.1016/j.ymthe.2017.07.016 [PubMed: 28822691]

10. Pleticha J, Malkmus SA, Heilmann LF, et al. High cerebrospinal fluid levels of interleukin-10 attained by AAV in dogs. *Gene Ther.* 2015; 22(2):202–208. DOI: 10.1038/gt.2014.96 [PubMed: 25354684]
11. Cavagnaro JA. Preclinical safety evaluation of biotechnology-derived pharmaceuticals. *Nat Rev Drug Discov.* 2002; 1(6):469–475. DOI: 10.1038/nrd822 [PubMed: 12119749]
12. Serabian MA, Huang Y, Serabian MA, Huang Y. *Pharmaceutical Sciences Encyclopedia*. Hoboken, NJ, USA: John Wiley & Sons, Inc; 2010. *Preclinical Safety Evaluation of Biopharmaceuticals*.
13. Anderson TD, Hayes TJ. Toxicity of human recombinant interleukin-2 in rats. Pathologic changes are characterized by marked lymphocytic and eosinophilic proliferation and multisystem involvement. *Lab Invest.* 1989; 60(3):331–346. [PubMed: 2784516]
14. Terrell TG, Green JD. Comparative pathology of recombinant murine interferon-gamma in mice and recombinant human interferon-gamma in cynomolgus monkeys. *IntRevExpPathol.* 1993; 34(Pt B):73–101. 0074-7718 (Print).
15. Sung MW, Chen S-H, Thung SN, et al. Intratumoral delivery of adenovirus-mediated interleukin-12 gene in mice with metastatic cancer in the liver. *Hum Gene Ther.* 2002; 13(6):731–743. DOI: 10.1089/104303402317322294 [PubMed: 11936972]
16. [Accessed February 1, 2018] Guidance for Industry Preclinical Assessment of Investigational Cellular and Gene Therapy Products. 2009. <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidance>
17. Martino AT, Herzog RW, Anegón I, Adjali O. Measuring immune responses to recombinant AAV gene transfer. *Methods Mol Biol.* 2011; 807:259–272. DOI: 10.1007/978-1-61779-370-7_11 [PubMed: 22034034]
18. Pleticha J, Maus TP, Jeng-Singh C, et al. Pig lumbar spine anatomy and imaging-guided lateral lumbar puncture: A new large animal model for intrathecal drug delivery. *J Neurosci Methods.* 2013; 216(1):10–15. DOI: 10.1016/j.jneumeth.2013.03.006 [PubMed: 23518340]
19. Yaksh TL, Rathbun ML, Dragani JC, et al. Kinetic and Safety Studies on Intrathecally Infused Recombinant-Methionyl Human Brain-Derived Neurotrophic Factor in Dogs. *Toxicol Sci.* 1997; 38(1):89–100. DOI: 10.1093/toxsci/38.1.89
20. Haurigot V, Marcó S, Ribera A, et al. Whole body correction of mucopolysaccharidosis IIIA by intracerebrospinal fluid gene therapy. *J Clin Invest.* 2013; 123(8):3254–3271. DOI: 10.1172/JCI66778
21. Tenenbaum L, Chtarto A, Lehtonen E, Velu T, Brotchi J, Levivier M. Recombinant AAV-mediated gene delivery to the central nervous system. *J Gene Med.* 2004; 6(SUPPL 1):S212–22. DOI: 10.1002/jgm.506 [PubMed: 14978764]
22. Gray SJ, Foti SB, Schwartz JW, et al. Optimizing promoters for recombinant adeno-associated virus-mediated gene expression in the peripheral and central nervous system using self-complementary vectors. *Hum Gene Ther.* 2011; 22(9):1143–1153. DOI: 10.1089/hum.2010.245 [PubMed: 21476867]
23. Paterna JC, Moccetti T, Mura A, Feldon J, Büeler H. Influence of promoter and WHV post-transcriptional regulatory element on AAV-mediated transgene expression in the rat brain. *Gene Ther.* 2000; 7(15):1304–1311. DOI: 10.1038/sj.gt.3301221 [PubMed: 10918501]
24. Castro MI, Eisenach JC. Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. *Anesthesiology.* 1989; 71(3):418–425. <http://www.ncbi.nlm.nih.gov/pubmed/2774269>. [PubMed: 2774269]
25. Eisenach J, Detweiler D, Hood D. Hemodynamic and analgesic actions of epidurally administered clonidine. *Anesthesiology.* 1993; 78(2):277–287. DOI: 10.1111/j.1399-6576.2005.00638.x [PubMed: 8439023]
26. Rose FX, Estebe JP, Ratajczak M, et al. Epidural, intrathecal pharmacokinetics, and intrathecal bioavailability of ropivacaine. *Anesth Analg.* 2007; 105(3):859–867. DOI: 10.1213/01.ane.0000278129.37099.9a [PubMed: 17717251]
27. Nalda-Molina R, Dokoumetzidis A, Charkoftaki G, et al. Pharmacokinetics of doripenem in CSF of patients with non-inflamed meninges. *J Antimicrob Chemother.* 2012; 67(7):1722–1729. DOI: 10.1093/jac/dks106 [PubMed: 22457313]

28. Gradert TL, Baze WB, Satterfield WC, Hildebrand KR, Johansen MJ, Hassenbusch SJ. Safety of chronic intrathecal morphine infusion in a sheep model. *Anesthesiology*. 2003; 99(1):188–198. DOI: 10.1097/00000542-200307000-00029 [PubMed: 12826859]
29. Johansen MJ, Satterfield WC, Baze WB, Hildebrand KR, Gradert TL, Hassenbusch SJ. Continuous intrathecal infusion of hydromorphone: safety in the sheep model and clinical implications. *Pain Med*. 2004; 5(1):14–25. DOI: 10.1213/01.ANE.0000120086.35289.9D [PubMed: 14996233]

FIGURE 1. Body weight (kg) for animals before and after vector administration. Vector was administered during week 0. Y680, square; Y683, diamond; Y991, triangle; Y994, letter Y.

FIGURE 2.

Secretion kinetics and xenogeneic immune response after intrathecal delivery of scAAV8 encoding hIL-10. Intrathecal administration of scAAV8 encoding hIL-10 resulted in expression of hIL-10 in the CSF in all animals (line graph, pg/ml). CSF hIL-10 levels peaked during week 2 and subsequently declined to baseline levels. The rise and fall of hIL-10 was associated with a xenogeneic immune response against the human IL-10 ortholog, as shown by the presence of anti-hIL-10 antibodies in the CSF and sera of all animals (inverted bar graph, pg/ml). Values displayed represent the mean concentration of each analyte for each time point measured from all animals ($n = 4$). Error bars represent standard error of the mean. CSF, blue; serum, red.

FIGURE 3.

Representative histological sections of cerebellum (A), forebrain (B), spinal cord dorsal root entry zone (C, arrowhead), and dorsal root ganglion (D) from animals that received intrathecal hIL-10. Meninges at cerebellum, spinal cord, and dorsal root ganglia lacked evidence of inflammation, including vascular infiltration, engorgement, or dilation (A, C, D, arrows). There were no signs of parenchymal infiltration by immune cells at all sites (A – D). H&E; magnification 5X.

FIGURE 4.

Peripheral biodistribution of scAAV8 after intrathecal delivery. There was minimal spread of the vector to the periphery, with less than 0.01 vector genome copies/diploid host genome in the peripheral organs of all animals ($n = 4$). The mean detectable value for liver was 0.0016 vector genome copies/diploid host genome. The presence of vector genomes was determined by qPCR and normalized to diploid host genomes (dotted line, detection limit of the assay). Y680, square; Y683, diamond; Y991, triangle; Y994, letter Y.

TABLE 1

Summary of experimental groups

Swine ID	Sex	Weight at week 0 (kg)	Vector-transgene	Vector dose (genome copies)
Y991	Male	23.0	scAAV8-hIL-10	1.8×10^{12}
Y994	Male	21.4	scAAV8-hIL-10	1.8×10^{12}
Y680	Male	25.0	scAAV8-hIL-10	3.5×10^{12}
Y683	Male	18.0	scAAV8-hIL-10	3.5×10^{12}

TABLE 2

Anti-AAV8 capsid antibody titers

Swine ID	CSF		Serum	
	Baseline	At sacrifice	Baseline	At sacrifice
Y991	<1:5	1:25	1:5	1:25
Y994	<1:5	<1:5	<1:5	1:125
Y680	<1:5	1:3,125	1:5	1:125
Y683	<1:5	1:125	1:5	1:125