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Safety Assessment of Intradiscal Gene Therapy II: Effect of Dosing and Vector Choice

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Abstract

Study Design—Clinical, biochemical, and histological analysis was performed following in vivo delivery of cDNA encoding various anabolic cytokines and marker genes to the lumbar epidural space of New Zealand white rabbits, using both adenoviral and adeno-associated viral vectors.

Objective—To mimic errant or misplaced doses of gene therapy in order to better ascertain the potential risks associated with alternative vectors and transgene products with regard to their application to problems of the intervertebral disc.

Summary of Background Data—Work done with several anabolic cytokines including bone morphogenic proteins and transforming growth factors, has demonstrated the potential of gene therapy. Recently, data has been published demonstrating that improperly dosed or delivered adenoviral-mediated gene therapy within the **subarachnoid** space can result in significant morbidity in rabbits. There are currently no studies examining the effect of these errors within the epidural space or using an adeno-associated viral (AAV) vector.

Methods—Using either adenoviral or adeno-associated viral vectors, **complementary DNA** (cDNA) encoding anabolic cytokines bone morphogenic protein-2 (BMP-2) and transforming growth factor-beta 1 (TGF- β 1) and marker proteins LacZ and green fluorescent protein (GFP) were injected into the epidural space of 37 New Zealand white rabbits at the **L5/6 level**. Rabbits were then observed clinically for up to six weeks, after which the rabbits were sacrificed in order to perform a comprehensive biochemical and histological analysis.

Results—Following adenoviral-mediated delivery of anabolic cytokine cDNA, up to eighty percent of rabbits suffered significant clinical, biochemical, and histological morbidity. Conversely, AAV-mediated delivery of any cDNA and adenoviral-mediated delivery of marker protein cDNA resulted in no clinical, histological, or biochemical morbidity.

Conclusion—Properly dosed and directed gene therapy seems to be both safe and potentially efficacious. This study suggests that side effects of gene therapy may be due to a combination of dosing, transgene product, and vector choice, and that newer AAV vectors may reduce these side-effects and decrease the risk of this technology.

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Introduction

Back pain has been identified as the leading cause of disability in people under the age of forty-five [1], and it has been estimated that up to eighty-five percent of Americans will experience back pain at some point in their life [2]. Pathology of the intervertebral disc (IVD) has been associated with the majority of chronic back pain, and degeneration of the disc, known as intervertebral disc disease (IDD), may play a large role in the pathogenesis of several other conditions including spinal instability and disc herniation. Recent studies have shown that a hallmark of disc degeneration is the decline in proteoglycan content within the nucleus pulposus [3,4,5]. Accordingly, in contrast to current treatment modalities that address only end-stage symptoms, there has been a growing interest in biological solutions to the problem of IDD targeting the disc itself.

Gene therapy is one potential biological therapy that has garnered tremendous interest in recent years. Certain proteins have been found to alter the balance between catabolism and anabolism of proteoglycans within the disc, playing major roles in the process of IDD, and providing targets for genetic manipulation [6,7]. Indeed, work done with several of these molecules has shown the distinct capacity of gene therapy to effect therapeutic change in cells of the IVD [8,9,10,11].

Despite the considerable promise of gene therapy, the potential for devastating side effects has also been realized [12], and application of this technology to the clinical setting will require a thorough investigation of various different safety issues, including strategies for dosing, delivery, and post-delivery regulation. One response to these concerns about safety has been the development of newer, safer vectors for gene delivery. Adeno-associated virus (AAV) is one such vector which has a decreased side-effect profile [13,14,15,16] and differs significantly from its adenoviral predecessor (Table 1). AAV has become a popular vector in more recent research designs and has already been shown capable of transducing cells of the IVD [17].

The proximity of the disc to vital neurovascular structures further magnifies these concerns about safety, particularly with respect to the treatment of non-lethal conditions such as IDD. In 2006 Wallach et al published data demonstrating that improperly dosed or delivered gene therapy within the subarachnoid space can result in significant clinical and histological morbidity in rabbits [18]. We have now designed a follow-up experiment to examine the effects of similar errors following placement of gene therapy into the *epidural* space, mimicking a misplaced injection or leakage of genetically transferred material outside of the disc. In addition we sought to determine if the decreased side effect profile of AAV would translate into less severe microscopic and clinical side effects.

Materials and Methods

Throughout the study New Zealand white rabbits were used under the guidelines set forth by the university's Institutional Animal Care and Use Committee (IACUC) and the Department of Laboratory Animal Resources (DLAR). All rabbits were females between nine and twelve months of age, weighing about five kilograms. In work done by our lab leading up to this study, 10^6 plaque forming units (pfu) of adenoviral vector was the lowest dose leading to a high rate of transgene expression and no associated inflammation [19], and was therefore considered the "therapeutic" dose, with 10^8 pfu considered a "high" dose. Due to the hundred-fold decrease in the infectivity of AAV 10^{10} particles (equivalent to 10^8 pfu of adenovirus) of this vector was considered to be a "therapeutic" dose. Unfortunately, due to limitations of vector construction, we were able to achieve only a tenfold increase of the

AAV vector (to 10^{11} particles), as opposed to the adenoviral groups in which the high dose was greater by a factor of 100.

Experimental Groups

A: Adenoviral Arm—Nineteen rabbits were divided into four experimental groups. Marker gene groups were created to examine zone of distribution of the vectors, and therapeutic transgene groups were created to examine the safety of the clinically plausible transgenes. One “therapeutic dose” group (N=4) received 10^6 plaque forming units (pfu) of the adenoviral-bone morphogenic protein-2 (Ad-BMP-2), and three “high dose” groups received 10^8 pfu of either Ad-BMP-2 (N=5), adenoviral-LacZ (Ad-LacZ) (N=4), or adenoviral-transforming growth factor-beta1 (Ad-TGF- β 1) (N=6).

B: AAV Arm—Eighteen rabbits were divided into four experimental groups. Two low dose groups received 10^{10} particles (“therapeutic dose”) of either the AAV-green fluorescent protein construct (AAV-GFP) (N=4) or the AAV-bone morphogenic protein construct (AAV-BMP-2) (n=5). Two high dose groups received 10^{11} particles of either AAV-GFP (N=4) or AAV-BMP-2 (N=5).

Vector Construction and Injection Preparation—The vectors used in the adenovirus group are first generation E1/E3 deleted, type 5, replication defective adenoviruses [19]. Complementary DNA (cDNA) encoding the desired gene product was inserted into the E1 region under the regulation of a human cytomegalovirus promoter. High viral titers were produced via permissive replication in the 293-human embryonic kidney cell line (American Type Culture Collection) [20].

AAV vectors were **single copy vectors** produced via the “triple transfection” technique described by Xiao in 1998 [21]. Desired cDNA was inserted into a pXX2 AAV serotype 2 packaging construct via a cytomegalovirus gene cassette. The new construct, termed pXX2- (desired cDNA), was then co-transduced with adenoviral helper plasmids pBHG10, pXX5, and pXX6. The final construct was incubated with human 293 cells, which were then lysed, releasing the ultimate viral-gene vector construct. Titers of vector were determined by manually counting blue cells after staining with 5-bromo-4-chloro-3-indolyl- β -galactosidase (X-Gal), and vector activity was tested prior to injection by transducing a fibroblast cell line with AAV-GFP and observing activity under fluorescent microscopy (Figure 1).

Injections for the adenovirus group consisted of a total volume of ten microliters (μ L). Hank’s balanced salt solution was combined with appropriate volumes of a viral stock solution to create the final injection volume containing either 10^6 or 10^8 pfu of the desired viral-gene construct.

Injections for the AAV group consisted of a total volume of twenty microliters, as more volume was necessary to achieve viral titers necessary for the high dose AAV groups. Injections were made from diluting appropriate amounts of stock solution in serumless medium, as the AAV stock solution itself was made of serumless medium. Stock solutions were stored in a freezer at -70 degrees Centigrade, and the same stock solution was used for each set of experiments. Just prior to usage, aliquots of stock solutions for each set of injections were thawed, mixed with necessary diluent, and stored on ice until the time of injection.

Surgical Technique—Rabbits were anesthetized, placed prone, and prepared and draped in a sterile fashion using betadine solution. A posterior, midline incision was made, followed by dissection to expose the posterior elements of the fifth and sixth lumbar vertebrae (L5

and L6). The caudal half of the L5 spinous process was then removed, exposing the ligamentum flavum between L5 and L6. A small window was then made in the midline of the ligamentum flavum, exposing the epidural space. Care was taken to avoid puncture of the dura mater, and prior to injection the area of the window was observed to rule out any leakage of cerebrospinal fluid indicative of a dural tear. The injections were then dripped into the epidural space using a twenty-five-gauge needle. Paraspinal musculature was laid over the window, and wounds were closed in a two-layer fashion. Rabbits were then kept under direct observation until fully awake and demonstrating normal neurological behavior. Two doses of cefazolin and ketoprofen were given postoperatively to limit risks of infection and post-surgical inflammation.

Postoperative Course and Evaluation—Animals were followed for up to six weeks postoperatively. The investigators, licensed veterinarians, and veterinary staff directly observed the animals twice daily for evidence of neurological or systemic symptoms. Evidence of neuropathology included self-inflicted skin lesions, diminished hind leg strength and use, and decreased levels of overall activity compared to non-study control rabbits. In addition, routine behaviors such as food and water intake and bowel habits were continuously monitored and charted throughout the postoperative period. Animals were sacrificed immediately after the observance of any altered behaviors, or ultimately at the six week time point if no ill effects were noted. Spinal cords with intact thecal sacs were then harvested for analysis.

After the entire spinal column was harvested, the area of injection was identified, and histological specimens were taken. Those from rabbits injected with Ad/LacZ were stained with 5-bromo-4-chloro-3-indolyl- β -galactosidase (X-Gal) to determine vector distribution patterns, whereas those from rabbits injected with AAV/GFP were cut by frozen section and analyzed by fluorescent light microscopy for evidence of gene expression. X-Gal staining was performed via the Sigma technique as follows [22]: cells and tissues were washed with phosphate-buffered saline (PBS) and fixed in 0.5% glutaraldehyde (Sigma) for ten minutes, followed by two rinses with PBS containing 1mmol/L magnesium chloride. The cells were then incubated with X-Gal substrate for 2 hours at 37 degrees Centigrade and then analyzed under a microscope. Also, after staining with hematoxylin and eosin (H&E), histological specimens from animals of each group were analyzed by a veterinary histopathologist blinded both from the type of injection and any resultant clinical morbidity. In addition, plasma and CSF samples were collected from representative animals of each group for ELISA quantification of transgene production in these extradural compartments.

Results

Adenoviral Arm

A: Clinical—None of the rabbits receiving a high dose of Ad/LacZ or a therapeutic dose of Ad/BMP-2 demonstrated any signs of clinical toxicity. Their behavior, feeding patterns, activity levels, and bowel and bladder habits all remained at their preoperative baseline. Three of the six rabbits receiving a high dose of Ad/TGF- β 1 exhibited grossly abnormal behaviors and signs of toxicity. Two developed bilateral flaccid paralysis of the hind extremities at one and two weeks, and one developed loss of sensation in bilateral hind extremities at week six, as evidenced by self-mutilating behavior. Only one of the five rabbits receiving a high dose of Ad/BMP-2 finished the six week course clinically well. Two from this group were found deceased during morning rounds at one and two weeks, and two others demonstrated the self-mutilating behavior indicative of sensory loss in the hind extremities at week five.

B: Histological—X-gal staining of tissue harvested from rabbits receiving LacZ revealed transgene expression not only in the meninges, but also in the dorsal root ganglia and the parenchyma of the spinal cord (Figure 2). H&E staining of tissues taken from these rabbits revealed largely normal meninges and spinal cords with only very mild epidural inflammation. Similar results were found in the rabbits injected with the therapeutic dose of Ad/BMP-2.

In contrast, histological examination of the specimens taken from rabbits receiving a high dose of Ad/BMP-2 was not benign. While tissue taken from the one rabbit remaining clinically well throughout the six-week course looked similar to controls, that taken from the two rabbits suffering hind extremity sensory loss revealed diffuse lymphocytic inflammation within the gray matter and central canal, as well as subacute infarction of focal regions within the dorsal spinal cord (Figure 3). In addition, slides from one of these subjects demonstrated chondrogenic metaplasia of the meninges (Figure 4) with adjacent lymphocytic inflammation within the epidural space.

Tissues taken from the high dose TGF- β 1 group showed quite dramatic histological changes. Even tissues taken from clinically healthy subjects were microscopically abnormal, and fibrotic thickening and lymphocytic infiltration of the meninges (Figure 5) occurred in all but one subject. In addition, slides taken from the rabbit suffering paralysis at week one showed actual parenchymal pathology, including lymphocytic infiltration of the white and gray matter of the spinal cord.

C: Biochemical—Plasma and CSF samples taken from clinically ill rabbits revealed changes consistent with their morbidity (Figure 6). In comparison to control values taken from clinically well animals, samples taken from the three clinically ill rabbits from the high dose Ad/ TGF- β 1 group contained significantly higher levels of transgene product (recombinant human TGF- β 1). Similarly, high levels of recombinant human BMP-2 were found in plasma and CSF samples obtained from the clinically ill rabbits from the high dose Ad/BMP-2 group.

AAV Arm

In stark contrast to the findings in the adenoviral arm of this study, virtually no deleterious effects were seen in rabbits injected with the AAV/GFP and the AAV/BMP-2 constructs, independent of dosage. All rabbits from all groups remained clinically well throughout the six week course, with no observations consistent with either local or systemic toxicity following the injections (Table 3). Histological samples taken from subjects of all groups were microscopically similar to normal tissue, with no transgene expression across the blood-brain barrier (Figure 7), and no evidence of dural or parenchymal pathology. Finally, plasma and CSF samples taken at two, four, and six weeks post-injection were not significantly different than pre-injection controls.

Discussion

In recent years there has been tremendous progress in the search for biological solutions to the problem of disc degeneration. Gene therapy has emerged as a promising therapeutic option with tremendous potential, and while researchers continue to study its efficacy, it is incumbent upon those seeking clinical application to fully evaluate its considerable risk profile. This experiment was done in the same vein as the safety study by Wallach et al, which demonstrated that while properly dosed and delivered gene therapy can effect dramatic therapeutic change within the IVD, misplacement or incorrect dosing can lead to devastating side effects. Just as the previous study examined effects of *subarachnoid* placement, our study sought to mimic misplacement or leakage of recombinant constructs

into the *epidural* space, a complication known to occur during other intradiscal procedures such as discography [23]. In addition, by comparing adenoviral and adeno-associated viral vectors, we sought to determine the role of vector choice in post-injection morbidity.

Prior to embarking on this study, we hypothesized that placement of genetic constructs into the epidural space would not result in extensive morbidity. Being that the epidural space is highly vascular, we postulated that the circulation would “wash away” injected material before the vector had an opportunity to transduce any cells, a theory that has been discussed in gene therapy literature [30]. Also the injection would be placed outside of the dura mater, separated from the spinal cord by the blood-brain barrier, limiting its ability to cause pathology within the spinal cord.

Several conclusions might be drawn from the somewhat surprising results from the adenoviral arm of our study. First, it appears that adenovirus is an extremely aggressive vector, capable of rapid and vigorous transduction of cells both in proximity and at a distance to the original site of its insertion. The presence of marker gene expression within the parenchyma of the spinal cord in our study supports the notion that adenovirus is capable of crossing the blood-brain barrier. That marker gene product appears exclusively within cells suggests that the viral particles themselves are taken in by cells, rather than simply transport of marker protein from remote sites. Transport of proteins would have likely resulted in the observance of marker gene in the extracellular matrix, which we did not see. While the precise mechanism by which the barrier is breached is beyond the scope of this paper, prior reports have noted an ability of viral vectors to gain access to the central nervous system via a retrograde axonal transport mechanism [24]. Our study perhaps lends support to this theory, and in addition suggests that adenoviral vectors are better able than AAV vectors to access the central nervous system from points of insertion outside the blood-brain barrier. Second, our study suggests that it is necessary for there to be a critical overdose of gene therapy outside of the disc to cause deleterious effects, as very little clinical or histological pathology was detected in any of the rabbits receiving a physiologic dose of Ad/BMP-2. As prior work has shown a 10^6 pfu dose to be sufficient in effecting therapeutic change in disc cells [25], our study suggests that an entire therapeutic dose of gene therapy can be misplaced into the epidural space without causing any clinical morbidity. Certainly this optimism must be tempered though, as very serious side effects were seen in the majority of rabbits receiving higher doses of Ad/TGF- β 1 and Ad/BMP-2, indicating that there exists a definite therapeutic index and highlighting the importance of proper dosing.

The paper by Wallach et al mentioned three potential mechanisms potentially responsible for the observed clinical morbidity. Our current study lends support to their conclusion that clinical and histological pathology is caused by a synergism between transgene product and viral vector. Five of six rabbits receiving a supratherapeutic dose of BMP-2 via an adenoviral vector suffered severe neurological problems, yet rabbits receiving a supratherapeutic dose of the same transgene via an AAV vector had no significant complications. Based on our current study, we suggest two ways in which the vector and the transgene combine to cause subject morbidity at both the macroscopic and cellular levels. First, our data indicate that an increased level of certain biologically active transgene products correlates with increased morbidity. Indeed, while a high dose of Ad-LacZ resulted only in a small amount of histological inflammation, the same dose of Ad-BMP-2 resulted in serious adverse effects. Yet our data also suggests that these effects cannot manifest unless the viral vector is capable of delivering this transgene to the tissues. As we observed, high doses of adenoviral constructs caused devastating side effects, while high doses of AAV constructs carrying the same transgene (BMP-2) resulted in neither clinical nor histological morbidity whatsoever. Though the exact pharmacokinetic profile of these vectors is not

known and is beyond the scope of this study, it appears that AAV may be a less aggressive vector than adenovirus, limiting its transduction efficiency in highly vascular areas such as the epidural space. Indeed, there is evidence in the literature to support this concept. A study done on New Zealand white rabbits looking at transgene expression following delivery by AAV and adenovirus to the jugular veins demonstrated a significantly more efficient response with an adenoviral carrier [26]. In our study, while extensive expression of marker gene occurred in the tissues of rabbits injected with adenoviral constructs, there was no marker transgene expression following delivery via AAV (Figure 5). We therefore suspect that while dilution by the epidural circulation may have prevented transduction of the surrounding cells by AAV, the more aggressive adenoviral vector was still able to function, and the consequences of supratherapeutic dosing was able to manifest. The variable transduction efficiency AAV demonstrates between different physiologic compartments in the body may point to a significant safety advantage whereby any vector delivered outside of the hypovascular target tissue (i.e. nucleus pulposus) is effectively neutralized by the hypervascularity of the surrounding tissues.

Second, we submit that the decreased antigenicity of AAV may help to limit the host immune response and lessen the damage done by lymphocytic infiltration into the areas of vector uptake. There is an abundance of literature that describes the differences in the immune response to adenoviral and adeno-associated viral vectors, and it seems that, particularly with respect to cell-mediated immune reactions, the response to AAV is considerably less aggressive [27,28, 29,30]. Clearly, as evidenced by the low levels of inflammation caused by high dose Ad-LacZ, the viral vector of choice is not likely to be the primary cause of deleterious effects. It should be noted however that we observed no histological evidence of inflammation whatsoever in the AAV arm of the study. Future studies involving these types of injections should examine typical circulatory depots, including the liver and lymphoid tissue, for any evidence of vector uptake.

While we have learned much from this project, it does have certain limitations. First, the total number of subjects in our study is small, owing to our attempt to limit the overall morbidity of our pilot study. While the lack of any adverse side effects in any of our AAV subjects would suggest that AAV is a safe vector, a direct comparison between it and adenovirus was limited by the available titer of our AAV vector. Unfortunately we were able to increase our dose of AAV by a factor of ten, compared to a 100-fold increase for adenovirus, and it would be reasonable to question whether we achieved a toxic dose of either AAV or the transgene product in our high dose AAV groups. Finally, with our study only spanning six weeks, it is possible that we are missing some later effects of the injections, particularly in those rabbits receiving AAV constructs. Future studies should be directed toward examining the potential long-term effects of misplaced or improperly dosed gene therapy.

In summary, gene therapy holds great potential to become a viable option in the biological treatment of intervertebral disc degeneration. While there do seem to be substantial benefits to the use of gene therapy, it has become clear that these do not come free of patient risk, and an increasing number of projects are being directed toward finding safer means by which to implement the technology [31,32]. Our current study seeks to shed some light not only on the benefits of genetic modulation, but also on the challenges it may pose when used to treat conditions of the spine. Prior to widespread clinical application, a precise therapeutic dose must be determined, as well as the best possible means by which to deliver this dose. In addition, we must develop a feasible method to consistently ensure the appropriate and accurate delivery of vector-gene constructs to the disc space. With persistent attention to issues of safety, research regarding the use of gene therapy will continue to progress,

bringing us closer to its clinical application to the problem of intervertebral disc degeneration.

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Figure 1.

Fluorescent light microscopy demonstrating intracellular green fluorescent protein within fibroblasts after transduction with our AAV-GFP construct. Note: the camera used to take the picture transformed the green color to a grayscale image, so the brighter the green, the more white the cells in the photograph.

Figure 2. Light microscopy image from a rabbit specimen demonstrating blue cells indicative of transgene expression in spinal cord parenchyma and meninges (image a) and the cells of the dorsal root ganglion (image b) following an epidural injection of adenoviral-LacZ marker gene construct.

Figure 3.
Light microscopy image from a rabbit specimen demonstrating an area of subacute infarct (yellow arrows) in the dorsal horn following an epidural injection of adenoviral-BMP-2.

Figure 4.

Light microscopy image from a rabbit specimen demonstrating chondrogenic metaplasia of cells within the meninges. The area of interest is enlarged within the square and the specific chondroid cells are enclosed within the circle.

Figure 5. (*Left*) Light microscopy image from a rabbit specimen demonstrating normal spinal cord and meningeal anatomy (dashed arrows). (*Right*) An image demonstrating fibrotic thickening of the meninges (solid arrows) and lymphocytic infiltration (*inset*) following epidural injection of adenoviral-TGF- β 1.

Figure 6.

(a) Plasma levels of transgene product taken from an ill rabbit following epidural injection of adenoviral-TGF- β 1. (b) Plasma levels of transgene product taken at the six-week time point following epidural injections of adenoviral-BMP-2. (c) Cerebrospinal fluid levels of transgene product taken at the six-week time point following epidural injections of adenoviral-BMP-2.

Figure 7.

Two fluorescent light microscopy images taken of the same section of rabbit spinal cord tissue following epidural injection of AAV-GFP. (*Left*) Nuclear stain showing a very cellular section of tissue. (*Right*) Green fluorescence image showing no transgene expression of GFP within the same section of spinal cord, evidenced by the lack of any green fluorescence in the sectioned tissue.

Table 1
Characteristics of Adenoviral and Adeno-associated Viral Vectors

This table lists some of the most important characteristics of both adenovirus and adeno-associated virus (AAV) with respect to their use as vectors in gene therapy.

	Adenovirus	AAV
Structure	Large (90nm diameter), nonenveloped icosahedron	Small (25nm diameter), nonenveloped icosahedron
Genome	Linear, double-stranded DNA	Encapsidated, single-stranded DNA
Packaging Capacity in Kilobases (Kb)	14Kb – 36Kb (high capacity)	Approximately 5.2Kb
Target Cell Type	Proliferating and Quiescent	Proliferating and Quiescent
Human Pathology	Respiratory, intestinal, and eye infections	None known
Immunogenicity	Strong humoral and cell-mediated immune response	Limited immune response

Table 2
Adverse Effects Using Adenoviral Vectors

This table depicts the types and percentages of clinical morbidity experienced by rabbits in the adenoviral arm of the study. Values in parentheses in the first column are the doses of injected vector-gene construct. Ratios in parentheses in the final column indicate the total number of sick rabbits over the total number of rabbits in the group.

	Parasthesias	Paralysis	Death	Total Complications
Therapeutic Dose BMP-2 (10⁶ pfu)	0%	0%	0%	0% (0/4)
High Dose BMP-2 (10⁸ pfu)	40%	0%	40%	80% (4/5)
High Dose TGF-β1 (10⁸ pfu)	17%	33%	0%	50% (3/6)
High Dose LacZ marker (10⁸ pfu)	0%	0%	0%	0% (0/4)

Table 3
Adverse Effects Using Adeno-Associated Viral Vectors

This table demonstrated that none of the rabbits in this group suffered any serious clinical morbidity. Values in parentheses in the first column are the doses of injected vector-gene construct. Ratios in parentheses in the final column indicate the total number of sick rabbits over the total number of rabbits in the group.

	Parasthesias	Paralysis	Death	Total Complications
Therapeutic Dose GFP (10 ⁸ pfu)	0%	0%	0%	0% (0/4)
Therapeutic Dose BMP-2 (10 ⁸ pfu)	0%	0%	0%	0% (0/5)
High Dose GFP (10 ⁹ pfu)	0%	0%	0%	0% (0/4)
High Dose BMP-2 (10 ⁹ pfu)	0%	0%	0%	0% (0/5)

Table 4
Histological Findings Following Injection with Adenoviral Vectors

This table lists the significant histological findings following administration of adenoviral vectors carrying cDNA for both marker and therapeutic proteins. Note that neither the therapeutic dose of Ad-BMP-2 nor the high dose of Lac-Z resulted in significant pathology, while the high doses of both Ad-TGF- β and Ad-BMP-2 resulted in markedly abnormal histology correlating with poor clinical outcomes.

	Histological Findings
Therapeutic Dose BMP-2 (10^6 pfu)	- very mild inflammatory changes - largely normal meninges
High Dose Lac-Z (10^8 pfu)	- very mild inflammatory changes - largely normal meninges
High Dose TGF-β1 (10^8 pfu)	- fibrosis and thickening of meninges - diffuse lymphocytic infiltration of meninges - parenchymal inflammation and damage
High Dose BMP-2 (10^8 pfu)	- only 1/5 subjects w/ normal histological findings - diffuse lymphocytic infiltration of gray matter and central canal - subacute infarctions in regions of the dorsal spinal cord - chondrogenic metaplasia in one subject