EXPLORATORY EVALUATION OF AR-42 HISTONE DEACETYLASE INHIBITOR IN THE TREATMENT OF VESTIBULAR SCHWANNOMA AND MENINGIOMA

IRBNet ID : 596699/14-078H

Version 8: 11 JUN 2019
Phase 0
IND Number: 114227
Drug : AR-42

Coordinating Center: The Massachusetts Eye and Ear Infirmary

PRINCIPAL INVESTIGATOR	**D. Bradley Welling, M.D., Ph.D.** Walter Augustus LeCompte Professor and Chair Harvard Department of OtolaryngologyDepartment of Otolaryngology-Head and Neck Surgery The Massachusetts Eye and Ear Infirmary Chief of Otolaryngology The Massachusetts General Hospital 243 Charles Street Boston, MA 02115 Administrative assistant: Suzanne Walker 617-573-4075 E-mail: brad_welling@meei.harvard.edu
CO-INVESTIGATORS	**Jaishri Blakeley, M.D., Johns Hopkins University** **Robert Jackler, M.D., Stanford University** **Brian Neff, M.D., Mayo Clinic, Rochester**
COLLABORATORS	**Ruwan Kiringoda, M.D., The Massachusetts Eye and Ear Infirmary**
LAB INVESTIGATORS	**Long-Sheng Chang, Ph.D., Nationwide Children's Res. Inst.** **Zhongfa Liu, Ph.D., The Ohio State University**
BIOSTATISTICIAN	Rong Guo, M.S.

Table of Contents

1. STUDY OBJECTIVES

1.1 Primary objectives

The primary objective of this study is to estimate the expression levels of phospho-Akt (p-AKT) and $p16^{INKA}$ after 3 weeks of oral AR-42 at 40 mg every other day, 3 times per a week for 3 weeks preceding surgery, as determined by immunohistochemistry in vestibular schwannomas (VS), meningiomas, and cutaneous schwannomas and control tissue samples from patients enrolled in the control group. This endpoint was chosen based on prior data from our published preclinical work and others showing marked reduction of phosphor-AKT in AR-42 treated VS and meningiomas *in vivo*.

1.2 Secondary objectives

The secondary objectives are:

1) Assess biological effects of AR-42 on the phosphoinositide 3 kinase (PI3K)/AKT signaling pathway, including the levels of p-AKT, total AKT, p-PRAS-40, total PRAS-40, p-S6 ribosomal protein, and p-4E-BP-1 by immunoblot and immunohistochemistry and compare to untreated samples from our control patients.

2) Assess biological effects of AR-42 on tumor proliferation (as assessed by Ki-67 proliferation index), cell cycle (as assessed by the expression of cyclins, CDK inhibitors, and mitotic checkpoint kinases), cell death (as assessed by cleaved caspase-3 and TUNEL staining), and angiogenesis (as assessed by the expression of VEGF and CD31) by immunohistochemistry and immunoblot after exposure to AR-42 and compare to untreated samples from our control patients.

3) Explore the utility of HR23B as a biomarker for sensitivity of VS and meningiomas to AR-42. HR23B was previously shown to be a biomarker for tumor sensitivity to HDACi-based therapy in cutaneous T cell lymphomas (Khan et al., 2010).

4) Perform NF2 gene sequencing (tumor and germ-line DNA) and Merlin protein expression in all VS and meningiomas and explore possible differences between sporadic and NF2-related tumors and baseline p-AKT activation and biological response to AR-42 based on NF2 mutational status and Merlin protein expression.

5) Assess any audiometric changes pre- and post- AR-42 administration by conventional pure tone and speech discrimination testing. A difference of 20% speech discrimination will be considered significant on a 50-word recorded NU word list.

6) Evaluate any volumetric tumor reduction after 10 doses of AR-42 in 10 study participants by magnetic resonance imaging. A decline of 20% by volumetric analysis will be considered a clinically significant reduction. Tumor reduction will not be assessed in additional participants if no tumor response is noted in these first 10 participants.

7) Determine the steady-state plasma and intra-tumoral concentrations of AR-42 at the time of surgical resection.

2. INTRODUCTION AND RATIONALE

2.1 *Neurofibromatosis type 2* (NF2) is a debilitating disease

NF2 is highly penetrant, autosomal dominant disorder with an incidence of approximately 1/40,000 (Bull. World Health Org. 1992). Patients with NF2 are at risk for multiple nervous system tumors. One-hundred percent of NF2 patients develop vestibular schwannomas (VS) and up to 60% develop meningiomas. Approximately 50%

develop subcutaneous schwannomas. NF2 is an extremely debilitating disease, leading to decreased life expectancy in those afflicted. Individuals with NF2 often show 8th-nerve dysfunction beginning in early childhood, including tinnitus, bilateral profound deafness, and ataxia. The mean age at presentation for NF2-associated vestibular schwannomas is 20-21 years of age (Evans et al., 1992; Parry et al., 1994). Due to deafness and bilateral facial nerve paralysis NF2 patients are socially isolated and employment is difficult, if not impossible. Brainstem compression and loss of lower cranial nerves which protect the airway are leading causes of death in NF2 patients.

2.2 NF2 tumor suppressor role remains elusive

The human *NF2* tumor suppressor gene was identified on chromosome 22q12 and mutations have been identified within the *NF2* gene in patients with VS, including both sporadic unilateral schwannomas and the less common NF2-associated bilateral schwannomas (Welling et al., 2008). Additionally, *NF2* mutations are frequently found in meningiomas (Bianchi et al., 1994, 1995). The *NF2* gene encodes a protein named 'merlin' for moesin, ezrin, and radixin-like protein (Rouleau et al., 1993; Trofatter et al., 1993). Unlike other tumor suppressors, merlin does not have any catalytic or DNA-binding domains. It belongs to the ezrin-radixin-moesin (ERM) family of cytoskeleton-associated proteins, but merlin is the only member of this family to possess tumor suppressor functions. Conditional biallelic inactivation of *Nf2* in Schwann cells or arachnoidal cells in mice leads to schwannomas or meningiomas, respectively (Giovannini et al., 2000; Kalamarides et al., 2002, 2011). In addition, merlin mediates contact-dependent inhibition of proliferation in confluent cells by restraining the membrane levels of multiple pro-mitogenic receptors, such as the epidermal growth factor receptor (EGFR) in fibroblasts and keratinocytes and ErbB2/3 in Schwann cells (Curto et al., 2007; Lallemand et al., 2009). Since the growth control of certain Schwann cells and meningeal cells is abrogated by *NF2* loss, it has been suggested that *NF2* deficiency disrupts some aspect of intracellular signaling that leads to a signal to proliferate, albeit slowly (McClatchey and Giovannini, 2005). However, the mechanism underlying the molecular pathogenesis of NF2-associated tumors is not completely understood. We (Welling et al., 2002; Lasak et al., 2002; Jacob et al., 2008) and others (Hanemann et al., 2006; Hansen et al., 2006; Rong et al., 2004; Doherty et al., 2008; Hilton et al., 2009; Lallemand et al., 2009) have identified several signaling pathways that are frequently deregulated in VS, including the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. In addition to slightly elevated AKT mRNA and protein levels, VS tumors express substantially higher levels of phosphorylated, activated AKT than paired vestibular nerve specimens (Jacob et al., 2008). Furthermore, activation of the PI3K/AKT pathway has been reported in meningiomas (Johnson et al., 2002; Mawrin et al., 2005). Since the PI3K/AKT pathway serves as a convergence point for many growth stimuli and, through its downstream substrates, controls cellular processes and responses such as cell survival, cell proliferation, insulin response, stress response, and differentiation (LoPiccolo et al., 2008), its activation likely contributes to tumorigenesis. Thus, the PI3K/AKT pathway is an attractive therapeutic target for VS, and small-molecule inhibitors of AKT signaling may have therapeutic potential in suppressing the growth of these tumors.

2.3 Like NF2-associated VS, sporadic VS can cause significant morbidity, and in some cases, mortality, due to brain stem compression

VS are histologically benign nerve sheath tumors that originate on the superior or inferior vestibular branches of cranial nerve VIII. The hallmark of NF2 is the development of bilateral VS, however, VS most commonly occur as sporadic unilateral solid tumors with an incidence of 40/100,000 (Evans et al. 1992). Patients with VS present with tinnitus, hearing loss and imbalance. The tumors may lead to deafness, facial nerve paralysis and brainstem compression, hydrocephalus, and death, if left untreated. The mean age at onset in our series is 49 years of age and there is a slight female predominance without ethnic predilection (Welling et al., 1998).

2.4 NF2-associated meningiomas are associated with a high risk of mortality

Meningiomas, which arise from meningothelial or arachnoid cap cells, constitute approximately 34% of primary intracranial brain tumors (Wiemels et al., 2010; Central Brain tumor Registry of the United States [CBTRUS], 2011). Meningiomas occur in up to 60% of NF2 patients and are often multiple. Meningiomas in NF2 patients are associated with a 2- to 3-fold increased risk of mortality, and their treatment is challenging (Goutagny and

Kalamarides, 2010; Baser et. al., 2002). Location-dependent cranial nerve palsy, seizures and brainstem compression may lead to paralysis, aspiration pneumonia and death. While NF2 patients develop both VS and meningiomas synchronously, these tumors can also occur sporadically in non-NF2 patients. Meningiomas affect lower cranial nerves by compression or invasion and may have similar devastating effects to VS.

2.5 Development of a medical therapy for VS and meningiomas

General principles to consider in treatment of VS and meningiomas include the importance of preservation of function of the brainstem, the facial nerve and lower cranial nerves for swallowing and airway protection, hearing and balance. Long-term quality of life is greatly affected. Current level of evidence in the literature regarding treatment outcomes in NF2 is predominantly grade IV (retrospective case series) (Bassim et al., 2010). Treatment options for NF2-associated tumors are presently limited to observation, surgical removal or stereotactic radiation.

Observation

Watchful waiting may be advocated with MRI to assess tumor growth rate along with audiometric studies to monitor hearing loss. Both VS and meningiomas are usually slow growing. The first complication associated with observation of VS is most often increased tinnitus and decreased hearing (Massick et. al., 2000). There is not good correlation with tumor size relative to hearing loss. Some tumors originate in the cerebellopontine angle where the tumors may grow without direct pressure being placed on the adjacent cranial nerves. If, however, the tumor growth occurs within the internal auditory canal, hearing may decline rapidly from pressure on the cochlear nerve or vascular compromise to the cochlea. At present, there are no good predictors or biomarkers of tumor growth.

Stereotactic radiation

Stereotactic radiation treatment has been proposed as a treatment for NF2-associated tumors with control rates of vestibular schwannomas from 66% to 81%. Hearing preservation rates at five years were reported at 48% (Mathieu et al., 2007) and serviceable hearing at 33% (Phi et al., 2009). In a consensus conference of 36 experts, we recommended caution in recommending radiation for NF2 patients given the young age of the NF2 patient population and the reports of secondary radiation induced malignancy (Evans, et. al., 2009; Mut et al., 2004; Balasubramaniam et al., 2007). The incidence of malignant change following stereotactic radiation to benign VS is presently less than 1%, however a disproportionate number of these are found in NF2 patients. Resulting malignant sarcomas or triton tumors are universally fatal when they occur. Twenty to 30 year follow up will be necessary to determine the true incidence of malignant change as shown with prior studies of radiation to the scalp for acne and to the tonsils for hypertrophy (Albright and Allday, 1967; Palmer et al., 1980). NF2 patients are predisposed by their tumor suppressor mutation to further malignancy. Radiation induced meningiomas are also a concern. Additionally, Friedman et al. (2005) reported much poorer surgical outcome in patients who failed stereotactic radiation. Fifty-percent of patients that had surgery following radiation suffered severe or complete facial paralysis.

Surgical resection

For VS, the advantages of surgical resection may include brainstem decompression, hearing and facial nerve preservation, but the success rate is variable depending upon a number of factors, including the aggressiveness and adherence of the tumor to the brainstem and cranial nerves, the preoperative hearing, the experience of the surgical team and the tumor size. Early removal of small tumors is more likely to succeed in functional preservation. However, hearing preservation in NF2-associated VS is not as high as non-NF2 associated VS and is reported between 35 and 65% (Welling et al., 1998; Slattery et al., 2007; Samii et al., 2008). Tumor recurrence or new cranial nerve schwannomas are more common in NF2. Complications of surgery include brainstem injury, facial nerve paralysis, hearing loss, cerebrospinal fluid leak, meningitis, post-operative bleeding, pneumocephalus, and death.

Surgical excision of meningiomas is complicated by the structures to which they are adherent. Complete excision can be very challenging. Recurrence occurs in up to 30% of meningiomas following resection as extensive dural attachments and the attempts to preserve adherent or encased vital structures may result in incomplete extirpation. Similar complications of surgical craniotomy occur with surgical excision of menigiomas

as with VS. Tumors located along the skull base are more likely to be incompletely excised and are associated with higher morbidity than tumors along the convexity of the cranial vault. Lower cranial nerve palsy and shared blood supply to the brainstem are especially hazardous.

Chemotherapy

At the present time, no FDA-approved drugs are available for the treatment of NF2-associated VS or meningiomas. A case series by Plotkin et al. (2009) with bevacizumab for VS shows some tumor shrinkage and hearing improvement in a portion of patients. Meningiomas do not appear to respond well to bevacizumab. Only seven of 40 meningiomas in 14 patients showed decreased volume over the course of treatment (Nunes et. al., 2011). Meningiomas are less radio-sensitive than VS. Together, these factors underscore the importance of developing an effective chemotherapeutic agent which will stop tumor growth or completely eradicate NF2-associated and sporadic VS and meningiomas. The ideal chemotherapeutic agent would target both.

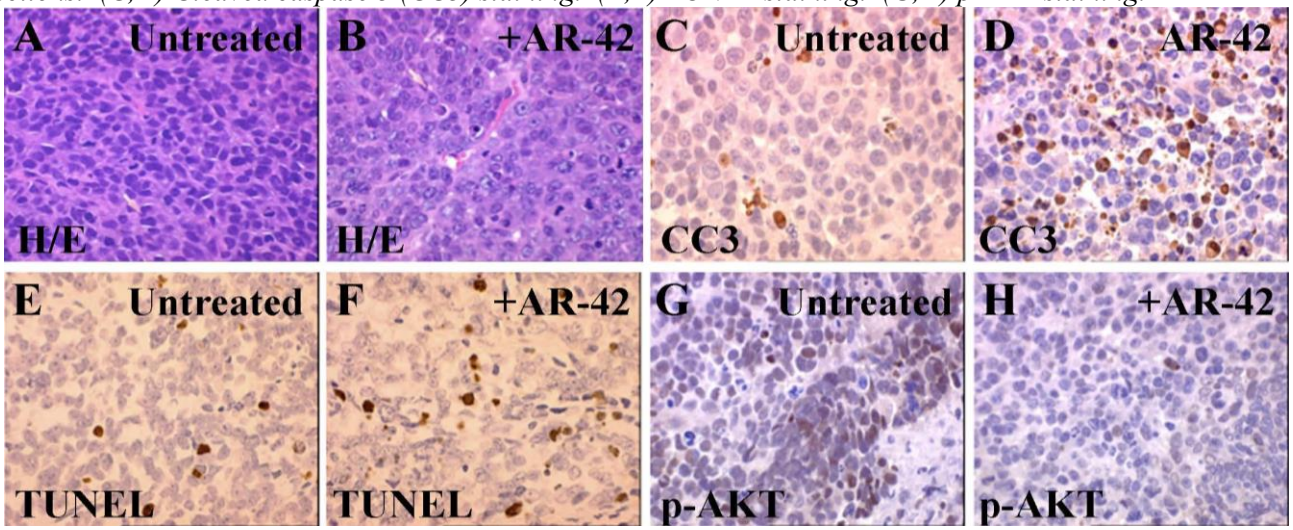
2.6 Histone deacetylase inhibition as a potential therapy for VS and Meningiomas

Histone acetylation is critical to epigenetic modifications occurring in many solid and hematological tumors (Cress and Seto, 2000; Marks et al., 2001). Histone proteins condense eukaryotic DNA 10,000 fold via the nucleosome, a histone octamer consisting of a histone H3 and H4 tetramer and two histone H2A and H2B dimers. These histone proteins are subject to post-translational modifications that regulate gene transcription by either inhibiting or facilitating tight interactions between the nucleosome and the DNA. Histone deacetylases (HDACs) remove acetyl groups from histone lysines and neutralize the positive charge on histone tails, thereby weakening interactions with negatively-charged DNA, creating a more "open" chromatin conformation, and altering transcription of growth-regulatory genes (Drummond et al., 2005; Bolden et al., 2006). Over-expressed or sustained HDAC activity has been reported in leukemia, lymphomas, and other types of cancers (Johnstone and Licht, 2003; Minucci and Pelicci, 2006). The aberrant HDAC activity in human neoplasms deacetylates the N-termini of core histones, resulting in a tightly-closed chromatin structure; therefore, tumor suppressor genes can be silenced by abnormal HDAC activity. Inhibitors of HDACs reverse this histone deacetylation, thus reactivating expression of silenced genes (Santini et al., 2007). Presently, more than a dozen HDAC inhibitors are being investigated in phase I/II clinical trials for solid and hematological tumors, with some showing efficacy and low pharmacotoxicity, including AR-42 (Drummond et al., 2005; Bolden et al., 2006; Minucci and Pelicci, 2006; Marks and Breslow, 2007; Lucas et al., 2010; Johnson et al., 2011).

AR-42 was developed at The Ohio State University (OSU) and was licensed to Arno Therapeutics, Inc. It is a novel hydroxamate-tethered phenylbutyrate derivative. AR-42 also down-regulates the AKT pathway by disrupting interactions between protein phosphatase-1 (PP1) and HDAC6, consequently allowing free PP1 to interact with and dephosphorylate AKT (Kulp et al., 2006). AR-42 has shown potent antitumor effects in multiple tumor types, at least in part, by inhibition of the PI3K/AKT pathway (Chen et al., 2005; Kulp et al., 2006; Lu et al., 2007; Sargeant et al., 2008; Yang et al., 2009; Bai et al., 2011; Lin et al., 2010; Zhang et al., 2011). We also found it to induce pro-apoptotic Bim and to decrease anti-apoptotic Bcl. AR-42 also induces cell cycle arrest by cyclin-dependent kinase inhibitors.

We recently reported AR-42 inhibition of the growth of human VS and *Nf2*-deficient mouse schwannoma cells with an IC_{50} of 500 nM and 250-350 nM, respectively (Bush et al., 2011). AR-42 also inhibited primary meningioma cells and benign meningioma Ben-Men-1 cells with IC_{50} values of 1.5 μM and 1.0 μM, respectively. AR-42 treatment induced cell cycle arrest at G_2 and apoptosis in both VS and meningioma cells. Also, AR-42 exposure decreased phosphorylated AKT in schwannoma and meningioma cells. *In vivo* treatment with AR-42 inhibited the growth of schwannoma xenografts, induced apoptosis, and decreased AKT activation (Figure 1). These results demonstrate a high potency of AR-42 against NF2-associated VS and meningiomas (Bush et al., 2011).

Figure 1.AR-42 treatment induced apoptosis and decreased phospho-AKT (p-AKT) staining in malignant schwannoma xenografts. (A,B) Hematoxylin/eosin (H/E) staining of untreated or AR-42-treated xenograft tumor sections. (C,D) Cleaved caspase 3 (CC3) staining. (E,F) TUNEL staining. (G,H) p-AKT staining.



We created a benign meningioma model by stereotactically implanting the skull base of SCID mice with a telomerase immortalized cell line, Ben-Men-1 (Puttmann et al., 2005) with a luciferase-expressing lentiviral vector (Burns et al., 2011). By bioluminescence imaging (BLI) we detected increasing luciferase activity in the brain region of these mice over time (Figure 2), establishing an intracranial meningioma model.

Figure 2. Ben-Men-1-LucB cells establish intracranial tumors that grow over time. Mice with Ben-Men-1-LucB cells ($5X10^5$) stereotactically injected into the skull base region were imaged at the indicated time.

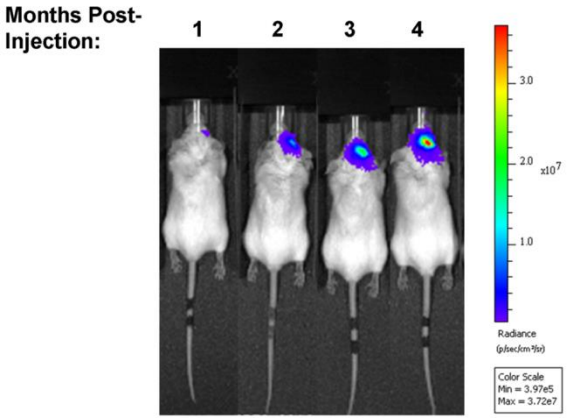


Figure 4. Ben-Men-1-LucB cells established intracranial tumors that grew over time. Mice with Ben-Men-1-LucB cells ($5X10^5$) stereotactically injected into the skull base region were imaged by BLI at the indicated time points.

2.7 AR-42 potently inhibits the growth of Ben-Men-1-LucB benign meningioma xenografts

To compare the *in vivo* efficacy of AR-42, we divided mice bearing intracranial Ben-Men-1-LucB xenografts into three groups one week after injection. The first group was fed normal diet, the second group received AR-42 at 25 mg/kg/day formulated into mouse chow, and the third group ate chow formulated with AR-12, a celecoxib derivative which we have also shown to inhibit the PI3K/AKT pathway, at 200 mg/kg/day (Research Diets, New Brunswick, NJ). We performed BLI to monitor tumor growth every month following treatment. Figure 3 showed that AR-42 potently inhibits the growth of Ben-Men-1-LucB benign meningioma xenografts. AR-42 treatment reduced the size of luciferase-expressing Ben-Men-1 meningioma xenografts by about 80~98% after five months. Importantly, the size of the Ben-Men-1-LucB xenograft was substantially reduced following AR-42 treatment for just one month. AR-12 also showed growth inhibitory activity on Ben-Men-1-LucB xenografts by 34~51% after one month of treatment; however, the tumors in mice receiving AR-12 continued to grow, albeit at a slower rate than the normal diet control group. We also performed MRI on mice treated for three months to confirm the growth inhibitory effect of AR-42 and AR-12 on intracranial Ben-Men-1-LucB xenografts (Figure 4). Histopathological examination showed that Ben-Men-1-LucB xenograft tumors grow along the meninges like benign meningiomas. The drug-treated tumors appeared much smaller than the untreated control (Figure 5). We are presently performing immunostaining for cleaved caspase 3 and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to confirm that the inhibition of tumor growth by AR-42 is mediated by apoptosis as that seen in schwannoma xenografts (Lee et al., 2009; Bush et al., 2011).

Figure 3. Growth inhibition of intracranial Ben-Men-1 benign meningioma xenografts by AR-42 and AR-12. SCID mice with established intracranial Ben-Men-1-LucB xenografts were divided into three groups (N=5). The first group (mouse #1 shown) was fed chow formulated with AR-42, the second group (mouse #2) ate normal diet, and the third group (mouse #3 and #4) received chow containing AR-12. At each indicated time points, BLI was performed.

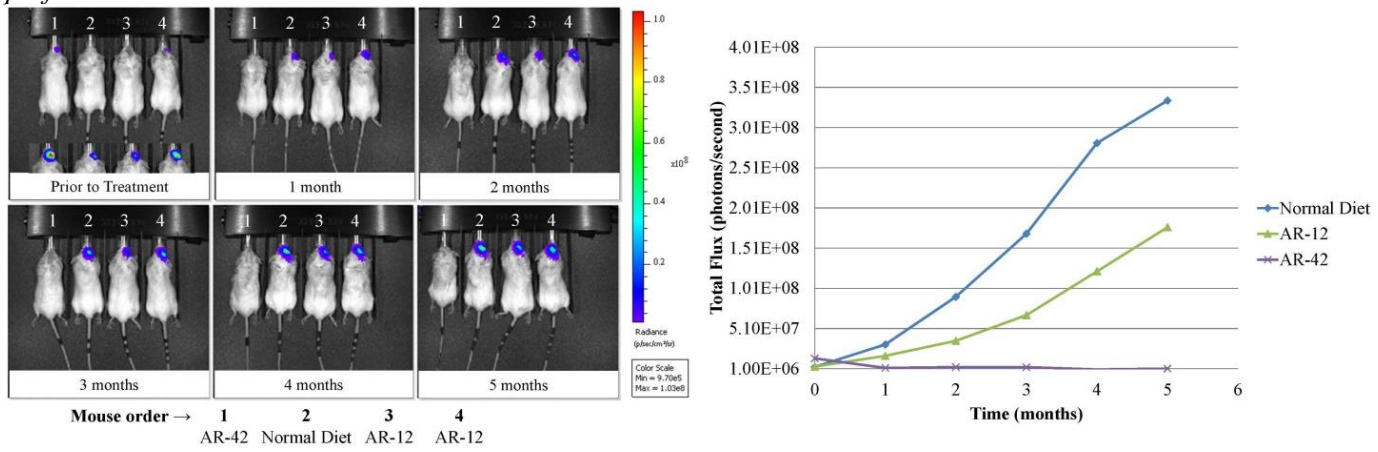
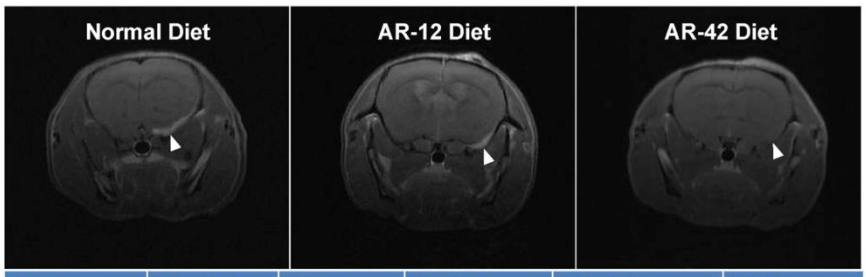


Figure 4. The effect of AR-42 and AR-12 on Ben-Men-1-LucB intracranial xenografts was confirmed by MRI. Multi-planar tumor volumes were calculated from manually-traced tumor areas on axial and coronal images. Comparing with total flux emitted from the tumor detected by BLI, a trend of reduction of tumor volumes in AR-42 or AR-12 treated mice was seen by MRI.



Treatment	Months of Treatment	Tumor Volume (mm^3)	% of Normal Diet Tumor Volume	Total Flux (photons/sec)	% of Normal Diet Total Flux
Normal Diet	4	6.7	100%	$9.46x10^6$	100%
AR-12	4	6.1	91%	$3.83x10^6$	40.5%
AR-42	4	0.0	0	$3.47x10^5$	3.7%

Figure 6. The growth inhibitory effects of AR-42 and AR-12 on Ben-Men-1-LucB intracranial xenografts were confirmed by MRI. Multi-planar tumor volumes were calculated from manually-traced tumor areas on axial and coronal images. Compared with total flux emitted from the tumor detected by BLI, a trend of reduction of tumor volumes in AR-42 or AR-12 treated mice was seen by MRI.

Figure 5. Ben-Men-1-LucB xenograft tumors grow along the meninges like benign meningiomas. Hematoxylin-eosin staining of sections of decalcified heads of xenograft-bearing mice fed normal diet or chow formulated with AR-42 for three months. Note that the xenograft tumor was much smaller in the mouse fed AR-42-containing chow.

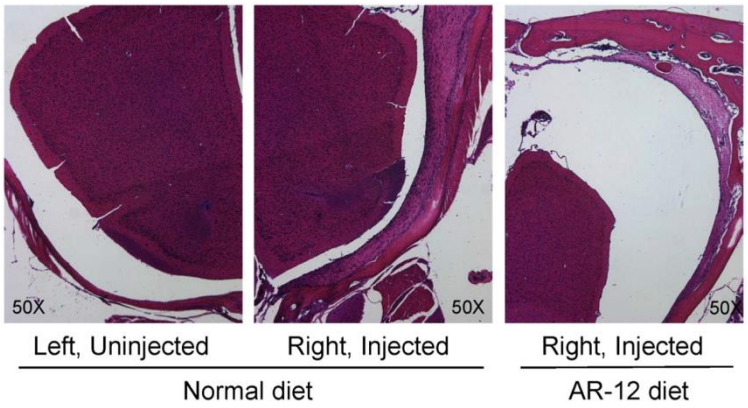
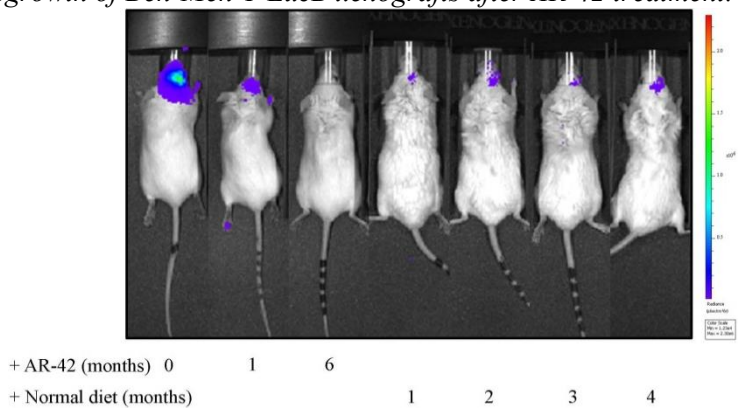


Figure 7. Ben-Men-1-LucB xenograft tumors grew along the meninges like benign meningiomas. Hematoxylin-eosin staining of sections of decalcified heads of xenograft-bearing mice fed normal diet or chow formulated with AR-42 for three months. Note that the xenograft tumor was much smaller in the mouse fed AR-42-containing chow.

To examine whether AR-42 effectively eliminated Ben-Men-1-LucB cells, xenograft-bearing mice that had been treated with AR-42 for six months were fed normal diet, and the residual tumor imaged every following month for four months. Although a residual tumor was detected and appeared to regrow over time (Figure 6), the tumor remained very small after four months on a normal diet.

Collectively, our results indicate that AR-42 possesses potent growth inhibitory activity in both schwannomas and meningiomas.

Figure 6. Minimal regrowth of Ben-Men-1-LucB xenografts after AR-42 treatment.



2.8 AR-42 passes blood-brain barrier and shows low toxicity profile

The dose of AR-42 which best corresponds to the severe toxic dose in 10% of rats (rat STD10) is 30 mg/kg (180 mg/m^2). In 28-day repeat dose toxicology studies in rats, a dose of 30 mg/kg administered orally every other day caused slight changes in bodyweight, significant decreases in red and all lineages of white blood cells (returned to control values after recovery) and histopathological changes (bone marrow, lymph nodes, spleen, thymus and

female and male reproductive tract). All histopathological changes, with the exception of testicular findings, showed evidence of total or partial recovery after 2 weeks. One-tenth of the rat STD10, or 18 mg/m^2, corresponds to a human dose of approximately 30 mg in an adult human.

A similar dose in beagle dogs of 1 mg/kg (20 mg/m^2) was not associated with severe toxicity when given p.o. q.o.d. for 7 or 28 days. However, dogs were determined to be a more sensitive species overall. The no observable effect levels (NOELs) in the 28-day dog study were 1 mg/kg in females and 0.3 mg/kg in males. The dose of AR-42 which best corresponds to the Highest Non Severe Toxic Dose (HNSTD) is 3 mg/kg (60 mg/m^2). In repeat dose studies in dogs, administration of 3 mg/kg p.o. every other day resulted in a slight decrease in body weight, decreases in red and white blood cell variables, vomiting and liquid feces. One-sixth of the dog HNSTD, or 10 mg/m2, corresponds to a human dose of approximately 20 mg in an adult human. Preclinical pharmacology conducted at OSU also showed that AR-42 passed the blood brain barrier in mice. In addition, mice fed AR-42 for 6 months behaved normally and did not showed overt weight loss. Blood-chemistry studies and organ histology performed after 3 and 6 months of AR-42 treatment demonstrated no clinically significant abnormalities (Jacob et al., 2012).

2.9 Clinical studies of AR-42

AR-42 is currently being studied in a phase I dose escalation study for relapsed or refractory multiple myeloma, chronic lymphocytic leukemia, and lymphoma and recurrent or advanced solid tumors. This is the first study of AR-42 in humans, and the study has demonstrated bioavailability of AR-42 in these patient populations. To date, 15 participants have been enrolled in the study. The study is currently enrolling participants into the 50 mg dose cohort in the hematologic malignancies arm. At this dose level, one patient experienced a grade 4 thrombocytopenia and therefore, this arm is now expanded to 3 more patients (a total of 6 patients).

Dose (mg)	Number of Participants
20	3
30	3
40	6
50	3

The recommended starting dose level for the solid tumors arm is one dose level less than the maximum tolerated dose (MTD) for the hematologic malignancies arm. At prior dose level (40 mg), a total of 6 patients were treated and one experienced a dose limiting toxicity (DLT) (Grade 3 thrombocytopenia). Based on these preliminary results to this point, 40 mg is proposed as the starting dose for the solid tumors arm, which may change to a higher starting dose, when the MTD for the hematologic malignancies arm is defined.

Serious adverse events (SAEs) have been reported. In the 40mg dosing cohort, one SAE was reported that was possibly related to AR-42, a grade 3 thrombocytopenia. Below is a summary of all SAEs reported in any dosing cohort, with SAEs possibly related to AR-42 listed in bold.

- 33 y Hodgkin's lymphoma patient on dose level 1a (20mg) had grade 3 blood bilirubin increase; unlikely r/t AR42 and r/t disease progression as evidence of CT scans and was removed from study for progressive disease.
- 74y multiple myeloma patient on dose level 1b (40mg) had grade 3 lung infection; unlikely r/t AR42 and r/t disease progression; was removed from protocol for disease progression and started on new treatment.
- **59y multiple myeloma patient on dose level 2b (50mg) had grade 4 platelet count decrease that was r/t AR42** (Dose Limiting Toxicity) and grade 2 confusion r/t his narcotic regimen. Was removed from study per PI decision for ongoing confusion and inability to obtain pain control with narcotic regimen.

- 67y multiple myeloma patient on dose level 2b (50mg) had grade 3 pain in extremity; unlikely r/t AR42 and r/t disease, she was continued on study as surgery was not an option and pain improved on pain medication.
- **71y multiple myeloma patient on dose level 2b (50mg) had grade 2 blurred vision, diplopia, muscle cramps and memory impairment that was possibly related to AR42;** she remained on study with dose reduction as toxicities reduced to grade 1 after AR42 was held.
- 61y multiple myeloma patient on dose level 2a (40mg) hospitalized with grade 3 diarrhea, grade 2 increased creatinine, and grade 3 dehydration; unlikely r/t AR42 and probable related to gastroenteritis, likely foodborne. He remains on study.
- **61y multiple myeloma patient on dose level 2a (40mg)** hospitalized with grade 2 sinus bradycardia, grade 3 non-cardiac chest pain, grade 2 creatinine increase unlikely related to AR42 and **grade 3 platelet count decrease possibly related to AR42**; He remains on study.

Other adverse events have been reported in the dosing cohorts (all Grade 1 or 2), but it is unknown if they are related to AR-42. These include:

- Anorexia
- Anemia
- Electrolyte abnormalities (hyponatremia, hypophosphatemia, hypoalbuminemia, hypermagnesemia, hyperglycemia, hypokalemia, hypercalcemia)
- Nausea, vomiting, diarrhea
- Dysgeusia
- Dry mouth
- Limb edema
- Pain (back, urinary tract, abdominal, extremities, myalgia, arthralgia)
- Increased creatinine, liver enzymes
- Neutropenia
- Leukopenia
- Thrombocytopenia
- Fatigue
- Prolonged QTc intervals
- Hyperhidrosis
- Constipation
- Insomnia
- Dyspnea
- Confusion

Preclinical and clinical results lead us to believe that an exploratory study to assess the potential of AR-42 in the treatment of the intracranial tumors associated with NF2 would be very useful. We have full support from ARNO Therapeutics who will provide us good manufacturing practice (GMP)-quality AR-42 for the proposed study. An IND application to the FDA for the exploratory evaluation of AR-42 in meningiomas and VS was filed by Dr. Brad Welling, and on January 20, 2012, the FDA concluded that it was reasonably safe to proceed with the study.

2.10 NF2 presents unique challenges for clinical testing

NF2-associated VS, meningiomas, and cutaneous schwannomas present several unique challenges. First, there are relatively few NF2 patients. Their tumors are primarily intracranial or along the spinal axis and thus are difficult to access for study; though a significant subset of NF2 patients do have peripheral nerve involvement and develop cutaneous schwannomas (Sperfield et al., 2002). Second, in spite of severe morbidity and mortality associated with

NF2, NF2 patients are unlikely to develop malignant diseases. The treatment aims and endpoints must be selected accordingly. We have experience in measuring not only volumetric tumor growth (Walz et al., 2012) with an outstanding imaging core, but also in measuring other surrogates, such as hearing (Massick et al., 2000). Hearing loss can result from vascular compromise or from an increased pressure on the cochlear nerve without demonstrated tumor growth, and thus is an important surrogate of functional significance. Third, the length of successful treatment is likely to be quite prolonged; therefore, drug safety and an extremely low side effect profile must be sought. Fortunately, the preliminary phase I data for AR-42 show a well-tolerated profile. Fourth, we have selected AR-42 to move forward to clinical trial very carefully because the patients are few and resources are precious, but this unique combination of histone mediated and non-histone mediated AKT pathway targeting by a single drug is entirely novel to the treatment of NF2-associated tumors. The preclinical and toxicity data to date is also very encouraging.

If AR-42 is found to have biological activity by showing suppressed p-AKT in VS, meningiomas, and cutaneous schwannomas and if the current Phase I AR-42 study continues to demonstrate minimal toxicity, we will seek to move forward into a Phase II study for efficacy. Successful completion of this study would aid in understanding the effects of AR-42 in NF2-associated tumor regulation in humans and may lead to substantial improvement over today's current approach to the treatment of NF2 with resulting improvement in the quality of life and longevity of our NF2 patients.

3. STUDY DESIGN AND DURATION

3.1 Study Design

This will be a multi-center, proof of concept phase 0 study to assess the suppression of p-AKT in VS, meningiomas, and cutaneous schwannomas by AR-42 in adult patients undergoing tumor resection. AR-42 is a small molecule which crosses the blood brain barrier (BBB) in rodents, but we are not certain yet if it will penetrate human VS. Meningiomas are outside the BBB, but seem to be unusually resistant to all current medical treatments. The primary endpoint of the bioactivity of suppression of p-AKT by AR-42 was selected as drug activity seems more informative than bioavailability. Our preclinical data and others have shown dose dependent suppression of p-AKT by AR-42 in both VS and meningiomas.

Addendum: Early results demonstrate intratumoral VS concentration of AR-42, therefore exploring the mechanism of action of AR-42 in peripheral schwannomas in patients with NF2 will add information of value and may allow more patient participation.

3.2 Definition of unacceptable toxicity

An unacceptable toxicity is defined as one of the following toxicities occurring during the cycle of AR-42.

Hematologic unacceptable toxicity criteria:

- Grade 3 thrombocytopenia (platelet count <50,000/μL) or anemia or grade 3 neutropenia (absolute neutrophil count [ANC] <1000/μL; as described by 1996 National Cancer Institute [NCI] Working Group Criteria) that does not resolve within 5 days.

Non-hematologic unacceptable toxicity: Any grade 3-4 non-hematologic adverse event (AE) (NCI Common Toxicity Criteria for Adverse Events [CTCAE] version 4) except as follows:

- Grade 3 or 4 laboratory abnormalities not associated with clinical sequelae, correctable within 24 hours, that does not lead to missing more than one dose of study drug.
- Grade 3 or 4 nausea or vomiting that resolves to grade 2 or less within 24 hours that does not lead to missing more than one dose of study drug.

- Liver function test abnormalities (aspartate transaminase [AST], alanine transaminase [ALT], bilirubin, or alkaline phosphatase) that resolve to <grade 1 within 5 days and that does not lead to missing more than one dose of study drug
- Any AE with clear evidence per the study PI to support an alternative causality other than study drug that does not lead to missing more than one dose of study drug.

3.3 Study accrual

p-AKT expression will be determined in up to 20 specimens total from AR-42 treated patients with the following tumor types: vestibular schwannomas (either NF2-associated or sporadic), meningiomas, and cutaneous schwannomas. A total of 20 subjects will be enrolled in the study. Control tissues of non-treated vestibular schwannomas and meningiomas will serve as comparators.

Given that NF2-associated VS and NF2-associated meningioma patients are rare, we plan to recruit patients from four high volume institutions with clinical trial expertise. We are expecting to enroll between 8-10 patients per year based upon current center surgical volume estimates. Patient recruitment should be completed within three years. Should these targets lag, we have another four centers who have agreed to participate if needed. We anticipate patient support from the NF2 communities and support groups also for this venture.

3.4 Duration of study for each patient

AR-42 will be administered three times per week beginning 3 weeks prior to resection. A total of ten doses, $\pm$ 1 dose at 40 mg/dose, will be self-administered orally by study participants at approximately the same time every day ($\pm$ 1 hour, preferably in the evening) 3 times per week for 3 weeks pre-operatively, with the last dose taken the night before resection. AR-42 should be taken at least 1 hour before or 2 hours after meals. The starting dose of 40 mg for this study of solid tumors was selected by pre-clinical and ongoing phase I data. The recommended starting dose level for the solid tumors arm in the ongoing phase I trial is one dose level less than the maximum tolerated dose (MTD) for the hematologic malignancies arm, which is currently 50 mg. Based on these preliminary results to this point, 40 mg is proposed as the starting dose for the solid tumors arm, which may change to a higher starting dose, when the MTD for the hematologic malignancies arm is defined.

Baseline assessments will all be done within 30 days of the start of AR-42 and will include: medical history, clinical examination, vital signs, concomitant medications, appropriate assessment of inclusion and exclusion criteria, comprehensive metabolic panel which includes liver function tests, serum chemistries including calcium, magnesium, and phosphorous, complete blood count, coagulation panel (PT/PTT), , pregnancy test, chest x-ray, ECG, audiogram, and Eastern Cooperative Oncology Group/World Health Organization (ECOG/WHO) performance status. A baseline MRI will also be required within 60 days of day 1 dosing of AR-42.

To monitor participant safety, a total of three monitoring assessments will be performed beginning one week after the first dose of AR-42, concluding with the last monitoring assessment at the pre-surgery visit. These assessments will include 12-lead ECGs at each visit, complete blood counts, and a monitoring of adverse events. A comprehensive metabolic panel (i.e. Chem 7) to also include liver function tests and serum chemistries for calcium, magnesium, and potassium will be completed at the last monitoring/ pre-surgery visit. To ensure that AR-42 administration will not adversely affect outcomes of surgical resection, subjects will undergo the last assessments within 4 days of the scheduled surgical resection. These assessments include clinical examination, vital signs, comprehensive metabolic panel which also includes liver function tests and serum chemistries for calcium, magnesium, and potassium, complete blood count, coagulation panel (PT/PTT), , pregnancy test, ECG, audiogram, and Eastern Cooperative Oncology Group/World Health Organization (ECOG/WHO) performance status. For the first 10 study participants enrolled in the study, an MRI will also be done within 4 days of the scheduled surgery. If no tumor reduction is noted in these participants, a MRI at this time point will not be required for the additional study participants that will be enrolled. Toxicity will be monitored and adverse event information collected. Toxicity will be assessed according to the NCI CTCAE V 4.0. If any changes in health status are noted that may affect a subject's safety and outcomes during surgery, surgery will be delayed.

During surgical resection of the tumor, tissue will be resected per clinical indications. The tumor tissue will first be sent to diagnostic pathology and when it is determined there is adequate tissue for diagnosis, additional samples will be taken and prepared as described in the study procedures section. The samples will be shipped to the participating laboratories (OSU Comprehensive Cancer Center (CCC) Pharmacoanalytical Shared Resource (PhASR) and Nationwide Children's Research Institute) for assessment of intratumoral drug concentration and assessment of intratumoral disease markers. During surgery, four blood samples will also be obtained and sent to the cooperating laboratory (PhASR) for determination of drug concentration and molecular analysis. Two blood samples will be taken prior to tumor removal and two will be taken at the time of tumor removal (while in the operating room). There will be no charge for collection of the blood and tumor samples since the collection will be done by study investigators who are also the treating surgeon/physician. The blood sample taken at the time of anesthesia induction will serve as the steady state measure as this will be drawn after 10 doses of AR-42. The sample taken at time of tumor resection will be used to confirm that essentially the same plasma levels are achieved indicating steady state. The plasma AR-42 concentration determined from the blood sample taken at the time of tumor resection will also be used to calculate the plasma to brain drug concentration ratio. The exact time of tumor resection and blood sampling will be recorded.

Subjects will take their last dose of AR-42 the night before surgery and no additional laboratory and surveillance studies will be completed after surgical resection. Patients will be evaluated within the context of their standard post-operative follow up which includes within 4 days of surgery and again at 2 weeks (+/- 10 days) after surgery. Toxicity will be monitored and adverse event information collected at within 4 days and 2 weeks (+/- 10 days) after surgery. Toxicity will be assessed according to the NCI CTCAE V 4.0. The total observation window for adverse events will be 2 weeks post-surgery. If there are any adverse events, the subject will be followed until recovery or stabilization or return to baseline condition if at all possible.

4. STUDY POPULATION

Patients eligible for enrollment in this study will be male and female adults diagnosed with a cutaneous schwannoma, a vestibular schwannoma, or a meningioma by MRI where surgical resection has been selected as the most appropriate treatment option by the treating physician and patient.

4.1 Inclusion criteria

- Patients with a cutaneous schwannoma, or a vestibular schwannoma, or a meningioma diagnosed by MRI where surgical resection has been selected as treatment.
- Patients diagnosed with NF2 must meet Manchester Criteria.
- Age $\geq$ 18 years of age
- Prior biologic therapy, chemotherapy, surgery or radiation is permitted.
    - Chemotherapy: Up to three prior cytotoxic chemotherapy treatments, in the metastatic setting, are allowed. Prior hormonal, biological or targeted therapies are not limited. At least 4 weeks need to have elapsed since last treatment and the patient must have recuperated from acute toxicities from the prior treatment.
    - Radiation Therapy: Prior radiation therapy is allowed. At least 1 year must have elapsed since last treatment, and the patient must have recovered from all toxicities to Grade 1 or less.
    - Surgery: Prior curative and palliative intent surgery is allowed. At least 3 weeks must have elapsed since last major surgical treatment and the patient must have recovered from surgery and any post-surgical complications. If photodynamic therapy has been performed, at least 4 weeks must have elapsed (to allow for full clearance of the photosensitizing agent). Minor surgical procedures, such as removal of skin lesions or MediPort and nephrostomy tube placements are not considered major surgery and treatment can start as soon as the surgical site is healed, per the treating physician's discretion.
- At the time of screening, the PI will review the patient's lab work to determine that the patient meets the eligibility criteria. The patient must have normal organ and marrow function as described below*:

- Leukocytes ≥ 3,000/mcL
- Absolute neutrophil count ≥ 1,500/mcL
- Platelets ≥ 100,000/mcL
- Total bilirubin < 1.5 mg/dL
- AST(SGOT)/ALT(SGPT) ≤ 5 x institutional upper limit of normal (ULN);
- Creatinine ≤ 1.5 x ULN OR creatinine clearance ≥ 50 mL/min by MDRD (original or abbreviated), OR measured creatinine clearance ≥ 50 ml/min
- Hemoglobin of at least 9 g/dL
- Normal serum magnesium (1.6 – 2.6 mg/dL), calcium (8.6 – 10.0 mg/dL), and potassium (3.5 – 5.1 mmol/L) levels.

*Any abnormal laboratory values will be reviewed by the PI to determine the appropriate treatment and continued study eligibility.

- Eastern Cooperative Oncology Group/World Health Organization (ECOG/WHO) performance status of 0-1.
- Patients must be able to swallow capsules.
- Patients must be able to read, understand and provide informed consent to participate in the trial.
- Females of childbearing potential (FCBP) must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL prior to starting AR-42. The effects of AR-42 on the developing human fetus are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

  A female of childbearing potential (FCBP) is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

- The patient must be willing to comply with fertility requirements as below:

  A. Male patients must agree to use an adequate method of contraception for the duration of the study and for 28 days afterwards
  B. Female patients must be either postmenopausal, free from menses ≥ 2 yrs, surgically sterilized, willing to use two adequate barrier methods of contraception to prevent pregnancy, or agree to abstain from heterosexual activity starting with screening and for 90 days afterwards.
  C. Patients must agree not to donate blood, sperm/ova during study participation and for at least 4 weeks after stopping treatment

4.2 Exclusion criteria

- Pregnant women are excluded from this study because the potential for teratogenic or abortifacient effects of AR-42 are not known. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with AR-42, breastfeeding should be discontinued if the mother is treated with AR-42.
- Pediatric patients are excluded from the phase 0 study as the effects of AR-42 are not known on children and there is no potential direct benefit to them.
- Patients with malabsorption or any other condition that in the opinion of the principal investigator could cause difficulty in absorption of drug.
- Patients requiring chronic corticosteroids (dose equivalent ≥ 20mg prednisolone).
- Concurrent use of complementary or alternative medicines that in the opinion of the principal investigator would confound the interpretation of toxicities and/or antitumor activity of the study drug.
- Patients with a "currently active" second malignancy that, in the opinion of the principal investigator, will interfere with patient participation, increase patient risk, or confound data interpretation.

- Patients with a mean QTcB > 450 msec in males and > 470 msec in females.
- Patients with long QT syndrome.
- Patients who are being treated for an active infection.
- Patients receiving the following concomitant medications:
  - Any other anti-neoplastic chemotherapy or biologic therapy during the study
  - Concomitant radiotherapy
  - Concomitant HDAC inhibitors (e.g. valproic acid) as class-specific adverse reactions may be additive
  - Use of granulocyte colony-stimulating factors including G-CSF, pegylated G-CSF or GM-CSF should follow ASCO guidelines for patients receiving anti-cancer therapy.
  - Drugs associated with QT/QTc prolongation (see Appendix A)
- Patients who are receiving concurrent anti-neoplastic therapy.
- Any other medical condition, including mental illness or substance abuse, deemed by the principal investigator to likely interfere with a patient's ability to sign informed consent, cooperate and participate in the study, or interfere with the interpretation of the results.
- Patients with significant cardiovascular disease, including a myocardial infarction or unstable angina within 6 months or unstable cardiac arrhythmias are not eligible for the study.
- Known HIV infection, as their immunosuppressive conditions may complicate potential pancytopenias seen with HDAC inhibitors and complicate evaluation of drug effect.

5. STUDY PROCEDURES

AR-42 will be administered three times per week beginning 3 weeks prior to surgery. A total of ten doses, $\pm$ 1 dose, at 40 mg/dose, will be self-administered orally by study participants at approximately the same time every day ($\pm$ 1 hour, preferably in the evening) 3 times per week for 3 weeks pre-operatively, with the last dose taken the night before surgery. The PI will instruct the patient on their medication regimen throughout the study. AR-42 should be taken at least 1 hour before or 2 hours after meals. The starting dose of 40 mg for this study of solid tumors was selected by pre-clinical and ongoing phase I data.

5.1 AR-42

5.1.1 Dosing schedule and cohorts

Treatment

AR-42 will be administered orally three times per week beginning 3 weeks prior to surgery for removal of a vestibular schwannoma, meningioma, or cutaneous schwannoma. A total of ten doses, $\pm$ 1 dose, at 40 mg/dose, will be self-administered by study participants at approximately 8:00pm ($\pm$ 1 hour) for 3 weeks pre-operatively, with the last dose being administered the night before surgery. For example, if surgery were scheduled for Tuesday, study participants would take the study drug on Mondays, Wednesdays, and Fridays for 3 weeks pre-operatively and one final dose on Monday, the evening before surgery. The PI is responsible for determining the dosing schedule and will include this in written directions. Study participants will receive written instructions regarding the correct dosing schedule and a Medication Log to record date and time medication was taken.

Doses of AR-42 should be taken on an empty stomach. Doses should be taken at least 1 hour before or 2 hours after meals. If a dose is missed or vomited up, the dose will not be made up. Study participants will be asked to record any missed doses or doses that were vomited up on the Medication Log.

5.1.2 Supportive care for patients receiving AR-42

Prophylactic use of growth factors is not allowed.

Nausea: Treat nausea with appropriate supportive care as required. The following are suggestions:

- Benzodiazepines: Lorazepam 0.25-1 mg PO or IV
- Dopamine blockade: Prochlorperazine 5-10 mg or Promethazine 12.5-25 mg by PO, IV, or IM.

- Steroid: Dexamethasone 4 mg PO or IV

Diarrhea: Treat diarrhea promptly with appropriate supportive care, including loperamide. Instruct patients to begin taking loperamide at the first signs of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day, or 3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then 2 mg after each unformed stool. Daily dose should not exceed 16 mg/day. Advise patients to drink plenty of fluids to help prevent dehydration caused by diarrhea. Avoid loperamide if there is the presence of blood or mucus in the stool or if diarrhea is accompanied by fever. If grade 3 or 4 diarrhea develops, hold treatment with AR-42.

Dysgeusia: Therapy will be provided to assure maintenance of adequate hydration in the setting of dysgeusia. Hydration with intravenous fluid may be given. Additionally, the use of popsicles and Gatorade may be helpful. Additional anti-emetics therapy may be given. If a dose is missed or vomited up, the dose will not be made up.

Hypokalemia or hypomagnesemia should be corrected to within normal limits prior to administration of AR-42, and weekly assessments during dosing of AR-42 will be conducted to monitor potassium and magnesium levels.

5.2 Dose delays and modifications

During treatment, patients who miss 2 or more doses of study drug due to non-compliance or AR-42 toxicity will discontinue study therapy, regardless of whether the toxicity causing the missed does is related or unrelated to AR-42. If there is any Unacceptable Toxicity (as defined in Section 3.2), the AR-42 will be stopped and the patient will be removed from the study.

5.3 Concomitant treatment restrictions

All patients should be treated for any medical conditions according to the best interests of the patients and acceptable community medical standards.

The following treatments are not permitted during the study:

- Any other anti-neoplastic chemotherapy or biologic therapy
- Concomitant radiotherapy
- Concomitant HDAC inhibitors (e.g. valproic acid) as class-specific adverse reactions may be additive
- Use of granulocyte colony-stimulating factors including G-CSF, pegylated G-CSF or GM-CSF should follow ASCO guidelines for patients receiving anti-cancer therapy
- Drugs associated with QT/QTc prolongation (see Appendix A)

The following are permitted:

- Anti-emetic treatment, with ativan, corticosteroids, and anti-5 HT3 agents.
- All supportive and palliative treatment if necessary (nutritional, transfusional support, pain control, etc.).

5.4 Schedule of tests and observations

5.4.1 Screening

Baseline assessments will all be done within 30 days of the start of AR-42 and will include: medical history, clinical examination, vital signs, concomitant medications, appropriate assessment of inclusion and exclusion criteria, comprehensive metabolic panel which includes liver function tests and serum chemistries for calcium, magnesium and potassium, complete blood count, coagulation panel (PT/PTT), urine or serum pregnancy test, chest x-ray, ECG, audiogram, and Eastern Cooperative Oncology Group/World Health Organization

(ECOG/WHO) performance status. A baseline MRI will also be required within 60 days of day 1 dosing of AR-42.

It should be noted that before a patient has been consented for the study, standard of care procedures may have been already conducted within 30 days prior to the screening visit. These results may then be used for baseline assessments in order to determine the patient's eligibility for the study. A standard of care MRI within 60 days prior to scheduled day 1 dosing of study drug may be used for screening/baseline assessments.

5.4.2 Evaluation during treatment

To monitor participant safety, a total of three monitoring assessments will be performed beginning one week after the first dose of AR-42 including the last assessment at the pre-surgery visit. The first two monitoring visits will include the following assessments: 12-lead ECGs at each visit, complete blood counts, comprehensive metabolic panel, review of concomitant medications, and monitoring of adverse events. These lab assessments may be accomplished locally to the patient with a phone interview for conmed review and AE assessment. To ensure that AR-42 administration will not adversely affect outcomes of surgical resection, subjects will undergo additional assessments at the third monitoring visit / pre-surgery visit within 4 days of the scheduled surgical resection. These assessments (to be accomplished at the research site) include clinical examination, vital signs, comprehensive metabolic panel which also includes liver function tests and serum chemistries for calcium, magnesium, and potassium, complete blood count, coagulation panel (PT/PTT), urine or serum pregnancy test, ECG, audiogram, and Eastern Cooperative Oncology Group/World Health Organization (ECOG/WHO) performance status.

For the first 10 study participants enrolled in the study, a MRI will also be done within 4 days of the scheduled surgery. If no tumor reduction is noted in these participants, a MRI at this time point will not be required for the additional study participants that will be enrolled.

If any changes in health status are noted that may affect a subject's safety and outcomes during surgery, surgery will be delayed. Tumor specimens will still be collected at the time of surgery to determine expression levels of phosphor-Akt (p-AKT) and $p16^{INKA}$ and assess biological effects of AR-42 on the phosphoinositide 3 kinase (PI3K)/AKT signaling pathway, tumor proliferation, cell cycle, cell death, and angiogenesis. Toxicity will be monitored and adverse event information collected at this visit. Toxicity will be assessed according to the NCI CTCAE V 4.0.

If the QTcB is prolonged to CTCAE grade 3 or higher, the patient will discontinue study drug and will be monitored until QTc intervals return to baseline.

5.4.3 Post-treatment follow-up

Patients will be evaluated within the context of their standard post-operative follow up which includes within 4 days of surgery and again at 2 weeks (+/- 10 days) after surgery. Evaluations include a clinical exam, vital signs, and ECOG/WHO performance status. Toxicity will be monitored and adverse event information collected at 4 days and 2 weeks after surgery. Toxicity will be assessed according to the NCI CTCAE V 4.0. The total observation window for adverse events will be 2 weeks post-surgery. If there are any adverse events, the subject will be followed until recovery or stabilization or return to baseline condition if at all possible.

All patients who discontinue the trial secondary to an adverse event thought to be related to protocol therapy (probably, possible, or definite) should be followed until resolution, stabilization, return to a baseline condition, or death.

5.4.4 Schedule of events

STUDY SCHEDULE OF EVENTS

	Baseline[1]	Monitoring Visit #1[2]	Monitoring Visit #2[2]	Monitoring Visit #3/ Pre-Surgery[3]	Surgery	Post-Surgery[4]	Off-Study[5]
CBC w/ differential and platelets	X	X	X	X			
Comprehensive Metabolic Panel[6]	X	X	X	X			
Serum or Urine Pregnancy Test[7]	X			X			
AE[8] Reporting	X	X	X	X		X	X
Concomitant Medication Review	X	X	X	X			
12-lead ECG	X[9]	X[9]	X[9]	X[9]			
Coagulation panel (PT/PTT)	X			X			
Chest x-ray	X						
H&P, Neuro Exam, Vital Signs	X			X		X	X
ECOG performance status	X			X		X	X
Audiogram	X			X			
MRI (CPT 70553)	X[10]			X[11]			
Intraoperative Blood and Tissue Collection					X[12]		

1 –Within 30 days prior to starting AR-42
2 –There will be three monitoring visits after the first dose of AR-42 has been taken. The third monitoring visit is combined with the pre-surgery visit.
3 –Within 4 days prior to surgery for the monitoring visit #3/ pre-surgery visit
4 –Within 4 days after surgery for the post-surgery visit
5 –Two weeks ± 10 days after surgery
6 –Standard peri-operative evaluations; comprehensive metabolic panel (i.e. Chem 12) to also include liver function tests and serum chemistries for calcium, magnesium, and potassium
7 –Only required for females that are, in the investigator's opinion, of childbearing potential
8 –AE= Adverse Events
9 –If the QTcB is prolonged to CTCAE grade 3 or higher, the patient will discontinue study drug and will be monitored until QTc intervals return to baseline.
10 -Done within 60 days of day 1 dosing of AR-42.
11 – Performed in first 10 study participants. Will not be required of additional study participants if tumor reduction not noted in the first 10
12 –Intraoperative blood (for steady state) and tumor sampling will be collected at the time of surgery

5.4.5 Tumor resection and sample collection

The treating surgeon will perform the clinically indicated surgical procedure as well as the specimen collection. To monitor the risk of increased surgical complications associated with AR-42, the treating surgeon will record any noted differences during surgical resection such as increased bleeding, tumor adherence to adjacent nerves, or increased difficulty in tumor removal. After tissue required for diagnostic pathology has been resected, additional tumor samples will be prepared for tissue sampling. Four tissue samples will be taken per tumor, two from the center of the tumor and two from the tumor capsule and snap-frozen immediately in the operating room ($\leq$15 minutes). Each sample will be approximately 5 mm^3 in size and will be placed into an individual cryotube labeled with a unique study number, time of collection, sampling location and then snap-frozen in liquid nitrogen. Efforts will be made to ensure only tumor tissue (not surrounding adjacent tissues) are frozen in the vials. The samples will be stored in a liquid nitrogen tank or -70ºC to a -80 ºC freezer until shipment. Tissue acquisition forms will be completed and shipped with the specimen. Shipment from the originating institution will occur only after the final diagnostic pathology has been reported. Once ready for shipment, the sample will be sent via overnight express on dry ice. Two samples will be sent to the OSU Pharmacoanalytical Shared Resource (PhASR) where they will be maintained at -70ºC to -80 ºC until used for drug concentration analysis and two will be sent to Nationwide Children's Research Institute for molecular analysis. Twelve unstained paraffin fixed slides will also be requested from each tumor sample as well. These will be used for standard histologic analysis and immunohistochemisty assessment to be performed at Nationwide Children's Research Institute. These specimens will be processed as soon as possible, and at least within the year from sample collection. Expression of proteins by immunoblot and immunohistochemistry to be measured will include 1) the PI3K/AKT signaling pathway including total AKT, phospho-Akt, p-PRAS40, total PRAS-40, p-S6 ribosomal protein, and p-4E-BP-1; 2) other implicated pathways such as the MAPK, ERK and total ERK, 3) cell cycle regulators Ac-Lysine, acetylated histone 2B (Ac-H2B), $p16^{INK4A,}$ p21, other suspected HDAC regulators such as HSP90; 4) HR23B as a potential biomarker for tumor sensitivity to AR-42; and 5) merlin. The biological effects of AR-42 on tumor proliferation (assessed by Ki-67 proliferation index), cell death (assessed by cleaved caspase-3 and TUNEL staining), and angiogenesis (assessed by expression of VEGF and CD31) will also be investigated. Protein expression levels and biological effects of AR-42 determined from study samples will be compared to untreated samples from our control patients. Tumor specimens will be examined according to our previous study (Bush et al., 2011).

NF2 gene mutation analysis will be performed via exon scanning and multiplex ligation-dependent probe amplification (MLPA), and merlin protein expression will be determined by immunoblot and immunohistochemistry.

Additionally, during tumor resection, four venous blood samples will be taken, two at the start of anesthesia on the day of surgery and two at the time of tumor removal (while in the operating room). The blood samples at the induction of anesthesia will serve as the steady state serum measure, and the samples taken at the time of tumor resection will be used to confirm that essentially the same plasma levels are achieved indicating steady state. The plasma AR-42 concentration determined from the blood samples taken at the time of tumor resection will be used to calculate the plasma to in-tumor drug concentration ratio. Each sample will be 6mL of blood, which will be drawn into heparinized plasma collection tubes. The specimens will be promptly mixed by gently inverting the Vacutainer tube several times and then be placed on wet ice.

The samples will be centrifuged at 1,100-1,300 x g for 10 min at 4°C within 15-30 min after collection. The plasma in each individual Vacutainer will be removed from the blood cells using a pipette and transferred into a separate labeled 4.5 mL self-standing polypropylene cryogenic tubes with external threads. The tubes will be placed on crushed dry ice until stored in a freezer maintained at -70°C. Complete sets of samples from one or more patients will be sent by batch by overnight mail on dry ice. The pharmacokinetic tissue and plasma assays will be performed at the Ohio State PhASR where assays for PK analysis of AR-42 in tumor and blood have been established and validated.

5.5 Study Records

Study data will be collected in a standardized case report form (CRF). The investigator or authorized study personnel will record all pertinent patient information, including patient identification number, tumor type and size, health history as well as information concerning drug administration, results of laboratory tests and audiograms, and toxicity data.

MEEI will be the central location for data processing and management utilizing the biostatistician on site.

All protocol-required information collected during the study will be entered by the investigator, or designated representative, in the appropriate study forms. The investigator, or designated representative, should complete the forms as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries will be completed immediately after the final examination. By design, an explanation must be provided for all missing data, altered data, and/or out of range data. The completed case report form will be reviewed and signed by the investigator named in the study protocol or by a designated sub investigator.

The Case Report Form will be provided for each subject; subjects will not be identified by name on any study documents. Any requested information that is not obtained as specified in the protocol should have an explanation for the omission noted on the applicable study form.

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, data sheets provided by the sponsor or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IRB review(s) and regulatory inspection(s), with direct access to all the required source records. Hard copies of any individual's study documents will be kept in separate subject binders in the locked office of the study coordinator at each site.

5.6 Selection Procedures

Potential participants will be identified and recruited by the study investigators and/or study coordinators at MEEI and any of the approved participating sites from their patient populations seen clinically for evaluation/management of their vestibular schwannomas, meningiomas, cutaneous schwannomas, and/or Neurofibromatosis Type 2. After clinical consultation with their physician, patients that elect surgical removal for management of their tumors will be informed of the study. If these patients are interested in participating, they will come to the clinical office for a screening visit and further discussion of the study. All treatment options including observation, surgical repair, and study participation will be presented to all eligible participants prior to obtaining informed consent. Recruitment and discussion of the study will be done in private, clinical offices. Physicians not included as study investigators may refer patients to approved study sites for enrollment in the study. If a physician identifies a potential participant, the physician can provide the participant's contact information to study team members (only after they have received the participant's permission) so that the study team can contact the potential participant and discuss the study.

Screening Procedures:

An inclusion/exclusion checklist worksheet for the study with detailed guidelines for the pre-study/screening evaluation to determine patient eligibility will be used by all participating sites. When a potential participant is screened for the study, the checklist will be completed and placed in the individual subject binder at the participating site where the participant was screened. A copy of the completed screening checklist will be forwarded to the study coordinator at the primary institution (MEEI).

A screening log will be distributed to all participating sites, and information from all potential participants screened must be documented on the screening log. Information collected on the screening log will include participant initials, demographics (gender, race, ethnicity), date of consent, checkbox to note if signed informed consent given

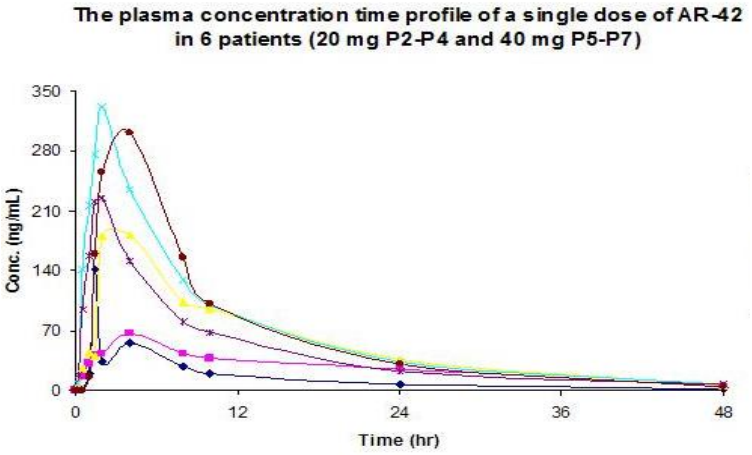
to subject, checkbox to indicate if subject enrolled, and reason for exclusion if subject not enrolled. The screening log will be kept in the regulatory binder for each site and will be sent to MEEI monthly for review by the study principal investigator (Dr. Welling) to monitor study accrual.

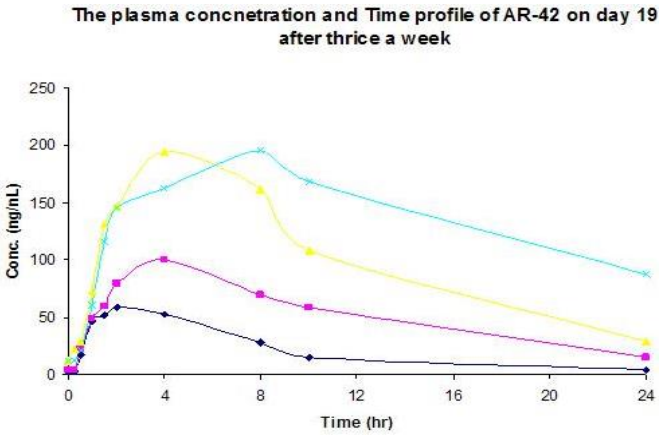
Determining Eligibility:
After all screening procedures are complete, the site investigator or delegated research staff will review the subject's full medical history to determine the subject's eligibility. There must be source documentation to support all requirements for determining eligibility. The subject's medical history and all relevant research screening tests and procedures must meet inclusion criteria and not meet exclusion criteria.

All tests, assessments, and procedures must be done within the protocol specified timeline. If the subject is deemed ineligible or wishes to not proceed with enrollment, then the delegated research coordinator or specialist will document the reason the subject was not enrolled in the trial and will update the site screening log appropriately.

6. PHARMACOKINETIC STUDIES

In the current Phase I study of AR-42, plasma PK studies and kidney excretion in all patients were conducted to assess inter-individual variability. Detailed pharmacokinetics with rich sampling (full profiles days 1-2 and 19-20, trough level on day 3 pre-dose) were conducted in all patients. Briefly, venous blood samples (6-mL heparinized tubes) have been collected in pre-chilled heparinized tubes and immediately placed in an ice/water mixture until centrifuged at the following specific time points: 15, 30 min, 1, 1.5, 2, 4, 8, 10 and 24 hours on day 1 cycle 1, and the pre-dose on day 3, 15, 30 min, 1, 1.5, 2, 4, 8, 10 and 24 hours on day 19 cycle 1. To understand the kidney excretion in the AR-42 elimination, the excretion of AR-42 in urine on day 1 has been collected at the following intervals: 0-4, 4-8, 8-10 and 10-24 hours after cycle 1 day 1 only. For patient's enrolled on the first two cohorts, pharmacokinetic profiling has revealed day 1 (Top plot) and day 19 (Bottom plot) profiles as shown below:





As a secondary endpoint, we will determine the steady-state plasma and intra-tumoral concentrations of AR-42 at the time of surgical resection after 10 days of dosing of AR-42.

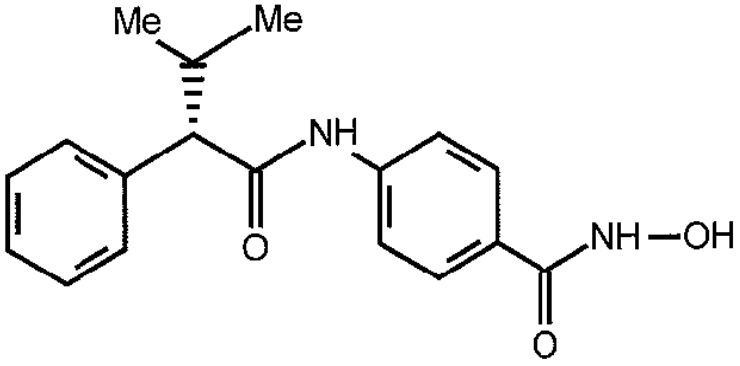
7. AR-42

7.1 Classification

Histone deacetylase inhibitor

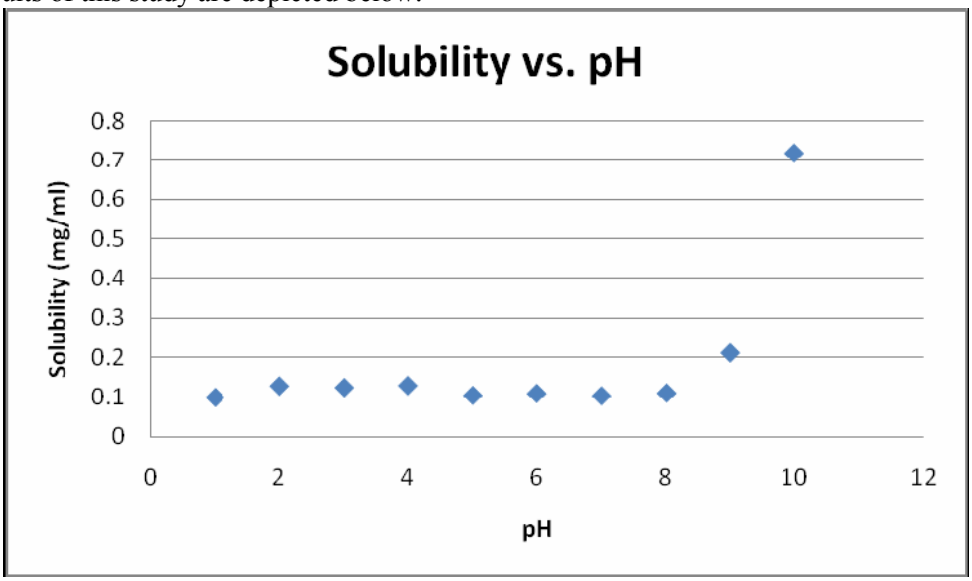
7.2 Chemical structure

AR-42, a free base formerly known as OSU-HDAC42 and NSC-D736012, is an orally bioavailable small molecule. The chemical name of AR-42 is N-Hydroxy-4-(3-methyl-2-(S)-phenyl-butyrylamino) benzamide. AR-42 is the chirally pure S-enantiomer of this compound. The empirical formula is $C_{18}H_{20}N_2O_3$, the molecular weight is 312.36, and the chemical structure is shown below:



7.3 Solubility and melting point

An equilibrium solubility study with the AR-42 free base in water produced a concentration of 0.16 mg/mL. The solubility of AR-42 free base in 50 mM aqueous buffer systems across the pH range from 1 to 10 was determined. The results of this study are depicted below:



Organic solubility:

The solubility of AR-42 was determined in common solvents and is summarized below.

Solvent	Solubility (mg/mL)
Acetonitrile	8.62
Isopropyl Alcohol	25.52
Methanol	54.27
Ethanol	37.10

Melting point: The melting point of AR-42 was determined by differential scanning calorimetry (DSC) and found to be 206.93°C.

7.4 Supply, handling and disposition, storage, and administration

ARNO Therapeutics, Flemington, NJ, will provide an adequate supply of AR-42 (histone deacetylase inhibitor) clinical trial material to conduct this clinical study. AR-42, capsules will be supplied in the dosage strengths below:

- 10 mg capsules: Forty-eight size 3 white opaque Coni-Snap capsules will be packaged into 120 cc wide mouth round high density polyethylene (HDPE) bottles. Each bottle will be capped with a 38 mm child resistant closure with FS M1/.035 pulp liner, induction sealed, and labeled. Study participants will take four 10 mg capsules per dose to achieve the study dose of 40 mg.

Handling and Disposition of Study Drug: All packaging and labeling operations will be performed according to the requirements of Directive 2001/20/EC[1] and in accordance with Good Manufacturing Practice for Medicinal Products. Each investigational site will work with their Investigational Drug Pharmacy Services to coordinate receipt, storage, dispensing, and return of AR-42.

Storage: The product is to be stored at 25°C and protected from moisture and light. Temperature excursions in the range from 15-30°C will be permitted.

Route of Administration: Oral. A one cycle supply of AR-42 will be dispensed at the start of treatment and study participants will receive written instructions regarding the correct dosing schedule. Study participants will also receive a medication dose log to record date and time medication was taken.

7.5 Pharmacokinetics in canines

A single non-Good Laboratory Practice (GLP) study to assess the comparative bioavailability of single (1 mg/kg) doses of orally or intravenously (iv) administered AR-42 was conducted in male dogs. Following both IV and oral administration, plasma concentrations of AR-42 were generally quantifiable up to 24 hours post-dose (i.e. the last sampling time point post-dose).

Following IV administration to 4 animals, the maximum plasma concentrations (C_{max}) ranged from 651 to 1140 ng/mL and were observed (T_{max}) at 0.0830 to 0.220 hours post-dose. Thereafter, plasma concentrations declined with a geometric mean apparent terminal half-life ($t_{1/2}$) of approximately 3.59 hours. The geometric mean apparent volume of distribution (V_{ss}) of AR-42 at steady-state was approximately 1280 mL/kg, which is considerably greater than total body water in dogs, indicating extensive distribution of AR-42 into tissues. The clearance (CL) of AR-42 was estimated to be approximately 406 mL/hour/kg, which is lower than hepatic and renal blood flow rates in dogs.

Following oral administration to 4 animals, C_{max} of AR-42 ranged from 147 to 398 ng/mL and were attained (T_{max}) at 0.480 to 4.00 hours post-dose in all animals. Thereafter, plasma concentrations declined with a geometric mean apparent terminal half-life of approximately 4.20 hours. The geometric mean bioavailability (F) of the oral formulation was estimated to be approximately 57.9%. The geometric mean absorption time (MAT) was 1.03 hours.

7.6 Toxicology in rats and canines

Rats	The MTD in rats receiving a single dose of AR-42 by oral gavage was determined to be 100 mg/kg in a non-GLP study. The MTD in rats dosed with AR-42 by oral gavage every other day for 7 days was determined to be 30 mg/kg in a non-GLP study. A 28-day GLP toxicity study in rats determined that the no adverse effect level (NOAEL) of AR-42 dosed orally by gavage once every other day for 4 weeks was 3 mg/kg in female animals. Due mainly to findings in the male reproductive system, neither a no effect level (NOEL) nor a NOAEL was established in this study for male animals. The severity of the findings noted in males when compared to females may have been a consequence of the increased systemic exposure noted in males over the 4-week dosing period.
Canines (non-GLP)	Two non-GLP studies of single and repeated (7-day) oral dosing of 1, 3, 10 and 30 mg/kg/day AR-42 to male and female dogs were completed. Plasma concentrations were generally measurable up to the last sampling time of 24 hours post-dose. C_{max} of AR-42 in male and female dogs were observed (T_{max}) at 0.5 to 2 hours post-dose. Thereafter, plasma concentrations of AR-42 declined with apparent $t_{1/2}$ ranging from 2.48 to 7.57 hours. Systemic exposure to AR-42, as measured by AUC and C_{max}, increased in an approximately dose-proportional manner. Following repeated administration, there was no appreciable accumulation of AR-42 in plasma, consistent with the short half-life measured in plasma relative to the dosing interval. There appeared to be no consistent or appreciable sex-related difference in systemic exposure to AR-42. The maximum tolerated single dose was considered to be 10 mg/kg. A 1 mg/kg dose of AR-42 was determined to be suitable for a subsequent multiple dose, 28-day toxicity study.
Canines (GLP)	In dogs dosed every other day for 28 days followed by a two week recovery period, the systemic exposure (C_{max} and AUC_{0-48}) to AR-42 increased in an approximately dose-proportional manner, and following repeated administration of AR-42 once every other day, there was no appreciable accumulation of AR-42 in male or female dogs. There also appeared to be no consistent or appreciable sex-related difference in the extent of systemic exposure to AR-42.

	Based on the changes in relative spleen and testes weight at 1.0 mg/kg, the oral NOEL in male dogs was considered to be 0.3 mg/kg/day. Based on the lack of any treatment-related findings in females, the NOEL was considered to be 1.0 mg/kg, the highest dose tested.

Cardiovascular, respiratory, and CNS safety:

Cardiovascular safety (*in vitro*, canine)	**Study ZNA20087.011 (*in vitro*)**: A study to assess the effect of AR-42, vehicle, or the reference substance E-4031 on hERG tail current recorded from HEK293 cells stably transfected with hERG cDNA was conducted using standard whole cell patch clamp methods. Compounds that inhibit hERG current have been shown to prolong the cardiac action potential and hence QT interval in man. AR-42 produced a concentration-dependent effect on hERG tail current recorded from stably transfected HEK293 cells. Exposure to 30 µM AR-42 produced no effect compared to the vehicle on hERG tail current; however, exposure to 100 µM produced an average 25% inhibition of tail current and exposure to 300 µM produced a transient potentiation of tail current followed by moderate inhibition (up to 50% in individual cell preparations). The decrease in tail current following treatment with 100 and 300 µM was found to be reversible. It is anticipated that 300 µM AR-42 is well in excess of the anticipated plasma levels that may be achieved in humans. The reference substance, E-4031, inhibited hERG tail current by approximately 90%, an effect consistent with its known activity. **Study ZNA20087.008 (canine)**: A cardiovascular safety study with telemetry in conscious dogs was conducted to assess the potential effects of ascending doses of AR-42 on arterial blood pressure, heart rate, and lead II electrocardiogram (ECG). Prolonged increases in heart rate (maximum increase approximately 64%) were noted after all dose levels of AR-42. There was no effect on blood pressure after administration of 1 mg/kg; however higher doses tended to produce decreases in arterial blood pressure of up to 20%. Evaluation of ECG parameters showed that QRS duration was not affected by AR-42 administration. Doses of 1 mg/kg AR-42 also had no effect on other parameters studied; however, higher doses were associated with decreases in RR interval and PR interval. The duration of the decreases in PR interval was dose-dependent, with a maximum decrease of approximately 16%. **At doses of 10 mg/kg AR-42, QTcF intervals were prolonged up to approximately 16%**. The ECG waveform and rhythm was unaffected by the administration AR-42.
Respiratory safety (rat)	A single study of AR-42 on the respiration rate and tidal volume in conscious rats was conducted. Oral administration of AR-42 at 10, 30 and 100 mg/kg did not significantly affect the respiration rate or tidal volume of male rats at any time point tested up to 1440 minutes post-dose.
Central nervous system safety (rat)	A single study was conducted to assess the effects of AR-42 in a 'Primary Observation Test' designed to detect effects on the gross behavioral and physiological state of rats. Oral administration of AR-42 at doses of 10 and 30 mg/kg did not cause rats to exhibit any changes from normal. Male Sprague-Dawley rats dosed orally with AR-42 at a dose of 100 mg/kg showed mild, transient CNS depression-like signs.

8. STATISTICAL CONSIDERATIONS

8.1 Safety endpoints

Safety will be assessed by evaluation of adverse events and clinical laboratory results and derived as data sets and frequency methods. The following safety measurements will be used.

- Adverse events by severity and causality as described below.

- Laboratory evaluations including comprehensive metabolic panel (which includes serum chemistries for magnesium, calcium, and potassium, and liver function tests), coagulation panel (PT/PTT), and CBC with differentials.
- Routine ECGs to monitor changes in QTc intervals.
- Clinically meaningful changes in vital signs including temperature, blood pressure, weight, pulse, and respiratory rate.

Adverse events will be described using the NCI CTCAE, V 4.0 criteria. Frequency and severity of adverse events according to body system and severity criteria will be described. In addition, frequency of grade 3 or 4 adverse events will be described separately. Causality will also be noted.

Laboratory assessments will also be described according to the NCI CTCAE, V 4.0 criteria, with separate descriptions for grade 3 or 4 laboratory abnormalities. Clinically significant laboratory abnormalities will be described as well.

8.2 Study design and sample size

For this multi-center, Phase 0 proof of concept study, 20 subjects will be enrolled in the treatment group. Differences in expression levels of phospho-Akt (p-AKT) and $p16^{INK4A}$ will be determined in five specimens from each of the following tumor types: 1) Non-NF2 associated VS tumors, 2) NF2-associated VS tumors, 3) Non-NF2 associated meningiomas, and 4) NF2-associated meningiomas. Additionally 10 control tissue from our control patients not receiving AR-42 will be analyzed. These sample sizes are consistent with phase 0 study designs. With 5 patients per group, we will have approximately 80% power to detect a 2 fold change, assuming a coefficient of variation of 0.30, and a type I error rate of 0.05. Since our analyses are exploratory we will not adjust for multiple comparisons.

Study enrollment and study treatment administration will be temporarily suspended if any patient dies from a drug-related AE. After a thorough review of the data by the study PI, investigational site PIs, and Data and Safety Monitoring Board, the study will be terminated if the death is determined to be drug-related.

8.3 Interim analysis and early stopping rules

Any unexpected deaths or SAEs possibly related to study treatment will be reported to the local Institutional Review Board (IRB). Trial accrual will be prematurely stopped if two or more unacceptable toxicities occur.

8.4 Statistical methods

The primary objective of this study is to estimate the expression levels of phospho-Akt (p-AKT) and $p16^{INK4A}$ in each of the four tumor types 1) NF2-related VS, 2) sporadic VS, 3) NF2-related meningiomas and 4) sporadic meningiomas after the described dosing of oral AR-42 as well as control samples from our control patients. Statistical analysis on the tumor samples for PK/PD will be descriptive and exploratory for this proof of concept study including summary statistics overall and by group for each variable as well as graphical displays for trend identification for use in planning future trials. P-AKT and $p16^{INK4A}$ will be evaluated as continuous variables in both AR-42 treated tumor samples and control samples. Of particular interest is the proportion of samples with complete inhibition of p-AKT and the increase in $p16^{INK4A}$ in AR-42 treated subjects. Because this is a phase 0 proof of concept trial with a small sample size we do not anticipate enough having enough power to see statistically significant differences between the groups and the controls. However, sufficient data will be gleaned to be extremely valuable in determining whether or not to move forward to the phase II clinical trial.

The secondary aims in this study include investigating the effect of AR-42 on acetylation of lysine (Ac-Lysine) and acetylated histone 2B (Ac-H2B) and on the phosphoinositide 3 kinase (PI3K)/AKT signaling pathway; tumor proliferation, cell cycle, cell death and angiogenesis; exploring HR23B as a biomarker for tumor sensitivity; performing NF2 gene mutation analyses; assessing audiometric changes pre and post AR-42; evaluating

volumetric changes in tumor size of the first 10 subjects; and determining the steady-state plasma and intra-tumoral concentrations of AR-42 at the time of surgical resection. For each of these secondary aims as well as baseline assessment variables we will use the appropriate summary statistics and graphical displays to summarize the variables collected for each of the four tumor types and the control samples. Since these are secondary aims no statistical testing will be performed.

8.5 Patient disposition and drug exposure

Patients' disposition will be summarized in the following manner:

- The number and percentage of patients selected, included, completed, withdrawn and lost to follow-up will be summarized using descriptive statistics.
- Major protocol deviations will be summarized.
- The reason for withdrawal (adverse events, major protocol deviation, non-medical reason) will be summarized.

9. PATIENT SAFETY

9.1 Monitoring of adverse events

Patients will be evaluated for toxicity if they have received at least one dose of AR-42. Significant adverse events (AEs) should be identified and recorded, then seriousness, expectedness, and causality will be assessed using the definitions that follow. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting.

An Adverse Event will be considered any unfavorable or unintended change in structure, function, signs, or symptoms temporally associated with the use of a medicinal product experienced by a person administered a pharmaceutical product, whether or not a causal relationship with the product has been established. Clinically significant laboratory abnormalities may be considered AEs if deemed appropriate by the Investigator. Worsening of a pre-existing condition is also considered an AE as is the discovery of an abnormal finding during physical exam that was not included in the medical history.

Subjects will be encouraged to spontaneously report any AE. Study personnel will ask open-ended questions to obtain information about AEs at every visit. Date and time of onset and resolution (if applicable) of the AE will be documented. All adverse events occurring after the initiation of the study treatment (treatment emergent adverse events) will be reported, including events present at baseline that worsened during the study.

Safety will be assessed by the investigator in the form of evaluation of adverse events and clinical laboratory results and derived as data sets and frequency methods. The following safety measurements will be used and conducted prior to AR-42 dosing and surgery.

- Adverse events by severity and causality as described below.
- Laboratory evaluations including comprehensive metabolic panel (which includes serum chemistries for magnesium, calcium, and potassium, and liver function tests) and CBC with differentials. For participants whose platelets decrease to less than 50,000/μL, AR-42 will be discontinued. Surgery may be delayed until platelet count is above 50,000/μL.
- Clinically meaningful changes in vital signs including temperature, blood pressure, weight, pulse, and respiratory rate.
- 12-lead ECG (triplicate).

To monitor any risk of increased surgical complications associated with AR-42, the treating surgeons will evaluate and record any noted intra-operative, short-term post-operative or intermediate post-operative differences and/or

complications such as increased bleeding, tumor adherence to adjacent nerves, or increased difficulty in tumor removal. A summary report of this information will be provided to the IRB after 5 participants have been accrued to the study. This information will also be provided to the research monitor for review.

9.2 Definitions of adverse events and causality

An **Adverse Event** is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment. Clinically significant laboratory abnormalities may be considered AEs if deemed appropriate by the Investigator. Worsening of a pre-existing condition is also considered an AE as is the discovery of an abnormal finding during physical exam that was not included in the medical history. For marketed products in the U.S., a **Serious Adverse Event** is any untoward medical occurrence that at any dose:

- Results in death (if the patient's death is suspected as being a direct outcome of the adverse event)
- Is life-threatening (the patient was at substantial risk of dying at the time of the adverse event or it is suspected that the use or continued use of the product would result in the patient's death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect (i.e., exposure to a medical product prior to conception or during pregnancy resulted in an adverse outcome in a child)
- Results in the development of drug dependency or drug abuse
- Requires intervention to prevent permanent impairment or damage
- Overdosage (regardless of adverse outcome) of any study medication. An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important.
- Pregnancy
- Is an important medical event, defined as a medical event(s) that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require medical or surgical intervention to prevent one of the other serious outcomes listed in the definition above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, or blood dyscrasias or convulsions that do not result in hospitalization.

The SAE reporting period begins once study drug treatment is initiated to within 30 days following cessation of treatment. AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to patients experiencing AEs that cause interruption or discontinuation of investigational product, or those experiencing AEs that are present at the end of their participation in the study. Such patients should receive post-treatment follow-up as appropriate. If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE entry for the event should be completed.

Additionally, any serious adverse event considered by an investigator to be possibly, probably, or definitely related to the investigational product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the IRB.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Note: The term "life-threatening" in the definition of "Serious Adverse Event" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

An **Unexpected Adverse Event** would either be unexpected for a similar HDAC inhibitor or differs because of greater severity or specificity.

Causality is a determination of whether there is a reasonable possibility that the drug may have caused or contributed to an adverse event. It includes assessing temporal relationships dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) or a lack of one or more likely causes.

The Investigator must attempt to determine if an adverse event is in some way related to the use of the study drug. This relationship should be described as follows:

Unlikely: The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug.

Possible: The event follows a reasonable temporal sequence from administration of the study drug or the event follows a known response pattern to the study drug *BUT* the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug or the event could be the effect of a concomitant medication

Probable: The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition *or* the event cannot be the effect of a concomitant medication

Definite: The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug

Unknown: Based on the evidence available, causality cannot be ascribed

9.3 Serious adverse events

Any Serious Adverse Events (SAE) as described in Section 9.2, including death due to any cause, which occurs during this study, must be reported immediately (within 24 hours) by confirmed email or phone call to the principal investigator or study coordinator in the central study office:

D. Bradley Welling, M.D., Ph.D.
Principal Investigator
The Massachusetts Eye and Ear Infirmary
Phone: 617-573-3632
Fax: 617-573-3415
Email: brad_welling@meei.harvard.edu

Amy Quinkert, PhD, CCRP
Study Coordinator
The Massachusetts Eye and Ear Infirmary
Phone: 617-573-4192
Fax: 614-293-7292
Email: amy_quinkert@meei.harvard.edu

When the principal investigator has determined that a Serious Adverse Event has occurred, the principal investigator is responsible for providing all Serious Adverse Events to the IRB within two working days of this determination. Additionally, the principal investigator will inform all participating investigators of an unacceptable toxicity or SAE at least possible related to AR-42 within 48-72 hours of its occurrence. This applies to initial and follow-up information.

Follow-up reports:

- The Investigator should take all appropriate measures to ensure the safety of the patients, notably he/she should follow up the outcome of any Serious Adverse Event and complete follow-up forms as necessary. The patient must be followed up until recovery, stabilization or return to baseline. This may mean that follow-up will continue after the patient has completed the trial and that additional investigations may be necessary.
- Any reportable Serious Adverse Events brought to the attention of the Investigator at any time after cessation of the trial and considered by him/her to be reasonably associated with medication administered during the period should also be submitted to the IRB.
- As with the initial submission to the IRB, the principal investigator is also responsible for providing all follow-ups of Serious Adverse Events to the IRB.

A copy of all 15 Day Reports and Annual Progress Reports will be submitted as required by FDA, or other local regulators. In addition, the FDA should be notified as soon as possible and in no case later than 7 calendar days after initial receipt, of all unexpected fatal or life-threatening suspected adverse reactions.

9.4 Data safety monitoring plan

The Data and Safety Monitoring Board (DSMB) as outlined in the DSMB charter will serve as the independent data and safety monitoring board for this study. The DSMB will meet annually to review progress of the study and will be available for emergent meetings if serious or unanticipated adverse events occur. The serious adverse events and responses will be reviewed by the DSMB.

The Data and Safety Monitoring Plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings and the discussion will be documented in the minutes. The PI of the trial will review toxicities and responses of the trial, where applicable, at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by DSMB. The PI will also submit a progress report (biannually for Phase II and quarterly for Phase I) that will be reviewed by the committee per the IRB of record as per the policies of the IRB. The DSMB Membership List is as follows:

Edwin Choy, MD, Chair and Research Monitor
MGH, Dana Farber/Harvard Cancer Center
Division of Hematology Oncology
55 Fruit Street
Boston MA 02114
Phone: (617) 643-0230
Fax: (617) 724-3166
Email: echoy@partners.org

Christopher Moertel, MD, Member
University of Minnesota
Department of Pediatrics
MMC 484
420 Delaware Street S.E.
Minneapolis, MN 55455
Phone: (612) 625-3229
Fax: (612) 626-2815
Email: moert001@umn.edu

Domenic Reda, PhD, Member (Statistician)
Director, Cooperative Studies Program Coordinating Center
Hines VA Hospital, CSPCC (151K)
5000 South 5th Avenue, Building 1, Room B240,
Hines, IL 60141-3030
Phone: 708-202-5853
Fax: 708-202-2116
Email: domenic.reda@va.gov

9.5 Reporting of adverse event information following study completion

Collection of safety information following the end of investigational product administration is important in assisting in the identification of possible delayed toxicities or withdrawal effects. All SAEs must be collected which occur within 30 days of discontinuation of dosing or completion of the patient's participation in the study if the last scheduled visit occurs at a later time.

9.6 Pregnancy statement and use in nursing women

All women of childbearing potential MUST have a negative pregnancy test. If the pregnancy test is positive, the patient must not receive any investigational product and must not be enrolled in the study.

Definition of childbearing potential: For the purposes of this study, a female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy (the surgical removal of the uterus) or bilateral oophorectomy (the surgical removal of both ovaries) or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time during the preceding 24 consecutive months).

Study participants must be willing to comply with fertility requirements as described below:

- Male participants must agree to use an adequate method of contraception for the duration of the study and for 28 days afterwards.
- Female participants must be either postmenopausal, free from menses $\geq$ 2 yrs, surgically sterilized, willing to use two adequate barrier methods of contraception to prevent pregnancy, or agree to abstain from heterosexual activity starting with screening and for 90 days afterwards.
- Participants must agree not to donate blood, sperm/ova during study participation and for at least 4 weeks after stopping treatment.

During the course of the trial, all patients of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition, a missed or late menstrual period should be reported to the treating physician. If a female patient or the treating physician suspects that the female patient may be pregnant prior to administration of study drugs, the study drugs must be withheld until the

results of a pregnancy test are available. If pregnancy is confirmed the patient must not receive study medications and must be withdrawn from the study. Throughout the entire pregnancy, additional contact should be made with the patient, and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 4 weeks.

If a male patient is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record abstraction, or a combination of these methods. All serious adverse event reports relating to the pregnancy, including spontaneous abortion, elective abortion and congenital anomalies, should be forwarded to the FDA.

It is not known whether AR-42 is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from AR-42, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

9.7 Patient withdrawal and study termination

A participant should be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the participant. If a participant does not return for a scheduled visit, every effort will be made to contact the participant. In any circumstance, every effort will be made to document participant outcome, if possible.

Participants should be removed from therapy if any of the following occurs:

- Adverse event: The occurrence of unacceptable toxicity indicating the need for cessation of treatment, defined as grade 3/4 arrhythmia.
- The physician feels it is in the best interest of the patient to stop treatment.
- The participant desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If a reason for withdrawal is given, it should be recorded in the case report form.
- Protocol violation: The participant's findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (eg. study drug compliance, returning for the specified number of visits).
- Participant is lost to follow-up. If a participant does not return for scheduled visits, every effort should be made to re-establish contact. In any circumstance, every effort should be made to document participant outcome, if possible.
- Participant becomes pregnant.
- Termination of the study.

The reason and date of discontinuation are to be documented in the participant's medical record and in the case report form.

The investigator should complete all end of treatment procedures when a participant withdraws from treatment. All participants who discontinue the trial secondary to an adverse event should be followed until resolution, stabilization or return to a baseline condition.

Participants who fail to complete treatment for reasons other than adverse event or unacceptable toxicity may be replaced. All participants who receive one or more doses of study treatment should be included in any safety analysis.

The investigator may discontinue the trial at any time. Reasons for early trial discontinuation may include, but are not limited to, unacceptable toxicity of study treatment, a request to discontinue the trial from a regulatory authority, or poor enrollment.

9.8 Research monitor

Edwin Choy, MD, will serve as the Chair of the DSMB and Research Monitor. Dr. Choy serves as an Assistant Professor of Medicine at the Harvard Medical School. Dr. Choy has several years of experience serving on a DSMB for similar research studies. Dr. Choy is the Director of Sarcoma Research at the Division of Hematology Oncology at MGH. He specialized in the medical management of patients with sarcomas, gastrointestinal stromal tumors, chordomas, and desmoid tumors. He works closely with a team of world class surgical, orthopedic, and radiation oncologists as well as connective tissue pathologists and radiologists to provide optimal care for his patients. He directs a clinical trials program at the MGH Center for Sarcoma and Connective Tissue Oncology that includes 9-12 active phase I-III clinical trials. He also maintains an active scientific research program that investigates diverse areas of sarcoma therapy ranging from the use of nanotechnology and other translational research tools aimed at developing new targeted therapies for treating cancers to preclinical studies using cell lines and animal models to better understand the molecular basis of sarcoma biology. His clinical expertise matched with his experience in data safety monitoring has equipped him to take on the position of Research Monitor for this study. In addition to attending the regular meetings of the DSMB, assessing any adverse events presented, and overseeing the overall maintenance of DSMB records and reporting, Dr. Choy is responsible for identifying and following through with the need to report to the IRB, when appropriate. Dr. Choy will work with the Principal Investigator, Dr. Welling, to ensure all reports are filed to the IRB in a timely and efficient manner in order to protect the safety of human subjects. As Research Monitor, Dr. Choy has the authority to stop the research protocol in progress, remove individual human subjects from the research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB assesses the report filed.

Dr. Choy is responsible for reviewing all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol. He will provide an unbiased written report of any events relating to these matters. This report will provide an overview of the event, including the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. In these reports, Dr. Choy will indicate whether he concurs with the details of the report provided by the principal investigator. Dr. Choy and Dr. Welling will ensure reports possibly or definitely related to participation and reports of events resulting in death are promptly forwarded to the USAMRMC ORP HRPO at usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil.

10. ETHICAL AND REGULATORY STANDARDS

10.1 Ethical principles

The study should be conducted according to the principles outlined by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments; the International Conference on Harmonization Guidelines for Good Clinical Practice; and FDA regulations regarding the conduct of clinical trials and the protection of human subjects.

10.2 Protocol compliance and protocol revisions

The study must be conducted as described in this approved protocol. The investigator should not implement any deviation or change to the protocol without prior review and documented approval from the IRB of an amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

10.3 Informed consent

The study investigator and/or delegated research staff at each site will obtain informed consent from each participant enrolled in the study, in accordance with the U.S. Food and Drug Administration (FDA) regulations 21 CFR 50.20 - 50.27, the laws and regulations of the state in which the investigation is being conducted, and should adhere to Good Clinical Practices and to the ethical principles that have their origin in the Declaration of Helsinki. Consent will only be obtained under circumstances that provide the prospective subject sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence.

All personnel involved with recruitment and obtaining informed consent will have completed training in the protection of human subjects in research and been approved by their IRB to participate in the study. Prior to the beginning of the trial, the investigator will have the IRB approval of the written informed consent form and any other written information provided to subjects. Subjects will not be consented until final IRB approval of these documents is received.

Obtaining Consent:

After study investigators have determined that an individual is eligible to participate, the study protocol, including all risks and potential benefits and investigational nature, will be explained to the participant by the site study investigators in a private clinical office with respect to the potential subject's privacy. The investigator will review and discuss details of the research study using the consent form as a guide. All basic elements of the consent form document, HIPAA, consent addendums and any additional relevant information will be presented in detail to the prospective subject. The information given to the subject shall be in a language understandable to the subject. No informed consent, whether oral or written, will include any exculpatory language through which the subject is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence. The information presented will not include complex, technical, highly specialized language or medical jargon that would not be understandable to potential subject. The PI will not coerce or unduly influence a subject to participate or to continue to participate in a trial.

Before informed consent may be obtained, the investigator will provide the subject ample time and opportunity to read, inquire about the details of the study and to decide whether or not to participate. All questions about the study will be answered to the satisfaction of the subject by site study investigators. The subject can chose to provide consent at the time of the office visit or take the consent home for review and discussion with others and have an opportunity to think about participation.

For patients to qualify to participate in this study, information such as the results of routine blood work necessary for surgery (including but not limited to chemistries, CBC, platelets, etc.) may be used to determine candidacy and may have been done within 30 days before the patient has consented into the study. A standard of care MRI done within 60 days of the scheduled day 1 of study drug may be used for screening/baseline assessments. The investigator or delegated research staff will obtain informed consent which will be documented by the use of a written consent form, approved by the IRB and signed and dated in pen, by the subject prior administering the first dose of the study medicine. This form will also be signed and dated in pen by the person obtaining consent and if necessary a witness to the consent process and maintained in an individual subject research binder.

All blanks on the consent form, consent addendum forms (if applicable) and HIPAA authorization form (if applicable) for subject name, subject initials, dates, signatures, yes/no check boxes for optional research procedures must be completed by the subject themselves if previously approved by the IRB. Delegated research staff may not complete these blanks for the subject.

The PI or delegated research staff will ensure that the subject expresses understanding of information presented on the clinical trial, that participation is voluntary, and that the subject can withdraw at any time without penalty or effects to their medical care. They will ensure that all of the subject's questions have been answered. Participants will also be made aware that by signing the consent form their personal health information and research records may be audited/reviewed by authorized study personnel.

By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to and understood by the subject, and that informed consent was freely given by the subject. The PI or delegated research staff will give a copy of all signed and completed forms to the subject. The investigational site should ensure that the subject understands that in order to participate on the clinical trial the subject must be eligible per the protocol's inclusion and exclusion criteria.
It is the responsibility of the investigator to obtain written informed consent from a patient before any study related procedures are performed. The Investigator will provide an informed consent in compliance with ICH GCP and U.S. FDA guidelines (21 CFR 50). The informed consent document must clearly describe the potential risks and benefits of the trial, and each prospective participant must be given adequate time to discuss the trial with the Investigator or site staff and to decide whether or not to participate. The informed consent must be approved by the IRB prior to being presented to a potential patient.

10.4 Institutional Review Board (IRB) approval

The principal investigator must obtain the approval of the protocol, the informed consent document and any other material used to inform the patient about the nature of the trial from the local IRB in the form of a written letter. On the approval letter, which must be signed by the chairperson of the IRB or the chairperson's designee, the following items should be clearly stated: trial title, protocol number and version, study-related documents (protocol, informed consent material, advertisement when applicable), IRB review date, and IRB decision. The trial should not start until a copy of this written approval has been received by the Investigator.

10.5 Additional responsibilities of the investigator

The investigator(s) agrees to perform the study in accordance with ICH Good Clinical Practice and FDA regulations. The Investigator is required to ensure compliance with respect to the investigational drug schedule, visit schedule and procedures required by the protocol.

The investigator should be able to recruit the required number of suitable patients and should have sufficient time to properly conduct and complete the trial. The Investigator should have available an adequate number of qualified staff and adequate facilities for the duration of the trial, and should ensure that all persons assisting with the trial are adequately informed about the protocol, the protocol-defined procedures, protocol therapy and trial related duties and functions..

The Investigator should be responsible for all trial-related medical decisions. During and following a patient's participation in a trial, the investigator should ensure that adequate medical care is provided to a patient for any adverse events related to the trial.

10.6 Confidentiality

It is the responsibility of the investigator to ensure that the confidentiality of all patients participating in the trial and all of their medical information is maintained. Case report forms must never contain the name of a trial patient. All case report forms and any identifying information must be kept in a secure location with access limited to the study staff directly participating in the trial. Personal medical information may be reviewed by a representative of the IRB, or of regulatory authorities in the course of auditing the trial. Every reasonable effort will be made to maintain such information as confidential.

10.7 Conflict of interest

AR-42 is a therapeutic that was generated at OSU and for which many laboratories at this same institution have performed pre-clinical studies supporting its use in different types of cancer. Ching-Shih Chen is the inventor of this compound. Both the inventor and the University (OSU) have the potential to benefit financially from AR-42 if the compound has clinical activity. Addressing conflict of interest relative to investigators is therefore very important. None of the clinical investigators involved in this trial have personal potential to financially gain from the success of this program. The pharmaceutical licensee (ARNO Therapeutics) will be supplying all of the study drug for completion of this trial. Safety issues relative to attribution of response will also be monitored by the DSMB and the Cancer Center IRB.

11. REFERENCES

Albright EC, Allday RW, 1967. Thyroid carcinoma after radiation therapy for adolescent acne vulgaris. JAMA 199:280-281.

Bai LY, Omar HA, Chiu CF, Chi ZP, Hu JL, Weng JR. 2011. Antitumor effects of (S)-HDAC42, a phenylbutryate-derived histone deacetylase inhibitor, in multiple myeloma cells. Cancer Chemother Pharmacol. 68:489-496.

Balasubramaniam, A., Shannon, P., Hodaie, M., et al. 2007. Glioblastoma multiforme after stereotactic radiotherapy for acoustic neuroma: case report and review of the literature. Neuro-Oncol. 9:447-453.

Baser ME, Friedman JM, Aeschliman D, Joe H, Wallace AJ, Ramsden RT, Evans DG. 2002. Predictors of the risk of mortality in neurofibromatosis 2. Am J Hum Genet. 71:715-723.

Bassim MK, Berliner KI, Fisher LM, Brackmann DE, Friedman RA. 2010. Radiation therapy for the treatment of vestibular schwannoma: a critical evaluation of the state of the literature. Otol Neurotol. 31:567-573.

Bates SE, Rosing DR, Fojo T, Piekarz RL. 2006. Challenges of evaluating the cardiac effects of anticancer agents. Clin Cancer Res. 12:3871-3874.

Bianchi AB, Hara T, Ramesh V, et al. 1994. Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types. Nat Genet. 6:185-192.

Bianchi AB, Mitsunnaga SI, Cheng JQ, Klein WM, Jhanwar SC, Seizinger B, Kley N, Klein-Szanto AJ, Testa JR. 1995. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (*NF2*) in primary malignant mesotheliomas. Proc Natl Acad Sci USA 92:10854-10858.

Bolden JE, Peart MJ, Johnstone RW. 2006. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 5:769-784.

Bull. World Health Org. 1992. Prevention and control of neurofibromatosis: Memorandum from a joint WHO/NNFF meeting. 70:173-182.

Burns SS, Akhmametyeva EM, Oblinger JL, Bush ML, Huang J, Senner V, Giovannini M, Chen C-S, Jacob A, Welling DB, Chang L-S. 2011. AR-42 and AR-12 potently inhibit the growth of NF2-deficient human meningiomas. Abstract presented to the 2011 CTF NF Conference, Jackson Hole, WY. Page 31.

Bush ML, Oblinger J, Brendel V, Santarelli G, Hrelec C, Davis J, Huang J, Akhmametyeva EM, Burns SS, Kulp S, Chen C-S, Giovannini M, Chang L-S, Welling D-B, Jacob A. 2011. HDAC42, A novel histone deacetylase inhibitor, as a potential therapy for vestibular schwannomas and meningiomas. Neuro-Oncology 13:983-999.

Central Brain Tumor Registry of the United States (CBTRUS), 2011. http://www.cbtrus.org/

Chen CS, Weng SC, Tseng PH, Lin HP, Chen CS. 2005. Histone acetylation-independent effect of histone deacetylase inhibitors on Akt through the reshuffling of protein phosphatase 1 complexes. J. Biol. Chem. 280:38879-38887.

Cheng H, Zhongfa L, Kulp SK, Chen C-S, Covey JM, Chan KK. Preclinical pharmacokinetic studies with s-HDAC-42 (NSC 736012), an inhibitor of histone deacetylase, by LC-MS/MS. Proceedings of the American Association of Cancer Research, 97th Annual Meeting, April 1-5, 2006, Washington, DC.

Committee IS. ICH Harmonized Tripartite Guideline: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs, E14. In: Committee IS, ed. International conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Geneva, Switzerland; 2005.

Cress WD, Seto E. 2000. Histone deacetylases, transcriptional control, and cancer. J. Cell. Physiol. 184:1-16.

Curto M, Cole BK, Lallemand D, Liu CH, McClatchey AI. 2007. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. J Cell Biol. 177:893-903.

Desai M, Li L, Desta Z, Malik M, Flockhart D. 2003. Variability of heart rate correction methods for the QT interval. Br J Clin Pharmacol. 55:511-517.

Doherty JK, Ongkeko W, Crawley B, Andalibi A, Ryan AF. 2008. ErbB and Nrg: potential molecular targets for vestibular schwannoma pharmacotherapy. Otol Neurotol. 29:50-57.

Drummond DC, Noble CO, Kirpotin DB, et al. 2005. Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol. 45:495–528.

Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Newton V, Harris R. 1992. A clinical study of type 2 neurofibromatosis. Q J Med. 84:603-618.

Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Teare D, Newton V, Strachan T, Ramsden R, Harris R. 1992. A genetic study of type 2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness, and confirmation of maternal transmission effect on severity. J Med Genet. 29:841-846.

Evans DG, Kalamarides M, Hunter-Schaedle K, Blakeley J, Allen J, Babovic-Vuskanovic D, Belzberg A, Bollag G, Chen R, DiTomaso E, Golfinos J, Harris G, Jacob A, Kalpana G, Karajannis M, Korf B, Kurzrock R, Law M, McClatchey A, Packer R, Roehm P, Rubenstein A, Slattery W 3rd, Tonsgard JH, Welling DB, Widemann B, Yohay K, Giovannini M. 2009. Consensus recommendations to accelerate clinical trials for neurofibromatosis type 2. Clin Cancer Res. 15:5032-5039.

Friedman RA, Brackmann DE, Hitselberger WE, Schwartz MS, Iqbal Z, Berliner KI. 2005. Surgical salvage after failed irradiation for vestibular schwannoma. Laryngoscope 115:1827-1832.

Funck-Brentano C, Jaillon P. 1993. Rate-corrected QT interval: techniques and limitations. Am J Cardiol. 72:17B-22B.

Giles F, Fischer T, Cortes J, et al. 2006. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. Clin Cancer Res. 12:4628-4635.

Giovannini M, Robanus-Maandag E, van der Valk M, et al. 2000. Conditional biallelic *Nf2* mutation in the mouse promotes manifestations of human neurofibromatosis type 2. Genes Dev. 14:1617-1630.

Goutagny S, Kalamarides M. 2010. Meningiomas and neurofibromatosis. J Neurooncol. 99:341-347.

Hanemann CO, Bartelt-Kirbach B, Diebold R, et al. 2006. Differential gene expression between human schwannoma and control Schwann cells. Neuropathol Appl Neurobiol. 32:605-614.

Hansen MR, Roehm PC, Chatterjee P, Green SH. 2006. Constitutive neuregulin-1/ErbB signaling contributes to human vestibular schwannoma proliferation. Glia 53:593-600.

Hilton DA, Ristic N, Hanemann CO. 2009. Activation of ERK, AKT and JNK signalling pathways in human schwannomas in situ. Histopathology 55:744-749.

Jacob A, Lee TX, Neff BA, Miller S, Welling DB, Chang L-S. 2008. Phosphatidylinositol 3-kinase/AKT pathway activation in human vestibular schwannoma. Otol Neurotol. 29:58-68.

Jacob A, Oblinger J, Bush ML, Brendel V, Santarelli G, Chaudhury AR, Kulp S, La Perle K, Chen C-S, Chang L-S, Welling DB. 2012. Preclinical validation of AR42, a novel histone deacetylase inhibitor, as treatment for vestibular schwannomas. Laryngoscope. 122(1):174-189.

Johnson MD, Okedli E, Woodard A, Toms SA, Allen GS. 2002. Evidence for phosphatidylinositol 3-kinase-Akt-p7S6K pathway activation and transduction of mitogenic signals by platelet-derived growth factor in meningioma cells. J Neurosurg. 97:668-675.

Johnson MD, O'Connell M, Pilcher W. 2011. Lopinavir inhibits meningioma cell proliferation by Akt independent mechanism. J Neurooncol. 101:441-448.

Johnstone RW, Licht JD. 2003. Histone deacetylase inhibitors in cancer therapy: Is transcription the primary target? Cancer Cell 4:13-18.

Kalamarides M, Niwa-Kawakita M, Leblois H, Abramowski V, Perricaudet M, Janin A, Thomas G, Gutmann DH, Giovannini M. 2002. Nf2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. Genes Dev. 16:1060-1065.

Kalamarides M, Stemmer-Rachamimov AO, Niwa-Kawakita M, Chareyre F, Taranchon E, Han ZY, Martinelli C, Lusis EA, Hegedus B, Gutmann DH, Giovannini M. 2011. Identification of a progenitor cell of origin capable of generating diverse meningioma histological subtypes. Oncogene 30:2333-2344.

Kahn O, Fotheringham S, Wood V, Stimson L, Zhang C, Pezzella F, Duvic M, Kerr DJ, LaThangue NB. 2010. HR23B is a biomarker for tumor sensitivity to HDAC inhibitor-based therapy. Proc Natl Acad Sci USA 107:6532-6537.

Kulp SK, Chen CS, Wang DS et al. 2006. Antitumor effects of a novel phenylbutyrate-based histone deacetylase inhibitor, (S)-HDAC-42, in prostate cancer. Clin Cancer Res. 12:5199-5206.

Lallemand D, Manent J, Couvelard A, et al. 2009. Merlin regulates transmembrane receptor accumulation and signaling at the plasma membrane in primary mouse Schwann cells and in human schwannomas. Oncogene 28:854-865.

Lasak JM, Welling DB, Salloum M, and Chang L-S. 2002. Deregulation of the Retinoblastoma Protein-Cyclin Dependent Kinase Pathway in Vestibular Schwannomas. Laryngoscope 112:1555-1561.

Lee TX, Packer MD, Huang J, Akhmametyeva EM, Kulp SK, Chen CS, Giovannini M, Jacob A, Welling DB, Chang L-S. 2009. Growth inhibitory and anti-tumour activities of OSU-03012, a novel PDK-1 inhibitor, on vestibular schwannoma and malignant schwannoma cells. Eur J Cancer 45:1709-1720.

Lin TY, Fenger J, Murahari S, Bear MD, et al. 2010. AR-42, a novel HDAC inhibitor, exhibits biologic activity against malignant cell lines via down-regulation of constitutively activated Kit. Blood 115:4217-4225.

LoPiccolo, J., Blumenthal, G.M., Bernstein, W.B., Dennis, P.A. 2008. Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. Drug Resist Updat. 11:32-50.

Lu YS, Kashida Y, Kulp SK, Wang YC, Wang D, Hung JH, Tang M, Lin ZZ, Chen TJ, Cheng AL, Chen CS. 2007. Efficacy of a novel histone deacetylase inhibitor in murine models of hepatocellular carcinoma. Hepatology 46:1119-1130.

Lucas DM, Alinari L, West DA, et al. 2010. The novel deacetylase inhibitor AR-42 demonstrates pre-clinical activity in B-Cell malignancies in vitro and in vivo. PLoS One 5:e10941.

Luo S, Michler K, Johnston P, Macfarlane PW. 2004. A comparison of commonly used QT correction formulae: the effect of heart rate on the QTc of normal ECGs. J Electrocardiol. 37 Suppl:81-90.

Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. 2001. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 1:194-202.

Marks PA, Breslow R. 2007. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol. 25:84-90.

Massick DD, Welling DB, Dodson EE, Scholfield M, Nagaraja HN, Schmalbrock P, Chakeres DW. 2000. Tumor growth and audiometric change in vestibular schwannomas managed conservatively. Laryngoscope 110:1843-1849.

Mathieu D, Kondziolka D, Flickinger JC, Niranjan A, Williamson R, Martin JJ, Lunsford LD. 2007. Stereotactic radiosurgery for vestibular schwannomas in patients with neurofibromatosis type 2: an analysis of tumor control, complications, and hearing preservation rates. Neurosurgery 60:460-468.

Mawrin C, Sasse T, Kirches E, Kropf S, Schneider T, Grimm C, Pambor C, Vorwerk CK, Firsching R, Lendeckel U, Dietzmann K. 2005. Different Activation of Mitogen-Activated Protein Kinase and AKT Signaling is Associated with Aggressive Phenotype of Human Meningiomas. Clin Cancer Res. 11:4074-4082.

McClatchey AI, Giovannini M. 2005. Membrane organization and tumorigenesis--the NF2 tumor suppressor, Merlin. Genes Dev. 19:2265-2277.

Minucci S, Pelicci PG. 2006. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6:38-51.

Mut M., Cataltepe O, Sovlemezoglu F, Akalan N, Ozgen T. 2004. Radiation-induced malignant triton tumor associated with severe spinal cord compression. Case report and review of the literature. J Neurosurg. 100(3 Suppl. Spine):298-302.

Nunes F, Merker V, Jennings D, Caruso P, Stemmer-Rachamimov A, Plotkin S. Meningioma response to bevacizumab: A retrospective analysis of treatment results in 14 NF2 patients. Children's Tumor Foundation Conference 2011, June 12, 2011, Jackson Hole, Wyoming.

Olsen EA, Kim YH, Kuzel TM, et al. 2007. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol. 25:3109-3115.

Palmer JA, Mustard RA, Simpson WJ. 1980. Irradiation as an etiologic factor in tumours of the thyroid, parathyroid and salivary glands. Can J Surg. 23:39-34.

Parry DM, Eldridge R, Kaiser-Kupfer MI, Bouzas EA, Pikus A, Patronas N. 1994. Neurofibromatosis 2 (NF2): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. Am J Med Genet. 52:450-461.

Phi JH, Kim DG, Chung HT, Lee J, Paek SH, Jung HW. 2009. Radiosurgical treatment of vestibular schwannomas in patients with neurofibromatosis type 2: tumor control and hearing preservation. Cancer 115:390-398.

Piekarz RL, Frye AR, Wright JJ, et al. 2006. Cardiac studies in patients treated with depsipeptide, FK228, in a phase II trial for T-cell lymphoma. Clin Cancer Res. 12:3762-3773.

Plotkin SR, Stemmer-Rachamimov AO, Barker FG 2nd, Halpin C, Padera TP, Tyrrell A, Sorensen AG, Jain

RK, di Tomaso E. 2009. Hearing improvement after bevacizumab in patients with neurofibromatosis type 2. N Engl J Med. 361(4):358-67. PMID: 19587327.

Puddu PE, Jouve R, Mariotti S, et al. 1988. Evaluation of 10 QT prediction formulas in 881 middle-aged men from the seven countries study: emphasis on the cubic root Fridericia's equation. J Electrocardiol. 21:219-229.

Püttmann S, Senner V, Braune S, Hillmann B, Exeler R, Rickert C, Paulus W. 2005. Establishment of a benign meningioma cell line by hTERT-mediated immortalization. Lab Invest. 85:1163-1171.

Rong R, Tang X, Gutmann DH, Ye K. 2004. Neurofibromatosis 2 (NF2) tumor suppressor merlin inhibits phosphatidylinositol 3-kinase through binding to PIKE-L. Proc Natl Acad Sci U S A. 101(52):18200-5. PMID:15598747.

Rouleau GA, Merel P, Lutchman M, et al. 1993. Alteration in a new gene encoding a putative membrane-organising protein causes neurofibromatosis type 2. Nature 363:515-521.

Samii M, Gerganov V, Samii A. 2008. Microsurgery management of vestibular schwannomas in neurofibromatosis type 2: indications and results. Prog Neurol Surg. 21:169-175.

Sandor V, Bakke S, Robey RW, et al. 2002. Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. Clin Cancer Res. 8:718-728.

Santini V, Gozzini A, Ferrari G. 2007. Histone deacetylase inhibitors: molecular and biological activity as a premise to clinical application. Curr Drug Metab. 8(4):383-393. Review. PMID: 17504226

Sargeant AM, Rengel RC, Kulp SK, Klein RD, Clinton SK, Wang YC, Chen CS. 2008. OSU-HDAC42, a histone deacetylase inhibitor, blocks prostate tumor progression in the transgenic adenocarcinoma of the mouse prostate model. Cancer Res. 68:3999-4009.

Shah MH, Binkley P, Chan K, et al. 2006. Cardiotoxicity of histone deacetylase inhibitor depsipeptide in patients with metastatic neuroendocrine tumors. Clin Cancer Res. 12:3997-4003.

Shah RR. 2005. Drugs, QTc interval prolongation and final ICH E14 guideline : an important milestone with challenges ahead. Drug Saf. 28:1009-1028.

Slattery WH 3rd, Fisher LM, Hitselberger W, Friedman RA, Brackmann DE. 2007. Hearing preservation surgery for neurofibromatosis Type 2-related vestibular schwannoma in pediatric patients. J Neurosurg. 106(4 Suppl):255-260.

Sperfield AD, Hein C, Schroder JM, Ludolph AC, Hanemann CO. 2002. Occurrence and characterization of peripheral nerve involvement in neurofirbomatosis type 2. Brain. 125: 996-1004.

Straus SM, Kors JA, De Bruin ML, et al. 2006. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol. 47:362-367.

Strevel EL, Ing DJ, Siu LL. 2007. Molecularly targeted oncology therapeutics and prolongation of the QT interval. J Clin Oncol. 25:3362-3371.

Trofatter JA, MacCollin MM, Rutter JL, et al. 1993. A novel Moesin-, Exrin-, Radixin-like gene is a candidate for the neurofibromatosis 2 tumor-suppressor. Cell 72:791-800.

Ueda H, Manda T, Matsumoto S, et al. 1994. FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. III. Antitumor activities on experimental tumors in mice. J Antibiot (Tokyo). 47:315-323.

Varterasian M, Meyer M, Fingert H, et al. 2003. Baseline heart rate-corrected QT and eligibility for clinical trials in oncology. J Clin Oncol. 21:3378-3379.

Walz PC, Bush ML, Robinett Z, Kirsch CFE, and Welling DB. 2012. Three-Dimensional Segmented Volumetric Analysis of sporadic vestibular schwannomas: Comparison of Segmented and linear measurements. Accepted, Otolaryngl HN Surg, e-Pub 15 May 2012.

Welling DB. 1998. Clinical manifestations of mutations in the neurofibromatosis type 2 gene in vestibular schwannomas (acoustic neuromas). Laryngoscope. 108:178-189.

Welling DB, Lasak JM, Akhmametyeva E, Pearl DK, Chang L-S. 2002. cDNA Microarray Analysis of Vestibular Schwannomas. Otol Neurotol. 23:736-748.

Welling DB, Packer MD, Akhmametyeva EM, Chang L-S. 2008. The Biology and Genetics of Vestibular Schwannomas. In *Surgery of the Cerebellopontine Angle*, Bambakidis, N., Megerian, C., and Spetzler, R. (Eds), B.C. Decker, Inc., Hamilton, Ontario, CA.

Wiemels J, Wrensch M, Claus EB. 2010. Epidemiology and etiology of meningioma. J. Neurooncol. 99:307-314.
Yang YT, Balch C, Kulp SK, Mand MR, Nephew KP, Chen CS. 2009. A rationally designed histone deacetylase inhibitor with distinct antitumor activity against ovarian cancer. Neoplasia 11:552-563.
Zhang S, Suvannasankha A, Crean CD, White VL, Chen CS, Farag SS. 2011. The novel histone deacetylase inhibitor, AR-42, inhibits gp130/Stat3 pathway and induces apoptosis and cell cycle arrest in multiple myeloma cells. Int J Cancer 129:204-213.

12. APPENDIX A: QT PROLONGATION – HISTORY, RATIONALE, MONITORING

The QT interval is an important variable in oncology drug development, but its significance in early-phase clinical trials is still uncertain. At least four HDAC inhibitors examined to date—including vorinostat (Olsen et al., 2007), panobinostat (Giles et al., 2006), and depsipeptide (Piekarz et al., 2006) have shown clinical evidence of QT prolongation in phase I and/or II studies. Although QT prolongation poses a risk of malignant cardiac arrhythmia with torsade de pointes and sudden cardiac death, it is important to note that, with a few exceptions such as depsipeptide, the QT abnormalities observed in HDAC inhibitor clinical trials have not translated into clinically meaningful events.

It is important to review the experience with Depispeptide. Depsipeptide (FK228), a cyclic peptide, is an HDAC inhibitor that has been variably associated with QT prolongation and rarely sudden cardiac death (Ueda et al., 1994; Bates et al., 2006). A phase I study of depsipeptide in solid tumor malignancies reported three asymptomatic cardiac arrhythmic events and one symptomatic episode of dose-limiting atrial fibrillation in four of 37 patients (Sandor et al., 2002). Depsipeptide's ability to induce QT toxicity appears to be heterogeneous and may vary based on the patient population. A phase II study at Ohio State University (PI Manisha Shah) investigated depsipeptide in 15 patients with metastatic neuroendocrine tumors. After the sudden death of one patient, the protocol was amended to include 24-hour telemetry after depsipeptide infusion. Subsequently, two patients had nonsustained ventricular tachycardia and prolonged QT was noted in three patients. The trial was terminated prematurely because of cardiac toxicity. Of note, most patients had been previously treated with octreotide and received antiemetic therapy with ondansetron, both of which are associated with at least minimal QT prolongation (Shah et al., 2006).

Evidence collected to date indicates that these QTc changes may be a class effect of HDAC inhibitors (Strevel et al., 2006) with the actual incidence and severity of QTc prolongation a function of the specific dose and schedule used for each HDAC inhibitor as well as the patient selection criteria.

In May 2005, the ICH issued the E14 guideline for the clinical evaluation of QT interval prolongation and proarrhythmic potential for nonantiarrhythmic drugs (Committee IS, 2005). The Thorough QT Study (TQTS), the fundamental recommendation of ICH E14, advises that every new drug undergo clinical assessment of its repolarization effects. The TQTS characterizes a drug's impact on the QT interval over an entire dose range (including maximum tolerated dose) and anticipates interactions predisposing to QT prolongation, and if a drug is found to prolong QT, determines its safest efficacious dose (Shah, 2005). TQTS participants are intended to be healthy volunteers. QTc prolongations of 60 ms or more over baseline or more than 500 ms are considered cautionary. Applying these guidelines to early oncology drug development is not immediately clear.

In two phase II studies, the appropriate reference values of QTc interval for adult patients with hematological or solid tumors, enrolled across various protocols were evaluated (Piekarz et al, 2006; Varterasian et al., 2003). Data indicated that the distribution of QTc interval duration was greater in these patients when compared with results from a trial with similar ECG methods conducted in healthy volunteers. This implies that if exclusion criteria of 450 ms for males and 470 ms for females (Straus et al., 2006) were considered for oncology trials, more than 10% of patients would be excluded from phase I or phase II studies because of marginally prolonged QTc interval at baseline.

Because of baseline tachycardia that is often seen in hematologic malignancies, we propose using correcting the QT interval for heart rate. Because heart rate is the principal determinant of repolarization length, many correction formulae have been developed to calculate a corrected QT interval (QTc) value corresponding to a QT value normalized at a heart rate of 60 beats/min (Funck-Brentano and Jaillon, 1993). The most widely used formula, in particular by automatic devices, has been proposed by Bazett but is known to overcorrect the QT interval at high heart rate and therefore could lead to a false diagnosis of prolonged QTc interval in patients with baseline tachycardia as is often seen in hematologic malignanies (Puddu et al., 1988; Desai et al., 2003; Luo et

al., 2004). Until other QT corrections such as the Fridericia formula are available in an automated fashion on ECG machines, we will use the Bazett formula (QTcB) in this protocol.

The following drugs should be avoided if possible (http://www.azcert.org/medical-pros/drug-lists/bycategory.cfm# obtained 28-Sep-09):

Generic Name	Brand Name	Class/Clinical Use	Comments
Amiodarone	Cordarone®	Anti-arrhythmic / abnormal heart rhythm	Females>Males,TdP risk regarded as low
Amiodarone	Pacerone®	Anti-arrhythmic / abnormal heart rhythm	Females>Males,TdP risk regarded as low
Arsenic trioxide	Trisenox®	Anti-cancer / Leukemia	
Astemizole	Hismanal®	Antihistamine / Allergic rhinitis	No Longer available in U.S.
Bepridil	Vascor®	Anti-anginal / heart pain	Females>Males
Chloroquine	Aralen®	Anti-malarial / malaria infection	
Chlorpromazine	Thorazine®	Anti-psychotic/ Anti-emetic / schizophrenia/ nausea	
Cisapride	Propulsid®	GI stimulant / heartburn	Restricted availability; Females>Males.
Clarithromycin	Biaxin®	Antibiotic / bacterial infection	
Disopyramide	Norpace®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Dofetilide	Tikosyn®	Anti-arrhythmic / abnormal heart rhythm	
Domperidone	Motilium®	Anti-nausea / nausea	Not available in the U.S.
Droperidol	Inapsine®	Sedative;Anti-nausea / anesthesia adjunct, nausea	
Erythromycin	Erythrocin®	Antibiotic;GI stimulant / bacterial infection; increase GI motility	Females>Males
Erythromycin	E.E.S.®	Antibiotic;GI stimulant / bacterial infection; increase GI motility	Females>Males
Halofantrine	Halfan®	Anti-malarial / malaria infection	Females>Males
Haloperidol	Haldol®	Anti-psychotic / schizophrenia, agitation	When given intravenously or at higher-than- recommended doses, risk of sudden death, QT prolongation and torsades increases.
Ibutilide	Corvert®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Levomethadyl	Orlaam®	Opiate agonist / pain control, narcotic dependence	
Mesoridazine	Serentil®	Anti-psychotic / schizophrenia	
Methadone	Dolophine®	Opiate agonist / pain control, narcotic dependence	Females>Males
Methadone	Methadose®	Opiate agonist / pain control, narcotic dependence	Females>Males
Pentamidine	Pentam®	Anti-infective / pneumocystis pneumonia	Females>Males

Pentamidine	NebuPent®	Anti-infective / pneumocystis pneumonia	Females>Males
Pimozide	Orap®	Anti-psychotic / Tourette's tics	Females>Males
Probucol	Lorelco®	Antilipemic / Hypercholesterolemia	No longer available in U.S.
Procainamide	Pronestyl®	Anti-arrhythmic / abnormal heart rhythm	
Procainamide	Procan®	Anti-arrhythmic / abnormal heart rhythm	
Quinidine	Cardioquin®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Quinidine	Quinaglute®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Sotalol	Betapace®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Sparfloxacin	Zagam®	Antibiotic / bacterial infection	
Terfenadine	Seldane®	Antihistamine / Allergic rhinitis	No longer available in U.S.
Thioridazine	Mellaril®	Anti-psychotic / schizophrenia	

13. APPENDIX B: MEDICATION LOG

MEDICATION LOG

Subject Study ID:_____

Date to Start Taking Study Medication:_____

1. **AR-42** should be stored at room temperature in the bottle dispensed.
2. You will take **AR-42** once a day on days specified below.
3. **AR-42** should be taken in the evening on an empty stomach; at least 1 hour before or 2 hours following a meal.
4. Record the date, time, and number of capsules taken at the time you take the medication.
5. If you make a mistake, please do not scribble out or use white out to make corrections. Draw a single line through the mistake, correct the mistake, and initial next to your correction.
   Example: ~~1:00pm~~ 2:30pm JD
6. If you have any comments or notice any side effects, please record them in the Comments column.
7. Please bring this form when you return the day of your surgery along with the study medication.

Day	Date	Time	Number of Capsules Taken	Comments
1				
3				
5				
8				
10				
12				
15				
17				
19				
22				

Number of capsules dispensed_____ **Number of capsules returned_____**

14. APPENDIX C: ELIGIBILITY/SCREENING CHECKLIST

PARTICIPANT ELIGIBILITY/SCREENING CHECKLIST

Participant Name: _____ DOB: _____

Medical Record #: _____ Site: _____

Date eligibility screened: _____

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown
Male						
Female						

Please ask each question to the patient being screened and circle the answer given. If the answer indicates that the patient is not eligible, you may suspend screening at that point in the questionnaire.

1. Is the patient 18 years of age or older?
   YES (continue) NO (not eligible)

2. For which of the following does the patient meet diagnostic criteria?
   NF2-Associated Vestibular Schwannoma (continue)
   Non NF2-Associated (sporadic) Vestibular Schwannoma (continue)
   NF2-Associated Meningioma (continue)
   Non NF2-Associated Meningioma (continue)
   None of the above (not eligible)

3. Has surgery for vestibular schwannoma or meningioma been determined to be clinically necessary by the treating physician?
   YES (continue) NO (not eligible)

4. Can this patient provide written informed consent?
   YES (continue) NO (not eligible)

5. Can this patient swallow tablets?
   YES (continue) NO (not eligible)

6. Is this patient currently pregnant or breast-feeding?
   YES (not eligible) NO (continue)

7. Does this patient have a known cardiovascular disease (myocardial infarction, angina, arrhythmia) requiring treatment?
   YES (not eligible) NO (continue)

8. Does the patient have a concurrent malignancy?
YES (not eligible) NO (continue)

9. Is the patient receiving concurrent anti-neoplastic therapy (chemotherapeutics or investigational agents, radiation, immunotherapy)?
YES (not eligible) NO (continue)

10. Has the patient received any prior biologic therapy, chemotherapy, surgery or radiation?
YES (continue to question 11) NO (continue to question 12)

11. For chemotherapy or biological therapy, have 4 weeks elapsed since the last treatment and patient has recovered from all toxicities to Grade 1 or less?
YES (continue) NO (not eligible)

For surgery, have 3 weeks elapsed since last major surgical treatment and patient has recovered from surgery and any post-surgical complications?
YES (continue) NO (not eligible)

For radiation therapy, has 1 year elapsed since last treatment and patient has recovered from all toxicities to Grade 1 or less?
YES (continue) NO (not eligible)

12. Does the patient have malabsorption or any other condition that could cause difficulty in absorption of the study drug?
YES (not eligible) NO (continue)

13. Does the patient require chronic corticosteroids (dose equivalent $\geq$ 20mg prednisolone)?
YES (not eligible) NO (continue)

14. Is the patient receiving treatment for an active infection?
YES (not eligible) NO (continue)

15. Does the patient have known HIV infection?
YES (not eligible) NO (continue)

16. Does the patient have any medical condition or illness that is likely to interfere with the patient's ability to cooperate and participate in the study (follow directions, complete study evaluations, etc.)?
YES (not eligible) NO (continue)

17. Is the patient receiving any of the following concomitant medications?
YES (not eligible) NO (continue)

- HDAC inhibitors (e.g. valproic acid)
- Granulocyte colony-stimulating factors including G-CSF, pegylated G-CSF or GM-CSF
- Drugs associated with QT/QTc prolongation (review Appendix A in protocol)

18. Is the patient currently using complementary or alternative medicines that in the opinion of the principal investigator could confound the interpretation of toxicities of the study drug?
YES (not eligible) NO (continue)

19. Is the patient of childbearing potential?
YES (continue to question 20) NO (continue to 22)

20. Has the patient agreed to use birth control/contraception for the length of this trial?
YES, method_____ (continue)
NO (not eligible)

21. Does the patient have a documented negative pregnancy test?
YES (continue) NO (not eligible)

22. Does the patient have QT/QTc prolongation documented by ECG (mean QTcB > 450 msec in males and > 470 msec in females)?
YES (not eligible) NO (continue)

23. Does the patient have an Eastern Cooperative Oncology Group/World Health Organization (ECOG/WHO) performance status of 0-1?
YES (continue) NO (not eligible)

24. Please provide documentation of the following (if any of these lab results are outside of the listed ranges or cannot be documented, then the patient is not eligible for the study):

- Leukocytes $\geq$ 3,000/mcL
- Absolute neutrophil count $\geq$ 1,500/mcL
- Platelets $\geq$ 100,000/mcL
- Total bilirubin < 1.5 mg/dL
- AST(SGOT)/ALT(SGPT) $\leq$ 5x institutional upper limit of normal (ULN)
- Creatinine $\leq$ 1.5x ULN, OR creatinine clearance $\geq$ 50 mL/min by MDRD (original or abbreviated), OR measured creatinine clearance $\geq$ 50 mL/min
- Hemoglobin of at least 9 g/dL
- Normal serum magnesium (1.6 – 2.6 mg/dL), calcium (8.6 – 10.0 mg/dL), potassium (3.5 – 5.1 mmol/L) levels

*If screening questionnaire indicates that the patient is eligible for the study, please proceed to obtaining informed consent. Once consent is obtained, please refer to the Study Manual for directions on enrolling the patient in the study.

Eligibility screening completed by: _____
(printed name)

_____ Date:_____
(signature

15. APPENDIX D: ELIGIBILITY/SCREENING LOG

PARTICIPANT ELIGIBILITY LOG

Site: _____ Page ____ of ____

In order to track enrollment numbers, please record all potential participants that are eligible for the study. Submit this form quarterly to the Central Study Office at The Massachusetts Eye and Ear Infirmary.

Participant Initials	Study ID #	Diagnosis NF2/non-NF2)	Date Screened	Eligible (Y/N)	If No, Why Not?	Screened By:
Example: A.B.	Example: 10006	Example: NF2 VS, MEN	Example: 01/01/01	Example: Y	Example: Pregnant	Example: John Smith