Published in final edited form as:

Otol Neurotol. 2018 June ; 39(5): e362–e369. doi:10.1097/MAO.0000000000001766.

A Xenograft Model of Vestibular Schwannoma and Hearing Loss

Christine T. Dinh, M.D.¹, Olena Bracho, B.S.¹, Christine Mei, B.S.¹, Esperanza Bas, Ph.D.¹, Cristina Fernandez-Valle, Ph.D.², Fred Telischi, M.D.¹, and Xue-Zhong Liu, M.D., Ph.D.¹

¹University of Miami Miller School of Medicine, Department of Otolaryngology, Miami, FL 33136

²University of Central Florida College of Medicine, Burnett School of Biomedical Sciences, Orlando, FL 32827

Abstract

Hypothesis—Microsurgical implantation of mouse merlin-deficient Schwann cells (MD-SC) into the cerebellopontine angle of immunodeficient rats will initiate tumor formation, hearing loss, and vestibular dysfunction.

Background—The progress in identifying effective drug therapies for treatment of Neurofibromatosis type II (NF2) is limited by the availability of animal models of VS that develop hearing loss and imbalance.

Methods—A microsurgical technique for implanting MD-SCs onto the cochleovestibular nerve of rats was developed. Ten Rowett Nude rats were implanted with either ~10⁵ MD-SCs expressing luciferase (N=5) or vehicle (N=5). Rats received bioluminescence imaging, auditory brainstem response testing, and were observed for head tilt every two weeks after surgery, for a total of 6 weeks. Tumors were harvested and processed with hematoxylin & eosin staining and immunohistochemistry was performed for S100.

Results—Rats implanted with MD-SCs developed significantly higher tumor bioluminescence measurements and hearing threshold shifts at multiple frequencies by the 4th and 6th weeks post-implantation, compared to control rats. Rats implanted with MD-SCs also developed gross tumor. The tumor volume was significantly greater than nerve volumes obtained from rats in the control group. All rats with tumors developed a head tilt, while control rats had no signs of vestibular dysfunction. Tumors demonstrated histological features of schwannoma and express S100.

Conclusion—Using this microsurgical technique, this xenograft rat model of VS develops tumors involving the cochleovestibular nerve, shifts in hearing thresholds, and vestibular dysfunction. This animal model can be utilized to investigate tumor-mediated hearing loss and perform preclinical drug studies for NF2.

Corresponding Author: Christine T. Dinh, M.D., Assistant Professor of Otolaryngology, University of Miami Miller School of Medicine, Address: 1120 NW 14th Street, Suite 579, Miami, FL 33136, Phone: (305) 243-1484; Fax: (305) 243-2009, ctdinh@med.miami.edu.

Sources of support that require acknowledgment: N/A

Disclosure of funding received for this work from an organization: N/A

Keywords

vestibular schwannoma; acoustic neuroma; hearing; auditory brainstem response; bioluminescence; tumor; neurofibromatosis type II; NF2

Introduction

Neurofibromatosis Type II (NF2) is a hereditary tumor disorder that predisposes individuals to develop multiple nervous system tumors, particularly bilateral vestibular schwannomas (VS) that arise from the Schwann cells of the cochleovestibular nerves.¹ Because VS are benign tumors involving the cochleovestibular nerve, these tumors have a propensity to cause progressive hearing loss (HL) and disabling imbalance.

NF2 is caused by an inherited or *de novo* mutation in the *Nf2* tumor suppressor gene on chromosome 22 that encodes the merlin tumor suppressor protein. Merlin regulates cell-cell adhesion and therefore, deficiencies in merlin can result in Schwann cell proliferation and tumorigenesis.² NF2 affects 1:60,000 people with an incidence of 1:33,000.³

Technological advances in diagnosis and treatment have improved the life expectancy overall in NF2 patients; however, this is without consideration of the substantial morbidity that occurs with this disease and its treatments.⁴ Microsurgical resection of these tumors often results in deafness and other neurological sequelae as these tumors demonstrate lobular growth patterns, faster growth rates, and poor cleavage planes.^{5–8} Radiosurgery is limited to small and medium-sized tumors and has several long-term side effects, such as variable tumor control rates, poor hearing outcomes, and higher rates of malignant transformation or secondary cancers in NF2 patients.^{9–13} Off-label use of select chemotherapeutic agents have been utilized with some effect on hearing preservation and tumor control; however, the benefits are often marginal, temporary, patient-dependent, and limited by drug side effects.
14–18

Optimal treatment regimens for each individual with NF2-associated VS *must* balance tumor control (to prevent intracranial complications) and quality of life (hearing preservation and balance). However, the development of effective drug therapies to treat NF2 is impeded by our progress in understanding how tumors mediate HL and activate compensatory strategies to evade drug cytotoxicity. In part, this is due to a paucity of relevant and readily available animal models that develop schwannoma-mediated HL for preclinical NF2 drug testing.

Current available animal models to study HL in VS include genetic and allograft murine models. Gehlhausen et al. (2015) described a genetically-engineered mouse model of NF2 that develops multiple paraspinal and cranial nerve schwannomas, as well as VS.¹⁹ These mice recapitulate the histological and biological changes seen in NF2 patients, including HL and vestibular dysfunction. However, this model develops tumors and HL over several months, which limits the utility of this animal model for testing and prioritizing potential drug therapies for NF2. Bonne et al. (2016) introduced an allograft model for VS created by orthotopic grafting of the SC4 Schwann cell line onto the cochleovestibular nerve by infusing cells embedded in Matrigel® into the cerebellopontine angle (CPA) through

microsurgical or stereotactic approaches.²⁰ This allograft model develops tumors as early as day 11 that can be detected with bioluminescence imaging (BLI) and magnetic resonance imaging (MRI). Mice also develop HL on auditory brainstem response (ABR) tests. However, the usefulness of this animal model for preclinical drug testing is limited by the short time (~21 days) to the development of adverse clinical outcomes that necessitate euthanasia in a majority of mice.

Our study is a proof-of-concept investigation that evaluates a microsurgical technique in immunodeficient rats for grafting mouse merlin-deficient Schwann cells (MD-SC) on the bioluminescent detection and development of tumors, HL, and vestibular dysfunction.

Methods

Animals

All animals used in this study were treated in compliance with the published Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (8th Edition). The study was performed in accordance with the University of Miami Institutional Animal Care and Use Committee (Protocol #16-187). Adult male Rowett Nude immunodeficient rats (250–300g) were obtained from Charles River Laboratories, Inc. Ten rats were utilized for this study. Five rats were randomized to the tumor implantation group and the remaining five rats were placed in the vehicle group. Rats were observed weekly for adverse clinical signs that require immediate intervention or euthanasia as enumerated in the Guidelines for the Welfare and Use of Animals in Cancer Research.²¹

Cell Line

A mouse Schwann cell line deficient in merlin (MTC-10+Luciferase) was obtained from Dr. Fernandez-Valle.²² This MD-SC line was created from Schwann cells that were isolated from neonatal NF2^{flox2/flox2} mouse sciatic nerves, purified with differential laminin adhesion, and transduced with Cre recombinase adenovirus at the second passage to express luciferase. The cell line possesses a deletion in exon 2 of the NF2 gene, expresses S100 on immunohistochemistry, and is deficient in merlin protein. Cells were grown on CellBind plates (Corning) in media containing 1:1 ratio of Dulbecco's Modified Eagle Medium (Gibco, #11965-084) and Ham's F-12 Nutrient Mix (Gibco, #11765-047), supplemented with 1% N2 supplement (Gibco, #17502-001).

Surgical Procedure for Cell Implantation

Rats were anesthetized using inhalational isoflurane (2–4% induction; 1–3% maintenance; with 1–2 L O₂/min). The hair on the vertex and behind the ears was shaved with an electric razor. The rats were then transferred to an isothermal pad and received isoflurane anesthesia using a nosecone. The right or left ear was randomly chosen for tumor implantation or vehicle. The surgical site was prepped with topical betadine, and the postauricular space was anesthetized with 1% lidocaine. An operating microscope was utilized to perform the microsurgical approach. The surgical procedure is outlined in Figure 1(A–G). A vertical incision on the dorsal-ventral axis was made behind the ear using a 15-blade scalpel. The platysma was identified and divided. The nuchal ridge and temporalis line were identified by

visualizing the attachments of the temporalis, trapezius, sternocleidomastoid, and digastric muscles. The facial nerve was dissected bluntly and traced to the stylomastoid foramen. The sternocleidomastoid muscle was dissected off of the temporalis line and retracted posteriorly to expose the mastoid process. The temporalis muscle was dissected off of the temporalis line to visualize the squamosal bone. Subsequently, using an electric drill (Stryker) with a small 2mm burr, the mastoid process, temporalis line, and squamosal bone were carefully drilled with copious irrigation. The sigmoid sinus was skeletonized and preserved as a landmark. The dura overlying the paraflocculus was decompressed and incised with a fine needle to allow for drainage of cerebrospinal fluid. The paraflocculus was carefully retracted superior-posteriorly to expose the root entry zone of the facial and cochleovestibular nerves. MeroGel® (Medtronic, Inc.), a bioresorbable packing made from esterified hyaluronic acid, was cut into 1×1 mm pieces and used as a substrate to deliver the cells to this area. In the vehicle group, 1×1 mm bioresorbable packing with Leibovitz's L-15 medium (Gibco, #21083-027) was placed on the facial and cochleovestibular nerves. In the tumor implantation group, the 1×1 mm bioresorbable packing was impregnated with $\sim 1 \times 10^5$ MD-SCs in L-15 medium. Subsequently, bone wax (Ethicon) was used to seal the craniotomy and prevent leakage of cerebrospinal fluid. The surgical site was closed in multiple layers using non-absorbable sutures. The rats received oral enrofloxacin (~ 50 mg/kg/day; ~ 0.25 mg/ml in drinking water) for 7 days, ketorolac (2 mg/kg) intraperitoneal twice daily for 4 days, and triple antibiotic ointment daily for total of 3 days. Food pellets soaked in Ensure and hydrogel packages were placed on the cage floor prophylactically for the first 7 days to aid in recovery.

ABR testing

Rats received baseline ABR tests prior to surgery and every two weeks after surgery for a total of 6 weeks. Prior to every ABR test, rats received otologic exams to confirm there were no tympanic membrane perforations or middle ear effusions. Rats were anesthetized with intramuscular ketamine (40 mg/kg) and xylazine (20 mg/kg) and placed in a soundproof room on an isothermal pad. Subdermal electrodes were placed on the vertex (reference), both mastoids, and the left hind leg (ground). Insert earphones that deliver sound stimuli were placed in both ear canals. Using a commercial system (SmartEP and SmartEP-ASSR, Intelligent Hearing Systems, Miami, FL, U.S.A.) customized for performing ABR tests in rats, quasi-steady state auditory evoked potentials (AEP) were collected for 4, 8, and 16 kilohertz (kHz) stimuli and steady state AEPs for 24 and 32 kHz stimuli as previously described.²³ High frequency transducers were utilized for 24 and 32 kHz frequencies and a high pass filter was utilized for 16, 24, and 32 kHz frequencies to minimize the influence of internal and external noise. Acoustic stimuli were presented as tone-pips to each ear at an average rate of 78.13 stimuli/s for 4, 8, and 16 kHz and 158 stimuli/s for 24 and 32 kHz frequencies. A minimum of 500 sweeps per frequency and decibel was delivered to the tested ear, while the contralateral ears were appropriately masked. Hearing threshold was defined as the minimum intensity in decibels (dB) required to produce an AEP response that was identifiable and consistent with a p-value < 0.05. Hearing thresholds were tested at 10 dB increments from 100 dB to 0 dB.

BLI

Rats underwent BLI using the Caliper/Xenogen IVIS® Spectrum platform at 2, 4, and 6 weeks after surgery. Awake rats were injected intraperitoneal with 30 mg/kg of D-Luciferin solution (Perkin Elmer, #122799). After 5 minutes, the rats were anesthetized with inhalational isoflurane anesthesia (2–4% induction; 1–3% maintenance; 1 L O₂/min) and placed in the IVIS platform in the prone position. The rats received continuous isoflurane through a nose cone. Bioluminescence imaging was obtained at ~10 minutes using the Living Image ® Software and total flux (p/s) and average radiance (p/s/cm²/sr) were measured.

Tumor Volume

Prior to immunohistochemistry, measurements were obtained from the cochleovestibular nerves and tumor specimens. Because the harvested nerves resembled a cylinder, the formula to determine volume of a cylinder was used [$\text{Volume}_{\text{nerve}} = \pi \times \text{radius}^2 \times \text{length}$]. Because the tumors represented an elliptical sphere, a formula that approximates volume of an elliptical spheroid was utilized [$\text{Volume}_{\text{tumor}} = (\pi \times \text{height} \times \text{width} \times \text{length})/6$].

Immunohistochemistry

When rats completed their 6th week ABR and BLI or met euthanasia endpoints, the rats were euthanized and temporal bones were harvested and fixed in 4% paraformaldehyde. Subsequently, the tumors or cochleovestibular nerves were harvested and embedded in paraffin. Five micrometer sections were obtained using a microtome and mounted on a slide. Antigen retrieval was performed with Tris-EDTA (pH 8.0). To confirm S100 expression, sections were permeabilized and blocked with 1% Triton X-100 and 5% donkey serum and then treated with 1:200 rabbit anti-S100 primary antibody (DAKO, A0311) overnight at 4 degrees Celsius, 1:200 donkey anti-rabbit secondary antibody (conjugated with Alexa-488; ThermoFisher) for 2 hours at room temperature, and DAPI (nuclear stain) for 15 minutes. Antifade medium was applied, a coverslip was placed, and slides were examined with a confocal microscope (Carl Zeiss LSM 700 Laser Scanning Confocal Microscope, Germany). Sections of paraffin-embedded tissues were also stained with hematoxylin and eosin.

Statistical Analysis

Audiometric, BLI, and tumor volume measurements obtained from rats implanted with MD-SCs and vehicle were displayed as mean $\pm$ standard deviation or mean $\pm$ standard error mean and analyzed using Mann Whitney U test. Significance was set at p-value less than 0.05. Bivariate correlation using Pearson's correlation coefficient was calculated to assess the strength of a linear correlation between end-point tumor volume, average of ABR threshold shifts across all tested frequencies, and BLI measurements.

Results

All control rats completed the study and obtained ABR testing and BLI at week 6. None of the control rats developed head tilt or weight loss. Two rats implanted with MD-SCs did not receive BLI at the six-week time-point as they met end-point criteria for euthanasia at the end of week 5 and beginning of week 6. Both rats developed severe head tilt, ataxia, and

rapid weight loss with time. One of these two rats completed ABR testing on the sixth week, and the audiometric data was included in the analysis. The remaining three rats implanted with MD-SC completed the six-week study and developed a mild head tilt without ataxia by the sixth week. These three rats that completed the study developed a gradual weight loss in the 5th and 6th week.

Auditory Brainstem Response

Rats implanted with MD-SCs developed significantly higher hearing thresholds on ABR testing at 4, 24, and 32 kHz during week 4 ($p=0.043$, $p=0.007$, and $p=0.025$, respectively) and at 16, 24, and 32 kHz during week 6 ($p=0.049$, $p=0.021$, $p=0.021$, respectively), compared to the vehicle group (Figure 2). Although higher thresholds were seen at 8 and 16 kHz during week 4 and at 4 and 8 kHz during week 6 in the MD-SC group, they were not statistically significant. Rats that were implanted with vehicle did not develop significant changes in ABR thresholds at all time points, when compared to baseline. When assessing shifts in ABR threshold from baseline, rats implanted with MD-SCs developed significantly larger ABR threshold shifts at 4, 8, 24, and 32 kHz during week 4 ($p=0.025$, $p=0.020$, $p=0.007$, and $p=0.011$, respectively) and 4, 24, and 32 kHz during week 6 ($p=0.046$, $p=0.024$, and $p=0.036$, respectively), when compared to control rats (Figure 3). The vehicle group did not develop significant ABR threshold shifts at all time-points.

Tumor Bioluminescence

The total flux and average radiance values of regions of interest (ROI) were normalized to background. At six weeks, rats in the vehicle group did not demonstrate visible bioluminescence (as determined by radiance measurements), while all rats implanted with MD-SCs developed a region of visible bioluminescence (Figure 4). All rats that demonstrated visible bioluminescent signal in the ROI developed detectable tumors in the CPA. Rats implanted with MD-SCs demonstrated significantly higher levels of total flux, compared to vehicle-treated controls, at weeks 2 and 4 ($p=0.032$ and $p=0.008$, respectively; Figure 5A). Significance was not achieved at 6 weeks, likely because BLI was not performed on two of the five rats due to development of adverse clinical signs.

Tumor Volume

All rats implanted with MD-SCs demonstrated visible bioluminescence on BLI and gross tumor formation. The harvested tumors were significantly larger in volume, when compared to nerve specimens obtained from the vehicle group ($p=0.036$; Figure 5B). The average volume was 501.6 mm^3 , whereas the average nerve volume was 1.8 mm^3 . The relationship between the end-point tumor or nerve volumes, average of ABR threshold shifts (across all frequencies tested), and BLI measurements was investigated. There was a weak positive correlation between tumor and nerve volumes and degree of HL ($r=0.381$) and a very weak negative correlation between BLI measurements and degree of HL ($r=-0.090$). However, there was a very strong positive correlation between tumor and nerve volumes and BLI measurements ($r=0.882$).

Immunohistochemistry

Several tumors demonstrated features commonly seen in schwannoma tumor, including areas of spindle-shaped cells with elongated nuclei, nuclear palisading (early Verocay bodies), areas of hypercellularity (Antoni A) and hypocellularity (Antoni B), as well as involvement of the cochleovestibular nerve fibers (Figure 6A–C). Select tumors were also processed using immunohistochemical techniques and demonstrated S100 positivity (Figure 6D).

Discussion

The development of effective therapies for NF2 patients is impeded by the availability of animal models of VS. Currently, the only allograft mouse model utilized for NF2 drug studies consists of injecting mouse MD-SCs or implanting human VS on the easily accessible sciatic nerve of immunocompromised mice.^{24–27} Although major advantages of this animal model are the ease and speed with which tumors form and drugs can be screened (typically 4–6 weeks), it does not recapitulate the normal microenvironment of NF2 schwannomas. Furthermore, hearing and vestibular function cannot be assessed. Recently, two other mouse models have been developed to investigate the effects of schwannoma formation on hearing and balance; however, they have not yet been exploited to assess the efficacy of potential drug therapies for NF2.

The mouse allograft model consists of infusion of a Schwann cell line (SC4-9*luc*), derived from *Nf2*-knockout mice and transformed to express luciferase, into the CPA of nude mice.²⁰ However, the stereotactic and microsurgical techniques utilized in this animal model are technically challenging and require years to master. Sham-injected mice also developed immediate shifts on ABR testing after implantation, that although improved partially with time, further depicts the challenges in reproducing this model by novice investigators. One advantage is that implanted mice develop tumors that involve the CPA and internal auditory canal, which is the relevant microenvironment for VS. In addition, it is a rapid method for developing VS animal models as tumor formation is seen on MRI as early as day 11. However, the life expectancy of nude mice implanted with SC4-9*luc* cells was ~21 days after surgical implantation, when adverse clinical signs required euthanasia. The short time period between tumor formation and development of adverse clinical signs limits the utility of this animal model for testing potential drug candidates for NF2. Fortunately, this mouse allograft model can be modified by implantation of human VS or less aggressive cell lines to better simulate normal growth patterns of VS and test candidate drug therapies.

The transgenic mouse model employs *Periostin-Cre*-driven inactivation of the *Nf2* gene by in-frame deletion of floxed exon 2.¹⁹ These mice develop schwannosis and schwannomas in paraspinal and cranial nerves, including the cochleovestibular nerve, over several months. Furthermore, mice develop progressive HL that starts at ~5 months of age. This mouse model is arguably the best model for investigating the molecular mechanisms behind tumor-mediated HL and tumorigenesis in NF2; however, it only depicts one variation of *Nf2* mutation seen in NF2 patients. Using the same techniques, different transgenic models can be developed in the future to investigate the effect of other variants in *NF2* on tumorigenesis, HL, and drug sensitivity. Although this animal model recapitulates the NF2 clinical picture,

a major disadvantage of this model is the cost of maintaining this animal model over several months and the prolonged time needed to assess whether a drug therapy is efficacious using a chemoprevention protocol (~12 months).

Our study describes an alternative xenograft animal model using mouse MD-SCs and immunodeficient rats. The MTC-10+luciferase cell line is a merlin-deficient mouse Schwann cell line that expresses a deletion in exon 2 of the *NF2* gene. This cell line has been used to investigate NF2-related molecular signaling and drug response *in vitro* and *in vivo*.^{22,24} We demonstrate that implantation of MTC-10+luciferase cells into the CPA of immunodeficient rats initiates tumor formation that involves the cochleovestibular nerve, HL, and vestibular dysfunction. Some tumors also expressed histologic features of schwannoma, such as Antoni A & B and Verocay bodies. The development of these tumors can be detected longitudinally with BLI and bioluminescence measurements can be detected at our earliest BLI time point (2 weeks). In addition, there was a very strong positive linear correlation between BLI values and tumor volume ($r = 0.882$), suggesting that BLI can be useful to track tumor progression *in vivo*. Furthermore, 60% of rats implanted with MD-SCs completed the entire 6-week study without adverse clinical endpoints, thus supporting the use of this animal model to assess efficacy of candidate drugs for NF2.

Another advantage of this xenograft model is that the rat's larger skull allows for non-traumatic surgical implantation of cells onto the cochleovestibular nerve without injury to inner ear structures. Absence of post-operative head tilt and ABR threshold shift in rats implanted with vehicle demonstrate the feasibility and reproducibility of the microsurgical approach. Furthermore, the rats implanted with MD-SCs developed head tilt and progressive HL that was statistically significant over several frequencies, making it a useful model to study VS. Although rats implanted with MD-SCs developed CPA tumors, there was a weak correlation between tumor and nerve volumes and the average of ABR threshold shifts at all tested frequencies ($r=0.318$). There was an even weaker correlation between the BLI values and degree of hearing loss ($r=-0.90$). The dissociation between tumor volume and growth with hearing loss in this xenograft model is a commonly observed phenomenon in patients with NF2-associated and sporadic VS.²⁸⁻³⁰ This finding represents a fortuitous advantage as this xenograft model will be useful in studying the pathophysiology of how tumors mediate HL. Lastly, head tilt toward the implanted side developed as early as 4 weeks after the implantation of MD-SCs and can be utilized as a functional measure of vestibular dysfunction and indirect measure of tumor formation.

This xenograft model of VS is versatile as primary human VS cells or tumor chunks can be implanted using similar microsurgical techniques, thereby allowing investigations into the effect of tumor heterogeneity on HL and tumor growth. In particular, it can be exploited to investigate how individual VS secrete growth factors or cytokines, such as tumor necrosis factor alpha, to initiate HL and to test potential otoprotective agents³¹. However, in order to monitor tumor progression with BLI, cells need to be transformed to express luciferase, which limits the utility of this imaging modality. In these circumstances, MRI can be substituted for BLI as the preferred technique for imaging. Another limitation of the proposed animal model is that immunodeficient rats incur higher costs than immunocompromised mice.

Because the xenograft model is not a genetic model, it cannot be used to study the natural history of NF2 as a hereditary tumor disorder. It is better suited for investigating sporadic isolated VS, which is a more common disorder than NF2³². Despite the limitation, the xenograft model is useful for initial *in vivo* screening of candidate drugs for NF2 VS and assessing drug penetration through the blood-brain and blood-nerve barriers. Utilizing the xenograft model as a screening method can quickly narrow the pool of best potential therapies that can be advanced for final preclinical drug testing in the NF2 genetic model. Because the genetic model develops tumors and HL over several months, it is not appropriate for screening and large-scale drug studies. Thus, this streamlined approach for filtering candidate drugs to top performing agents will enable efficient identification of effective therapies for NF2 clinical trials. Furthermore, the immunocompetent genetic model is likely better at predicting drug tolerance and tumor response in NF2 patients than immunodeficient animals.

In conclusion, we describe a microsurgical approach for the implantation of MD-SCs expressing luciferase into the CPA of immunodeficient rats. This xenograft model develops tumors expressing schwannoma histological features as well as auditory and vestibular dysfunction. These findings support the use of this xenograft model in NF2 preclinical trials to assess the impact of candidate drugs on tumor burden, HL, and balance, and prioritize drug therapies for clinical investigations.

References

1. Evans DGR. Neurofibromatosis type 2 (NF2): A clinical and molecular review. *Orphanet J Rare Dis.* 2009; 4:16. [PubMed: 19545378]
2. Petrilli AM, Fernandez-Valle C. Role of Merlin/NF2 Inactivation in Tumor Biology. *Oncogene.* 2016; 35(5):537–548. [PubMed: 25893302]
3. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *Am J Med Genet A.* 2010; 152A(2):327–32. [PubMed: 20082463]
4. Otsuka G, Saito K, Nagatani T, Yoshida J. Age at symptom onset and long-term survival in patients with neurofibromatosis Type 2. *J Neurosurg.* 2003; 99(3):480–3. [PubMed: 12959433]
5. Jääskeläinen J, Paetau A, Pyykkö I, Blomstedt G, Palva T, Troupp H. Interface between the facial nerve and large acoustic neurinomas. Immunohistochemical study of the cleavage plane in NF and non-NF2 cases. *J Neurosurg.* 1994; 80(3):541–7. [PubMed: 8113868]
6. Sobel RA. Vestibular (acoustic) schwannomas: Histologic features in neurofibromatosis 2 and in unilateral cases. *J Neuropathol Exp Neurol.* 1993; 52:106–13. [PubMed: 8440992]
7. Samii M, Matthies C, Tatagiba M. Management of vestibular schwannomas (acoustic neuromas): auditory and facial nerve function after resection of 120 vestibular schwannomas in patients with neurofibromatosis 2. *Neurosurgery.* 1997; 40(4):696–705. [PubMed: 9092842]
8. Nowak A, Dziedzic T, Czernicki T, et al. Strategy for the surgical treatment of vestibular schwannomas in patients with neurofibromatosis type 2. *Neurol Neurochir Pol.* 2015; 49(5):295–301. [PubMed: 26377980]
9. Baser ME, Friedman JM, Aeschliman D, et al. Predictors of the risk of mortality in neurofibromatosis 2. *Am J Hum Genet.* 2002; 71:715–23. [PubMed: 12235555]
10. Meijer OW, Vandertop WP, Lagerwaard FJ, Slotman BJ. Linear accelerator-based stereotactic radiosurgery for bilateral vestibular schwannomas in patients with neurofibromatosis type 2. *Neurosurgery.* 2008; 62(5 Suppl):A37–42. [PubMed: 18580779]
11. Sun S, Liu A. Long-term follow-up studies of Gamma Knife surgery for patients with neurofibromatosis Type 2. *J Neurosurg.* 2014; 121(Suppl):143–9. [PubMed: 25434947]

12. Subach BR, Kondziolka D, Lunsford LD, Bissonette DJ, Flickinger JC, Maitz AH. Stereotactic radiosurgery in the management of acoustic neuromas associated with neurofibromatosis Type 2. *J Neurosurg.* 1999; 119(Suppl):815–22.
13. Kim BS, Seol HJ, Lee JI, et al. Clinical outcome of neurofibromatosis type 2-related vestibular schwannoma: treatment challenges. *Neurosurg Rev.* 2016; 39(4):643–53. [PubMed: 27142681]
14. Plotkin SR, Merker VL, Halpin C, Jennings D, McKenna MJ, Harris GJ, Barker FG 2nd. Bevacizumab for progressive vestibular schwannoma in neurofibromatosis type 2: a retrospective review of 31 patients. *Otol Neurotol.* 2012; 33(6):1046–52. [PubMed: 22805104]
15. Slusarz KM, Merker VL, Muzikansky A, Francis SA, Plotkin SR. Long-term toxicity of bevacizumab therapy in neurofibromatosis 2 patients. *Cancer Chemother Pharmacol.* 2014; 73(6): 1197–204. [PubMed: 24710627]
16. Mautner VF, Nguyen R, Knecht R, Bokemeyer C. Radiographic regression of vestibular schwannoma induced by bevacizumab treatment: sustain under continuous drug application and rebound after drug discontinuation. *Ann Oncol.* 2010; 21(11):2294–5. [PubMed: 20860987]
17. Blakeley J. Development of drug treatments for neurofibromatosis type 2-associated vestibular schwannoma. *Curr Opin Otolaryngol Head Neck Surg.* 2012; 20(5):372–9. [PubMed: 22931905]
18. Karajannis MA, Legault G, Hagiwara M, et al. Phase II trial of lapatinib in adult and pediatric patients with neurofibromatosis type 2 and progressive vestibular schwannomas. *Neuro Oncol.* 2012; 14(9):1163–70. [PubMed: 22844108]
19. Gehlhausen JR, Park S-J, Hickox AE, et al. A murine model of neurofibromatosis type 2 that accurately phenocopies human schwannoma formation. *Hum Mol Genet.* 2015; 24(1):1–8. [PubMed: 25113746]
20. Bonne NX, Vitte J, Chareyre F, et al. An allograft mouse model for the study of hearing loss secondary to vestibular schwannoma growth. *J Neurooncol.* 2016; 129(1):47–56. [PubMed: 27177628]
21. Workman P, Aboagye EO, Balkwill F, et al. Guidelines for the welfare and use of animals in cancer research. *Br J Cancer.* 2010; 102(11):1555–1577. [PubMed: 20502460]
22. Petrilli AM, Fuse MA, Donnan MS, et al. A chemical biology approach identified PI3K as a potential therapeutic target for neurofibromatosis type 2. *Am J Transl Res.* 2014; 6(5):471–93. [PubMed: 25360213]
23. Bas E, Bohorquez J, Goncalves S, et al. Electrode array-eluted dexamethasone protects against electrode insertion trauma induced hearing and hair cell losses, damage to neural elements, increases in impedance and fibrosis: A dose response study. *Hear Res.* 2016; 337:12–24. [PubMed: 26892906]
24. Fuse MA, Klingeman Plati S, Burns SS, et al. Combination Therapy With c-Met and Src Inhibitors Induces Caspase-Dependent Apoptosis of Merlin-Deficient Schwann Cells and Suppresses Growth of Schwannoma Cells. *Mol Cancer Ther.* 2017; 16(11):2387–2398. [PubMed: 28775147]
25. Giovannini M, Bonne NX, Vitte J, et al. mTORC1 inhibition delays growth of neurofibromatosis type 2 schwannoma. *Neuro-oncology.* 2014; 16(4):493–504. [PubMed: 24414536]
26. Licciulli S, Maksimoska J, Zhou C, et al. FRAX597, a small molecule inhibitor of the p21-activated kinases, inhibits tumorigenesis of neurofibromatosis type 2 (NF2)-associated Schwannomas. *J Biol Chem.* 2013; 288(40):29105–29114. [PubMed: 23960073]
27. Jacob A, Oblinger J, Bush ML, et al. Preclinical validation of AR42, a novel histone deacetylase inhibitor, as treatment for vestibular schwannoma. *Laryngoscope.* 2012; 122(1):174–189. [PubMed: 22109824]
28. Sughrue ME, Kane AJ, Kaur R, et al. A prospective study of hearing preservation in untreated vestibular schwannomas. *J Neurosurg.* 2011; 114(2):381–385. [PubMed: 20486891]
29. Jethanamest D, Rivera AM, Ji H, Chokkalingam V, Telischi FF, Angeli SI. Conservative management of vestibular schwannoma: predictors of growth and hearing. *Laryngoscope.* 2015; 125(9):2163–8. [PubMed: 25647714]
30. van de Langenberg R, de Bondt BJ, Nelemans PJ, Dohmen AJ, Baumert BG, Stokroos RJ. Predictors of volumetric growth and auditory deterioration in vestibular schwannomas following in a wait and scan policy. *Otol Neurotol.* 2011; 32(2):338–44. [PubMed: 21150682]

31. Dilwali S, Landegger LD, Soares VY, Deschler DG, Stankovic KM. Secreted factors from human vestibular schwannomas can cause cochlear damage. *Sci Rep.* 2015; 5:18559. [PubMed: 26692421]
32. Evans DG, Moran A, King A, Saeed S, Gurusinghe N, Ramsden R. Incidence of vestibular schwannoma and neurofibromatosis 2 in the North West of England over a 10-year period: higher incidence than previously thought. *Otol Neurotol.* 2005; 26(1):93–7. [PubMed: 15699726]

Figure 1.

Surgical Approach to the Cerebellopontine Angle in Rat. (A) The right auricle is retracted anteriorly and a retroauricular incision is made along the dorsal-ventral axis. The platysma (P) is identified and transected to expose deeper neck muscles. (B) The nuchal ridge (solid line) and temporalis line (dotted line) are identified as well as adjacent muscles, including the trapezius (Tp), temporalis (T), sternocleidomastoid (SCM), and digastric (Dg) muscles. (C) The main trunk of the facial nerve (FN) is exposed in order to identify the location of the stylomastoid foramen (*). The SCM is detached from the temporalis line and retracted posteriorly to expose the mastoid process (MP). (D) The temporalis muscle is detached from the temporalis line and retracted anteriorly to expose the squamosal bone (S). (E) The mastoid process, temporalis line, and squamosal bone (outlined by the diamond) are drilled carefully layer by layer. (F) The sigmoid sinus (SS) is skeletonized and the bone over the parafoolculus (Pf) is decompressed to expose the overlying dura. (G) The dura is incised to decompress the cerebrospinal fluid and the parafoolculus is retracted superior-posteriorly to expose the root entry zone of cranial nerve VII and VIII (#). After bioresorbable packing impregnated with merlin-deficient Schwann cells was placed on the cranial nerves, bone wax was used to seal the craniotomy site and the surgical site is closed with non-absorbable suture.

Figure 2.

Auditory Brainstem Response (ABR) Thresholds. When MD-SCs were implanted in the cerebellopontine angle of Rowett Nude rats, rats developed significant hearing threshold shifts on ABR testing at 4, 24, and 32 kHz during week 4 and at 16, 24, and 32 kHz at week 6, compared to the vehicle group. Rats that were implanted with vehicle did not develop significant shifts in ABR thresholds at all weeks compared to baseline. Bars represent mean values. Error bars represent standard deviation. * $p<0.05$. ** $p<0.01$.

Figure 3.

Auditory Brainstem Response (ABR) Threshold Shifts. Rats implanted with MD-SCs developed significantly larger shifts in hearing thresholds (from baseline) on ABR testing at 4, 8, 24, and 32 kHz during week 4 and 4, 24, and 32 kHz at week 6, compared to the vehicle group. Rats that were implanted with vehicle did not develop significant shifts in ABR thresholds at all weeks compared to baseline. Bars represent mean values. Error bars represent standard deviation. * $p < 0.05$. ** $p < 0.01$.

Figure 4. Tumor Bioluminescence Imaging. Representative bioluminescence images are shown in rats implanted with vehicle and MD-SCs when study end-point measures were reached. No bioluminescence was detected in the vehicle group.

Figure 5.

Tumor Bioluminescence and Volume. (A) Rats implanted with MD-SCs demonstrated significantly higher levels of total flux at weeks 2 and 4, compared to vehicle-treated controls. Significance was not achieved at 6 weeks, likely because bioluminescent imaging was not performed on two of the five rats due to development of adverse clinical signs. (B) The tumors that were harvested from rats implanted with MD-SCs were significantly larger in volume than the nerve specimens that were harvested from the vehicle group. Bars represent mean values. Error bars represent standard error mean. * $p < 0.05$.

Figure 6.

Immunohistochemistry of Tumors. (A) Tumors demonstrated areas of hypercellularity (Antoni A; #) and hypocellularity (Antoni B; *). (B) Regions of palisading nuclei (early Verocay bodies; black dotted box) are seen. (C) Tumors involve the cochleovestibular nerve (arrow, junction between disease nerve above and tumor below). (D) Tumors demonstrate S100 positivity (green) and nuclear staining (DAPI; blue).