Schwannoma gene therapy by adeno-associated virus delivery of the pore-forming protein Gasdermin-D

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Abstract
Schwannomas are peripheral nerve sheath tumors associated with three genetically distinct disease entities, namely sporadic schwannoma, neurofibromatosis type-2, and schwannomatosis. Schwannomas are associated with severe disability and in cases lead to death. The primary treatment is operative resection that itself can cause neurologic damage and is at times contra-indicated due to tumor location. Given their homogenous Schwann-lineage cellular composition, schwannomas are appealing targets for gene therapy. In the present study, we have generated an adeno-associated serotype 1 virus (AAV1)-based vector delivering N-terminal of the pyroptotic gene Gasdermin-D; (GSDMDNterm) under the control of the Schwann-cell specific promoter, P0. We have demonstrated that AAV1-P0-GSDMDNterm injection into intra-sciatic schwannomas reduces the growth of these tumors and resolves tumor-associated pain without causing neurologic damage. This AAV1-P0-GSDMDNterm vector holds promise for clinical treatment of schwannomas via direct intra-tumoral injection.

Introduction
Schwannomas are slow growing tumors of Schwann cell origin that develop along peripheral nerves [1]. In ~60% of the cases these lesions arise in children and young adults and new tumors continue to develop in multiple locations throughout the patient’s life [2]. Schwannomas are associated with a variety of disabling neurological symptoms, both positive (gain of function) and negative (loss of function). Pain, deafness, motor dysfunction including paralysis and even death are among these symptoms [3]. In addition, while they initially present as benign and non-invasive tumors, they can then develop into malignant peripheral nerve sheath tumors [4]. Conventional treatment of these tumors includes surgical resection and radiotherapy. Operative resection is not always possible due to the risk of neurologic damage and radiotherapy is limited by risk of malignant transformation in NF2-deficient schwannomas [5]. Clinical trials testing cancer chemotherapeutics, such as anti-angiogenic compounds and small molecules, are ongoing. However, the efficacy of the tested drugs has been limited and transient [6].

The typically slow growth and mitotic index of schwannomas makes these tumors an appropriate target for gene therapy. Further, gene therapy via direct tumor injection is minimally invasive compared with operative resection. There are very few reported preclinical schwannoma gene therapy studies. Intra-tumoral injection of a herpes simplex virus-1 (HSV-1) ampiclon vector expressing caspase-1 (interleukin-β-converting enzyme; ICE) under the Schwann cell-specific P0 promoter led to tumor regression in a xenograft model in which a human schwannoma cell line was implanted subcutaneously [7]. In further development of this strategy, we utilized adeno-associated viral vector serotype-1 (AAV1) expressing a pro-apoptotic caspase-1 gene under the control of the Schwann-lineage cell-specific promoter, P0 to target intra-sciatic schwannomas in another xenograft model [8]. These studies together demonstrated that the P0 promoter, which is selectively expressed in Schwann and schwann-lineage cells (including schwannoma tumor cells), prevented transgene expression in neurons, and tumor treatment was not associated with any neural toxicity. Thus, gene therapy utilizing tissue-specific delivery of
transgene capable of selectively killing schwannoma tumor represents a promising treatment.

Gasdermin-D (GSDMD), a member of the gasdermin protein family, is an essential mediator of inflammasome-mediated pyroptosis in human and mouse cells [9–11]. Inflammasome activation and assembly with caspase-1, caspase-11, caspase-4, and caspase-5 and apoptosis-associated speck-like protein containing a CARD (ASC) results in GSDMD cleavage and induction of pyroptotic cell death. The N-terminal fragment of GSDMD (GSDMDNterm) is sufficient to induce pyroptotic cell death [10, 11]. The mechanism of GSDMDNterm induction of cell death is still not fully elucidated. Pyroptosis had long been demonstrated to involve the formation of a plasma membrane pore, and subsequent osmotic rupture of the host cell [12]. It is likely that GSDMDNterm either promotes the formation of this pore or itself has pore-forming activity [13].

Here we report on the synthesis of a GSDMDNterm transgene and its AAV1-mediated delivery as a pro-pyroptotic protein by AAV-1 under the Schwann cell specific promoter, P0. Our data show that exogenous expression of GSDMDNterm in cultured cells of the human schwannoma cell line, HEI-193, induces cell death. Intra-tumoral injection of AAV1-rp0-GSDMDNterm into intra-sciatic schwannomas in both xenograft human and syngeneic mouse schwannoma models led to increased tumor cell death and decreased tumor cell proliferation, which in turn was associated with a significant reduction in tumor burden. There was no evidence that this treatment was associated with toxicity. This work demonstrates that GSDMDNterm is a promising transgene for schwannoma gene therapy.

**Materials and methods**

**Animals**

All animal experimentation was approved by and conducted under the oversight of the Massachusetts General Hospital (MGH, Boston, MA) Institutional Animal Care and Use Committee. Animals, nu/nu mice and FVB/N, were kept on a 12:12 light-to-dark cycle with ad libitum access to food and water. Animal health was evaluated daily.

**Cell culture and transfection**

The HEI-193 human schwannoma cell line (from D.J. Lim, House Ear Institute, Los Angeles, CA) was established from a schwannoma in a patient with NF2, immortalized with human papillomavirus E6/E7 genes and grown as described [14, 15]. Mouse 08031-9 schwannoma cells (from Dr. Marco Giovannini, Univ. of California, Los Angeles, CA) were grown as described [16]. Cells were infected with lentivirus encoding Fluc and mCherry for bioluminescence imaging and IHC, respectively [7]. HEI-193 cells were transfected with lipofectamine 2000 (Invitrogen, Carlsbad, CA) per the manufacturer’s instructions. Skin fibroblasts were kindly provided by (Dr. Christopher Bragg laboratory, Massachusetts General Hospital, MA, USA). Briefly, skin biopsies were performed by standard procedures [17]. Tissue explants were transferred to culture dishes containing fibroblast growth medium [Dulbecco’s Modified Eagle Medium (DMEM) with 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin] and placed under sterile coverslips to promote cell attachment. Cultures were maintained in a humidified incubator at 37 °C and 5% CO2 with medium exchanges every 3–4 days as fibroblasts migrated from the explant. Cultures were expanded by trypsinization, collecting cells by centrifugation, and resuspending in growth medium. Skin fibroblasts were transfected using Human Dermal Fibroblast Nucleofector Kit (Amaxa Biosystems) per the manufacturer’s instructions. All tissue culture media and supplements were obtained from Life Technologies (Grand Island, NY, USA). All cell lines were confirmed to be free of contamination, including mycoplasma, prior to experimental use.

**Cell viability and toxicity assay**

The Cell Titer-Glo luminescent cell viability assay (Promega, Madison, WI) was used to determine the number of viable cells in culture based on ATP level quantification, an indicator of metabolically active cells. Measurement of the luminescence was conducted with a luminometer (Dynex Technologies, Chantilly, VA) per the manufacturer’s instructions. Cell death was measured by the LDH assay using CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI) per the manufacturer’s instructions.

**Cell death detection by Flow Cytometry**

HEI-193 cells were transfected with pAAV-CBA-GSDMDNterm, pAAV-CBA-GFP or exposed to lipofectamine alone then stained with annexin V-conjugated to fluorescein isothiocyanate (FITC) and propidium iodide (PI) by using the annexin-V-FITC staining kit (Invitrogen, USA) per manufacturer’s instructions. The cells were analyzed on the FACS Aria II platform (BD Bioscience).

**AAV vectors**

The AAV vector plasmid self-complementary (sc)AAV-CBA-GSDMDNterm was derived from scAAV-CBA-BGHpA
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(Dr. Miguel Sena-Esteves, University of Massachusetts Medical Center, Worcester, MA). The rat P0 promoter has been previously described [18]. scAAV-rP0-GSDMDNterm and scAAV-rP0-null plasmids were derived from the plasmid scAAV-rP0-ICE [8]. These plasmids carry two AAV inverted terminal repeat (ITR) elements, one wild type and one in which the terminal resolution site was deleted, as described [19], generating a vector that is packaged as a self-complementary, double-stranded-like molecule. scAAV-rP0-null was generated by digesting the ICE transgene out of scAAV-rP0-ICE using EcoRV and then religating the blunt site. scAAV-rP0-GSDMDNterm plasmid was generated by inserting PCR-amplified mouse GSDMDNterm (828 bp) into scAAV-rP0-null that was linearized with ECORI. GSDMDNterm (828 bp) sequence was described previously [11] and synthesized by Genscript, USA. Resulting plasmid clones were screened by restriction digest and sequencing for correct GSDMDNterm orientation in relation to the P0 promoter. The primers that were used for GSDMDNterm amplification contain an ECORI restriction site, the sequences are: Forward GATCAGAATTTGGACCCATGCCCCTCGG and reverse CATGTTCAATCTCAATCTGACAGGAGAC. All AAV vectors carry the bovine growth hormone polyadenylation signal. The identity of all the cloned transgenes was confirmed by sequencing. AAV1 serotype (AAV1) vectors were produced by transient co-transfection of 293T cells by calcium phosphate precipitation of vector plasmid (scAAV1-rP0-GSDMDNterm), adenoviral helper plasmid pAdΔ6, and a plasmid encoding the AAV1 cap gene (pXR1), as previously described [20]. Viral vectors titers (genome copies [GC]/ml) was determined by real time TaqMan PCR amplification with primers and probe specific for the bovine growth hormone polyadenylation signal. AAV1 vectors were stored at −80 °C until use.

**Generation of tumors and vector injection**

Sciatic nerve schwannomas were generated by direct injection of HEI-193FC human or 08031-8FC mouse schwannoma cells into the left sciatic nerve of isoflurane-anesthetized mice, as described [21]. HEI-193FC or 08031-9FC cells were trypsinized and rinsed with cold PBS, and 30,000 (or 10,000 for 08031-9) cells in a volume of 0.5 µl of PBS were injected into the sciatic nerve of athymic nude mice (nu/nu, 5–7-week-old males; National Cancer Institute [NCI]), or syngeneic FVB/N mice (5–7-week-old males; Charles River Laboratories), respectively, using a glass micropipette and a gas-powered microinjector (IM-300; Narishige, Tokyo, Japan). Tumors were injected with AAV1-rP0-GSDMDNterm, AAV1-rP0-null or PBS (n = 8/group) either 2-weeks’ post HEI-193 tumor-cell implantation or 4-days post 08031-9 tumor cell implantation, in each case with 2 × 1010 vector GC in 2 µl PBS, targeting the location of the sciatic nerve where tumor cells were implanted. Tumor growth was monitored blindly by in vivo bioluminescence imaging at weekly intervals for HEI-193 and twice a week for 08031-9, as described [21]. Briefly, mice were injected intraperitoneally with the Fluc substrate d-luciferin, and, 10 min later, signal was acquired with a high efficiency IVIS Spectrum (Caliper Life Sciences, Hopkinton, MA)

**Behavioral analysis**

Behavioral testing utilized the von Frey method for pain/mechanical sensitivity, the Hargreaves plantar test for Pain/Thermal sensitivity, and by rotarod for gross motor function, all according to published methods [22, 23]. Nu/nu mice were used for the behavioral experiments (n = 8/group). All animals were habituated to the behavioral apparatus for 1 week before testing for baseline. Three baseline measurements on three separate days preceded the first injection. Mice were then tested the day after each injection and twice per week for 8 weeks. Mechanical sensitivity of the hind paw was measured by determining withdrawal thresholds assessed with von Frey filaments employed to determine mechanical sensitivity of the plantar surface of the hind paw, as described [8]. The 50% threshold for each paw withdrawal was calculated, as previously described [24]. Thermal sensitivity of the hind paw was measured by determining withdrawal latency assessed with Hargreaves plantar test, as described [25]. A rotating rod apparatus (Columbus Instruments, Columbus, OH) was used to assess motor performance. Mice were placed on the elevated accelerating rod beginning at 1 rpm/min for two trials per day twice per week. Animals’ fall latency (in seconds) was scored, as described [8].

**Histological and immunohistochemical analysis**

Six-weeks post tumor implantation and 4-weeks post virus injection, animals were terminally anesthetized with isoflurane (3%) and killed by decapitation. Sciatic nerves were removed and snap frozen for hematoxylin and eosin (H&E) and immunohistochemical staining, as described [26]. The sciatic nerves were kept in OCT blocks at −80 °C. Sections were stained with H&E in accordance with routine protocols. Proliferation marker staining was performed using...
antibody against Ki67 (Abcam, Cambridge, MA). Briefly, sections were dried at room temperature (RT) overnight. They were fixed in pre-chilled acetone at 4 °C for 10 min, allowed to dry, and then immediately stained. Sections were washed in PBS, blocked with serum-free protein block (Dako, Carpinteria, CA) and quenched for peroxidases in dual endogenous enzyme block (Dako). Sections were washed in PBS then incubated with anti-Ki67 primary antibody for 1 h at room temperature, then washed in PBS and incubated with horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature. Sections were washed in PBS and incubated with DAB solution (Dako). Counterstaining was accomplished by dipping sections in ethanol and xylene before mounting in Cytoseal (Richard Allan Scientific, San Diego, CA) and covered with cover slips for microscopic visualization. In vivo apoptosis staining was assessed using the TACS® 2 TdT-DAB in Situ apoptosis Detection Kit (Trevigen, Gaithersburg, MD). Fifteen micrometre sections (cryostat) of fresh-frozen nerves fixed with 3.7% formaldehyde after mounting on slides was stained and visualized with diaminobenzidine (DAB) under light microscope according to the manufacturer’s instructions.

Data analysis

All data are presented as group averages ± standard error of the mean (SEM). All baseline behavioral values represent average of all measurements obtained before injection. Data were analyzed with GraphPad Prism and Microsoft Excel. Two-tailed t-test and repeated-measure analysis of variance (ANOVA) were utilized, as described [27]. p < 0.05 was accepted as significant.

Results

Over-expression of GSDMDNterm leads to death of schwannoma cells in vitro

To assess the effect of exogenous expression of GSDMDNterm on the schwannoma cell lines, we have generated an AAV plasmid construct to express the pore forming GSDMDNterm, the end-product and effector of inflammasome pathway activation (Fig. 1a). We expressed GSDMDNterm under either constitutive promoter CBA or the Schwann cell specific promoter, P0 (Fig. 1b); pAAV-CBA-GSDMDNterm was used to over-express GSDMDNterm in cultured schwannoma cell lines, while pAAV-rP0-GSDMDNterm was used for AAV1 packaging and production for the in vivo testing.

Transfection of human the schwannoma cell line, HEI-193, with pAAV-CBA-GSDMDNterm led to cell death as indicated by increased LDH release and decreased ATP-based viability (Fig. 2a). Microscopic examination of the transfected cells confirmed the

Fig. 1  a A schematic diagram of the inflammasome pathway and activation of Gasdermin-D. Inflammasome activation leads to assembly with ASC and caspase-1 which results in cleavage of pro-caspase-1 to its active form. The activated caspase-1 causes IL-1β and IL-18 maturation and cleavage of gasdermin-o to its active N-terminal form which generates pyroptotic cell death. b Schematic diagram of the self-complementary AAV vector encoding the mouse GSDMDNterm gene under the control of the CBA or rat P0 promoter. GSDMDNterm cleavage site is at arrow, inflammatory caspases cleave mouse gasdermin-D at site 276 between aspartic acid (D) and glycine (G). AAV-rP0-GSDMDNterm was used for AAV viral vectors production for in vivo application. ITR inverted terminal repeats, PA polyadenylation signal

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We further confirmed the GSDMDNterm induced cell death after transfection of mouse schwannoma cell line, 08031-9, with pAAV-CBA-GSDMDNterm (Supplementary Figure 1). To examine whether GSDMDNterm over-expression induces cell death in non-tumor tissue, we transfected normal human dermal fibroblasts with pAAV-CBA-GSDMDNterm and measured LDH release and ATP-based viability. We did not find a difference in either parameter in comparison to cells transfected with the control plasmid, pAAV-CBA-GFP or non-transfected cells (Fig. 2c). Furthermore, there was no altered morphology in the cells transfected with GSDMDNterm construct compared to control (Fig. 2d). There was no significant difference in transfection efficiency of HEI-193 and cultured human dermal fibroblasts (Supplementary Figure 2). Flow cytometric analysis demonstrated significantly more double Annexin V and propidium iodide (PI) staining of GSDMDNterm-transfected HEI-193 cells, indicative of cell death, compared with GFP-transfected or lipofectamine exposed cells. GSDMDNterm over-expression resulted in ~45% dead cells, compared with 6% for CBA-GFP transfected and lipofectamine exposed cells (graph on right).

**Fig. 2**  

**a** Lactate dehydrogenase (LDH) release cytotoxicity and ATP-based viability of human HEI-193 were measured 24 or 72 hours, respectively, post pAAV-CBA-GSDMDNterm transfection, pAAV-CBA-GFP transfection or lipofectamine exposure alone.  
**b** GSDMDNterm over-expression causes altered morphological pattern and noticeable detached cells compared with the GFP transfected cells and lipofectamine control. Western blot of GSDMDNterm protein after transfection of HEI-193 demonstrated GSDMDNterm expression (31 KDa) in pAAV-CBA-GSDMDNterm transfected cells, but not in pAAV-CBA-GFP transfected or lipofectamine exposed cells.  
**c** Lactate dehydrogenase (LDH) release cytotoxicity and ATP-based viability of normal human dermal fibroblasts 24 or 72 hours, respectively, after GSDMDNterm over-expression by transfection did not show any difference from the control GFP or non-transfected cells.  
**d** GSDMDNterm did not induce any morphological alteration or resulted in detached cells upon transfection.  
**e** Flow cytometric analysis demonstrated significantly greater double Annexin V and propidium iodide (PI) staining of GSDMDNterm-transfected HEI-193 cells, indicative of cell death, compared with GFP-transfected or lipofectamine exposed cells. GSDMDNterm over-expression resulted in ~45% dead cells, compared with 6% for CBA-GFP transfected and lipofectamine exposed cells (graph on right).
Intra tumoral injection of AAV1-rP0-GSDMDNterm controls schwannoma growth

For in vivo testing, we designed adeno-associated viral vector for delivery of GSDMDNterm under the control of the rat P0 promoter. We have previously demonstrated that use of the P0 promoter restricts transgenes expression to Schwann lineage cells, including schwannoma tumor cells, and prevents expression in and toxicity to neuronal tissue [7, 8]. Thus, we have generated two different AAV1 vectors, the experimental vector containing GSDMDNterm transgene under control of the rat P0 promoter; it is denoted “AAV1-rP0-GSDMDNterm” and a control vector lacking a transgene sequence downstream of rP0 (AAV1-rP0-null). We first tested efficacy of AAV1-rP0-GSDMDNterm in a human xenograft schwannoma model we developed [21]. In this model, schwannomas are generated in athymic nude (nu/nu) mice via intra-sciatic implantation of a firefly luciferase (Fluc)-expressing variant of the NF2 patient-derived schwannoma cell line, HEI-193. Two independent experiments were conducted comparing intra-tumoral injection of AAV1-rP0-GSDMDNterm with either AAV1-rP0-null or phosphate-buffered saline (PBS) vehicle as controls. We also developed an allograft murine schwannoma model by implanting mouse 08031-9 schwannoma cells [16] in which we expressed firefly luciferase (Fluc) into the sciatic nerve of syngeneic FVB/N male mice. Tumor growth was assessed non-invasively using in vivo bioluminescent imaging [21]. Repeated measures ANOVA revealed that intra-tumoral injection of AAV1-rP0-GSDMDNterm significantly reduced tumor growth in both human schwannoma xenografts and mouse schwannoma allograft tumors, respectively, compared to their respective AAV1-rP0-null or PBS control groups (Fig. 3).

The therapeutic effect of GSDMDNterm delivery on tumor growth in the xenograft model was confirmed by hematoxylin and eosin (H&E) staining of nerve sections harvested at the end of the study (6-weeks post implantation and 4-weeks post virus injection); a clear difference between experimental and control groups in tumor cell burden was evident (Fig. 4). The staining showed abundant hematoxylin-positive HEI-193 tumor cells in PBS-injected mice, but scant numbers of tumor cells following AAV1-rP0-GSDMDNterm injection. Additionally, evaluation of tumors injected with AAV1-rP0-GSDMDNterm showed increased dead cells and decreased Ki67 staining, the latter suggesting decreased proliferation (Fig. 4).
Intra tumoral injection of AAV1-rP0-GSDMDNterm does not cause neuronal toxicity

Delivery of pore-forming proteins such as GSDMDNterm into schwannoma tumor may carry the risk of neurotoxicity. To investigate whether the GSDMDNterm gene therapy is associated with toxicity to injected nerves, we have conducted behavioral tests. Three behavioral tests were performed. Thermal sensitivity was assessed using Hargreaves’ Method in which an infrared heat source stimulates the hind paw ipsilateral to tumor; and withdrawal latency is the primary dependent measure (Fig. 5a) [25]. Mechanical sensitivity – which can detect allodynia or decreased sensation – was tested using von Frey filaments to establish withdrawal threshold of the hind paw ipsilateral to the tumor-containing sciatic nerve (Fig. 5b) [22]. Finally, gross motor performance was assayed by the accelerating rotarod test as a measure of neurotoxicity, (Fig. 5c) [23].

Intra-tumoral AAV1-rP0-GSDMDNterm inhibited tumor growth (Fig. 3a). Tumor growth itself lead to sensitization to thermal stimulation (Fig. 5a, PBS control group) which is interpreted as development of pain sensitivity in the tumor bearing limb. Interestingly, as indicated by repeated measure ANOVA $[F (1,120) = 65.3, p < 0.01]$ AAV1-rP0-GSDMDNterm treatment was associated with normalization of heat sensitization (Fig. 5a). Von Frey withdrawal threshold and gross motor performance did not change following treatment with AAV-rP0-GSDMDNterm treatment compared with PBS controls (Fig. 5b, c, respectively). Taken together, these behavioral data indicate lack of toxicity of AAV-rP0-GSDMDNterm treatment in the HEI-193 xenograft schwannoma model.

Discussion

We have previously shown that over-expression of procaspase-1 can control experimental schwannomas using an AAV1-based gene therapy vector [8]. In the experiments described here, we report on the use of a novel transgene,
specifically the N-terminal of gasdermin D (GSDMDNterm) under the control of the schwann-lineage specific promoter, P0, in an AAV1-based schwannoma gene therapy; the vector is denoted “AAV1-rP0-GSDMDNterm.” GSDMD, like caspase-1, constitutes a primary component of the inflammasome pyroptotic cell death pathway. To our knowledge, this is the first study to propose GSDMDNterm as a transgene that could be used in a schwannoma gene therapy.

GSDMD was identified as a substrate for the inflammatory caspases 1/4/5/11 [11]. Pyroptotic cell death was shown to be dependent upon Cleavage of GSDMD and the release of the GSDMDNterm which produces pores in both artificial liposomes and the plasma membrane of cells [28].

Our in vitro data show that over-expression of GSDMDNterm in the human NF2 schwannoma cell line, HEI-193, induces cell death as measured by ATP-based viability, LDH release and PI/annexin flowcytometric analysis. In contrast, GSDMDNterm did not cause cell death when over-expressed in normal human dermal fibroblasts. However, it is unclear why GSDMDNterm was not toxic to the normal dermal fibroblasts. These observations suggest the selective killing of schwannoma tumor cells compared to healthy cells following exogenous over-expression of GSDMDNterm. Thus, we have generated pre-clinical data supporting a potentially effective gene therapy strategy for the treatment of schwannomas. Our therapeutic approach utilizes intra-tumoral injection of an AAV1 vector for delivery of a GSDMDNterm transgene. Tissue specificity and hence neuronal protection is generated using the Schwann-lineage cell specific promoter, P0. Previous work from our lab demonstrated that there is essentially no expression of P0-controlled transgenes in sensory neurons [8, 29]. Intra-tumoral injection of AAV1-rP0-GSDMDNterm into mouse sciatic nerve demonstrated tumor cell death without signs of neuronal axons toxicity. Behavioral testing, both sensory and motor, supported safety of AAV1-rP0-GSDMDNterm.

Although we have tested the therapeutic outcome of AAV1-rP0-GSDMDNterm using both xenograft and allograft mouse schwannoma models, preclinical assessment of this product requires further studies in our immune-competent schwannoma mouse model. This includes investigation of potential innate and adaptive immune responses associated with AAV1-rP0-GSDMDNterm treatment, and any effects of these responses on efficacy or toxicity.

In summary, we have identified GSDMDNterm as potentially therapeutic transgene with promise for schwannoma gene therapy and generated a therapeutic strategy that utilizes a novel mechanism that does not interfere or overlap with current schwannoma treatments. Translation of this AAV1-rP0-GSDMDNterm schwannoma gene therapy to clinical application could reduce the need for surgical resection and its associated morbidity. Thus, AAV1-rP0-GSDMDNterm holds
the promise of filling a major unmet clinical need for the schwannoma patients and their families.

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Compliance with ethical standards
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