ORIGINAL RESEARCH

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Early phase clinical studies of AR-42, a histone deacetylase inhibitor, for neurofibromatosis type 2-associated vestibular schwannomas and meningiomas

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Abstract

Objectives: Two pilot studies of AR-42, a pan-histone deacetylase inhibitor, in human neurofibromatosis type 2 (NF2), vestibular schwannomas (VS), and meningiomas are presented. Primary endpoints included safety, and intra-tumoral pharmacokinetics (PK) and pharmacodynamics (PD).

Methods: Pilot 1 is a subset analysis of a phase 1 study of AR-42 in solid tumors, which included NF2 or sporadic meningiomas. Tumor volumes and treatment-related adverse events (TRAEs) are reported (NCT01129193).

Pilot 2 is a phase 0 surgical study of AR-42 assessing intra-tumoral PK and PD. AR-42 was administered for 3 weeks pre-operatively. Plasma and tumor drug concentrations and p-AKT expression were measured (NCT02282917).

Results: Pilot 1: Five patients with NF2 and two with sporadic meningiomas experienced a similar incidence of TRAEs to the overall phase I trial. The six evaluable patients had 15 tumors (8 VS, 7 meningiomas). On AR-42, tumor volume increased in six, remained stable in eight, and decreased in one tumor. The annual percent growth rate decreased in eight, remained stable in three, and increased in four tumors. Pilot 2: Four patients with sporadic VS and one patient with meningioma experienced no grade 3/4 toxicities. Expression of p-AKT decreased in three of four VS. All tumors had higher AR-42 concentrations than plasma.

Conclusions: AR-42 is safe. Tumor volumes showed a mixed response, but most slowed growth. On a 40-mg regimen, drug concentrated in tumors and growth pathways were suppressed in most tumors, suggesting this may be a well-tolerated and effective dose. A phase 2 study of AR-42 for NF2-associated tumors appears warranted.

Level of Evidence: 1b, 4.

KEYWORDS
AR-42, histone deacetylase inhibitor, meningioma, neurofibromatosis type 2, vestibular schwannoma

1 | INTRODUCTION

1.1 | Neurofibromatosis type 2 is a debilitating disease

Neurofibromatosis type 2 (NF2) is a highly penetrant, autosomal dominant disorder with an incidence of approximately 1/40 000.[1,2] The mean age at presentation is 20-21 years.[3] Patients develop multiple nervous-system tumors, primarily bilateral vestibular schwannomas (VS), but up to 60% also develop meningiomas.[4] Mutations in the *NF2* tumor suppressor gene cause schwannomas and meningiomas to arise from Schwann cells, and meningothelial or arachnoid cap cells, respectively.

VS disrupt cranial nerve VIII resulting in tinnitus, hearing loss, and vestibular disequilibrium. Deafness and facial nerve paralysis lead to social isolation and communication challenges. Meningiomas or lower cranial nerve schwannomas can cause aspiration, cranial nerve paralysis, and brainstem compression. NF2 shortens life expectancy, particularly in patients with truncating *NF2* mutations or meningiomas.[5,6] Sporadic VS and meningiomas may cause similar debilitating symptoms. Meningiomas are the most common intracranial tumor, constituting 38.2% of all primary intracranial tumors.[4]

1.2 | Improved therapy for VS and meningiomas is urgently needed

Current treatment options for NF2-associated tumors are limited to observation, surgery, or stereotactic radiation.[7] Given the risk of secondary malignancy, radiation is seldom used first-line in children,[8,9] but may be considered in adults. Meningiomas are less radiosensitive than VS, but radiation is still utilized when surgical resection is incomplete or impractical.[10] If tumors grow following radiation, surgical tumor removal from cranial nerves and the brainstem may be complicated by adhesion to adjacent neural structures.

Surgical resection of VS and meningiomas may be necessary to relieve brainstem compression and threat of acute hydrocephalus. Hearing and facial nerve preservation may not be possible. Complications can also include brainstem injury, cerebrospinal fluid (CSF) leak, meningitis, hemorrhage, pneumocephalus, and death. Lower cranial nerve palsies and shared blood supply to the brainstem are especially hazardous. Up to 30% of patients have recurrence following resection of meningiomas.[10] Surgical intervention in a genetic disease is, at best, a temporizing treatment.

No FDA-approved drugs are available for the treatment of NF2-associated tumors despite numerous trials.[11-21] Off-label, bevacizumab has been most widely used, showing decreased VS volumes and hearing improvement in 30%-50% of patients,[14,22] but toxicities prevent long term use. Additionally, meningiomas do not respond to bevacizumab.[23] It is evident that current treatment options for NF2-associated tumors are inadequate.

1.3 | Development of new therapies for NF2 has been difficult

Development of targeted treatments for NF2-associated VS and meningiomas has been challenging in both preclinical and clinical studies. Primary VS and meningioma cells in culture have a limited lifespan and mouse xenograft models of VS do not show consistent

growth.[24,25] Genetically engineered mice with *Nf2*-knockout in Schwann cells do not consistently produce VS.[26,27] Two meningioma mouse models have been successful. *Nf2* inactivation in arachnoidal cells or meningeal precursor cells leads to meningioma in mice[28,29] and *NF2*-deficient Ben-Men-1 cells expressing luciferase have been successfully implanted into the inner table of SCID mice crania.[30]

Clinical trials are difficult to perform because there are relatively few patients with NF2, endpoints must be selected carefully, and any drug candidate must be well tolerated to be considered for long courses of suppressive treatment in this slowly progressive disease in a young patient population.

1.4 | Histone deacetylase inhibitors suppress tumor growth

To assess the activity of 19 small molecules in NF2, isogenic VS- and meningioma-related cell lines with or without *NF2* expression were used. Two of the agents with greatest activity across schwannoma and meningioma cell models were HDAC inhibitors (HDACis): panobinostat, a pan-HDACi, and CUDC-907, a PI3K and HDAC inhibitor.[31]

The PI3K/AKT pathway is activated in human VS and meningiomas.[32-37] Since it is a convergence point for many growth stimuli, and it controls downstream cellular processes such as cell survival, cell proliferation, insulin response, stress response, and differentiation,[38] the PI3K/AKT pathway is an attractive therapeutic target for VS.

AR-42 is an orally bioavailable pan-histone deacetylase inhibitor (HDACi) with tumor inhibitory effects on schwannomas and meningiomas in vitro and in vivo.[39,40] AR-42, also known as REC-2282 or OSU-HDAC42, was formerly licensed by Arno Therapeutics, Parsippany, NJ and now is licensed by Recursion Therapeutics, Salt Lake City, UT. AR-42 was shown to inhibit the growth of human VS and *Nf2*-deficient mouse schwannoma cells with a half-maximal inhibitory concentration (IC_{50}) of 500 nM and 250-350 nM, respectively.[39] It also inhibited primary meningioma cells and *NF2*-deficient benign meningioma Ben-Men-1 cells with IC_{50} values of 1.5 and 1.0 μM, respectively. AR-42 induced cell-cycle arrest at G_2/M, triggered apoptosis, and decreased p-AKT levels in both VS and meningioma cells. In vivo AR-42 inhibited the growth of schwannoma xenografts, induced apoptosis, and decreased AKT activation. Additionally, AR-42 markedly diminished meningioma tumor volumes in a Ben-Men-1 xenograft mouse model[40] and tumors did not return when followed 6 months post-treatment. Collectively, these results demonstrate a high potency of AR-42 against NF2-associated VS and meningiomas.

In a first-in-human, phase I, open-label, 3 + 3 dose-escalation study, AR-42 was investigated for safety and tolerability in patients with multiple myeloma, and T- and B-cell lymphomas.[41] The maximum tolerated dose (MTD) was 40 mg three times weekly for 3 weeks of a 28-day cycle. Subsequently, a phase I, 3 + 3 dose-escalation study was performed with single-agent AR-42 in a cohort of patients with advanced solid tumors to evaluate safety, the MTD, pharmacokinetics (PK), and preliminary clinical activity.[1] The study enrolled 5 patients

with NF2, 2 patients with sporadic non-NF2 meningiomas, and 10 patients with various solid tumor malignancies. Patients received AR-42 30, 60, or 80 mg orally three times weekly for 3 weeks of a 28-day cycle. The two dose-limiting toxicities (DLTs) were grade 3 thrombocytopenia and grade 4 psychosis. The recommended phase II dose was 60 mg daily, three times weekly for 3 weeks of a 28-day cycle. The most common treatment-related adverse events (TRAEs) were cytopenias, fatigue, and nausea. The best response was stable disease in 53% of patients (95% CI: 26.6-78.7). A post-hoc subset analysis of the patients with NF2 and non-NF2 meningiomas showed a median progression-free survival of 9.1 months (95% CI: 1.9—not reached) by RECIST criteria. It was concluded that AR-42 was safe and well tolerated, and that further studies may be considered in combination with other agents in advanced solid tumors.[1]

Here, we present two early-phase in-human clinical studies of AR-42 in patients with NF2 and sporadic meningiomas and VS. Pilot 1 is a post-hoc subset analysis of the patients with NF2-associated tumors and sporadic meningiomas from Collier et al.[1] Pilot 2 is a phase 0 surgical study to determine intra-tumoral PK and pharmacodynamics (PD) based on blood samples and surgically resected meningiomas and VS.

2 | MATERIALS AND METHODS

2.1 | Pilot 1

2.1.1 | Study design

Pilot 1 is a post-hoc subset analysis of the open label phase I trial noted above of AR-42 in patients with advanced solid tumors, including NF2-associated VS and meningiomas, to determine safety and tolerability of AR-42 in this patient population.[1] The secondary objective was to analyze change in tumor volumes with AR-42. This was a 3 + 3 dose escalation study starting at 30 mg orally three times weekly for 3 weeks followed by 1 week off every 28-day cycle, as previously published. Toxicities were graded based on the Common Terminology Criteria for Adverse Events v4.0. AR-42 was provided by Arno Therapeutics (Parsippany, NJ). The study was approved by the Ohio State University Institutional Review Board (Protocol 2010C0006, approved 3/24/2010) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

2.1.2 | Eligibility criteria

To enroll, patients with CNS tumors had to be at least 18 years old and have NF2-associated or sporadic VS or meningiomas with ≥15% volumetric growth of at least one tumor on pre-enrollment MRI. Additional requirements were an Eastern Cooperative Oncology Group (ECOG) performance status 0-1, normal organ function, and life expectancy >12 months. Exclusion criteria included chemotherapeutic intervention within 4 weeks, major surgery within 3 weeks, malabsorption, or pregnancy.

2.1.3 | Tumor volumetric measurement

Response was monitored with magnetic resonance imaging (MRI). Three-dimensional tumor volumes were measured at least as frequently as (a) within 2 years prior to study entry; (b) at the time of study entry; (c) at end of study; and (d) post-treatment up to 2 years. Volumetric measures were created using Visage 7 Enterprise Imaging software (version 7.1.15; San Diego, CA) with manual and auto-segmentation. Additional contouring in the axial, sagittal, and coronal planes and 3-D image overlay for verification of segmentation accuracy were performed.

Percent tumor volume change was calculated between the start and end of treatment. By the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS)[42] criteria for tumor assessment with MRI and volumetric analysis, a 20% volume decrease indicates a partial response, and a 20% volume increase indicates progressive disease. Estimated annual percent growth rates were calculated by dividing the percent volume change by the time between measurements in years. The pre-treatment, on-treatment, and post-treatment annual percent growth rates were compared with a Wilcoxon matched-pairs signed-rank test using R version 3.6.0. A nominal P-value ≤.05 was considered statistically significant.

2.2 | Pilot 2

2.2.1 | Study design

Pilot 2 is a phase 0 study of subjects undergoing planned surgical resection of VS or meningioma to evaluate tumor penetration by drug and intra-tumoral growth pathway suppression. The primary objective was to measure expression levels of p-AKT in VS and meningiomas compared with control untreated VS and meningioma tissue samples from our tissue bank. Secondary objectives included measurement of plasma and intra-tumor AR-42 concentrations. Subjects took AR-42 40 mg orally three times per week for 3 weeks prior to tumor resection. The study was started prior to completion of the solid tumor phase I trial; therefore, 40 mg was selected based on the MTD in the phase 1 study of AR-42 for hematologic malignancies.[41] Study drug was provided by Arno Therapeutics. The study was approved by the Massachusetts Eye and Ear Institutional Review Board (Protocol 2018A019856, approved 10/01/2014) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

2.2.2 | Eligibility criteria

To enroll, patients with a VS and/or meningioma diagnosed by MRI with planned surgical resection had to be greater than 18 years of age and have normal organ and bone marrow function. ECOG performance status of 0-1 and the ability to swallow capsules were required. Females of childbearing potential were required to have a negative serum or urine pregnancy test and be willing to comply with contraception. Exclusion criteria included pregnancy, breastfeeding, malabsorption, chronic corticosteroid use, second malignancy, prolonged QT, active infection, anti-neoplastic systemic therapy, or concomitant radiotherapy.

2.2.3 | Pharmacokinetics and pharmacodynamics

Venous blood samples were collected at the time of induction of anesthesia and again at tumor removal, about 3-4 hours from surgery start. Plasma samples were prepared by centrifugation at 4°C. Upon tumor removal, each VS was divided into a peripheral capsule (P) and a tumor center (C). Plasma and tumor samples were rapidly frozen and kept at −70°C until PK analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at The Ohio State University Comprehensive Cancer Center Pharmaco-analytical Shared Resource, as previously described.[25] Plasma and tumor AR-42 concentrations were estimated by comparison to a standard curve of known drug concentrations. Serum levels were compared using a t-test with $P \leq .05$ considered statistically significant.

The Supplementary Methods describe in detail the preparation of tumor tissue lysates and quantitation of pAKT, total AKT, p-S6, total S6, p-ERK, and total ERK using fluorescent Western blotting.

3 | RESULTS

3.1 | Pilot 1

3.1.1 | Patients

Five patients with NF2 and two patients with sporadic meningiomas were enrolled between June 2012 and November 2013. Demographics are shown in Table 1. Only one patient (Patient 1.5) had previously received radiation. Enrolled patients received AR-42 either 60 mg (n = 6) or 80 mg (n = 1) orally three times weekly for 3 weeks followed by 1-week off every 28-day cycle. Median duration of treatment was 109 days (range 11-838), about 4 cycles. Patient 1.3 was non-compliant and was dismissed from the study after only six doses, and thus was not evaluable for tumor volume change. Patient 1.5 withdrew consent for mild toxicities after 3 months. Patient 1.7 did not complete cycle 1 due to a DLT and was included only in the safety analysis of the reported phase 1 trial, though tumor volumetric analysis is presented here. The remaining four patients discontinued due to progression.

3.1.2 | Safety and tolerability

Seven patients with NF2 or sporadic meningioma were evaluable for toxicity. Collier et al previously reported the adverse events for the full phase I trial.[1] Table 2 shows the TRAEs for only the subset of

TABLE 1 Patient demographics

Pilot	Patient #	Age	Sex	Weight (kg)	Ethnicity	Diagnosis	Dose (mg)	Days on AR-42	Number of doses
1	1.1	33	F	76.2	Caucasian	NF2	60	838	234
	1.2	48	F	78.5	Caucasian	NF2	60	85	26
	1.4	28	M	76.2	Caucasian	NF2	60	276	89
	1.5	59	F	55.8	Caucasian	Sporadic meningioma	60	133	33
	1.6	20	F	62.2	Caucasian	NF2	60	59	18
	1.7	20	F	47.6	Asian	NF2	80	11	5
2	2.1	69	F	97.1	Caucasian	Sporadic VS	40	21	9
	2.2	40	M	120	Caucasian	Sporadic VS	40	21	11
	2.3	64	M	100.7	Caucasian	Sporadic VS	40	21	9
	2.5	30	M	90.7	Caucasian	Sporadic VS	40	21	8
	2.7	64	M	82.6	Caucasian	Sporadic meningioma	40	21	8

Abbreviations: NF2, neurofibromatosis type 2; VS, vestibular schwannomas.

patients with NF2 and sporadic meningioma. The most common TRAEs of any grade were thrombocytopenia (n = 7), nausea (n = 6), fatigue (n = 6), and diarrhea (n = 6). One of the two DLTs in the phase I study was a grade 4 psychiatric disorder, possibly related to AR-42, at the 80 mg dose level in Patient 1.7 with NF2. This DLT occurred after 10 days of treatment, or 5 doses. Drug was terminated and she came off study. Her psychosis resolved with termination of AR-42, initiation of an anti-psychotic and a benzodiazepine, and treatment of a urinary tract infection. One patient (Patient 1.5) required a dose reduction for fatigue, which was ultimately attributed to hypophysitis from previous cranial irradiation. There were no reported arrhythmias or QT prolongations of any grade. AR-42 was generally well tolerated at 60 mg among the patients with NF2 or sporadic meningioma, though the incidence of toxicities may not be acceptable for chronic use in this young patient population.

3.2 | Efficacy

Tumor volumes and annual percent growth rates prior to entry on study, while on study, and following drug termination are demonstrated in Figure 1. Six patients were evaluable for response and together had 15 evaluable tumors (8 VS and 7 meningiomas). Pre-treatment, 13 of 15 tumors demonstrated volumetric growth of 20% or more as shown in Table S1. As shown in Table 3 and Figure S1, while on AR-42, six tumors showed volume growth of 20% or greater, eight showed stable disease, and one showed a partial response with a 24% decrease in volume. There was no appreciable difference between the response of VS and meningiomas to AR-42. Five VS were stable and three progressed. Four meningiomas were stable or regressed, and three progressed. Analysis of the change in the estimated annual rate of tumor growth from pre-treatment to on-treatment showed that eight tumors had reduced growth rates (>20% decrease in growth rate), three had stable growth rates (± <20% change in growth rate) and four had increased growth rates (>20% increase in growth rate).

The median reduction in annual growth rate was −54%, but the pre-treatment and on-treatment growth rates were not significantly different ($P = 0.259$).

After termination of AR-42, four tumors were not available, two because of surgery and two because long-term follow-up was not available. Two patients had progression requiring a tumor to be excised shortly after coming off study. The tumor excised from Subject 1.6 was a WHO-grade II meningioma. Subject 1.4 had his left VS re-excised and the adjacent stable posterior fossa meningioma was also removed, found to be WHO-grade I. Of the 11 remaining evaluable lesions, when compared to growth rates on AR-42, 4 tumors rebounded in growth rate (increased more than 20%), 4 were not changed, and 3 decreased. Pre-treatment (median = 60%) to post-treatment (median = 10%) estimated annual growth rates were not statistically different ($P = .083$). Nor did on-treatment (median = 22%) to post-treatment estimated growth rates differ ($P = .760$).

Though preservation of hearing was not a prespecified endpoint, standard of care audiograms were monitored for the five patients with NF2. The two patients with meningiomas did not have involvement of the eighth cranial nerve and were not monitored with audiograms. Audiometric results are summarized in Table S2. Subject 1.2 was profoundly deaf in both ears prior to study initiation and no change was noted with treatment. Three patients (1.1, 1.4, and 1.7) had profound deafness in one ear with measurable hearing in the contralateral ear that remained stable on AR-42. Of note, Patients 1.1 and 1.4 had radiographically stable disease on AR-42 and remained on treatment for approximately 27.5 and 9 months, respectively. Patient 1.7 only received AR-42 briefly prior to experiencing a DLT in cycle 1. Conversely, Patient 1.6, who had radiographic progression after 2 months on AR-42, also had a decline in hearing while on treatment from good hearing in both ears at initiation of the study with 17 db pure tone average (PTA) to 55 db PTA with a loss of discrimination from 100% to 0%. Though the numbers are small in this post hoc analysis, it appears that preservation of hearing was associated with radiographic assessment.

TABLE 2 Treatment-related adverse events

Adverse event	Pilot 1 (60-80 mg)					Pilot 2 (40 mg)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total	Grade 1	Grade 2	Grade 3	Grade 4	Total
Thrombocytopenia	1	5	1		7	3				3
Fatigue	2	3	1		6	3				3
Nausea	5		1		6	2				2
Diarrhea	5		1		6	1				1
Anemia	3	2			5	2				2
Increased creatinine	3	2			5					
Leukopenia	4				4	2	1			3
Neutropenia	2	2			4	1				1
Anorexia	2	1	1		4					
Confusion	3				3	4				4
Lymphopenia		2	1		3	1				1
Myalgia	3				3	1				1
Hypoalbuminemia	2	1			3					
Low serum phosphate	1	1	1		3					
Taste change	2				2	1				1
Hypocalcemia	2				2					
High alanine aminotransferase (ALT)	2				2					
Increased INR	1		1		2					
Weight loss	1		1		2					
Emesis	2				2					
Constipation	2				2					
Urinary frequency	2				2					
Hypernatremia	2				2					
Headache		1			1	1	2			3
Dyspepsia	1				1	2				2
Facial weakness	1				1	2				2
Rash	1				1	1				1
Hypokalemia	1				1					
High aspartate aminotransferase (AST)	1				1					
Psychotic disorder			1		1					
Vestibular disorder		1			1					
Hematuria	1				1					
Urinary tract infection		1			1					
Upper respiratory infection		1			1					
Thrombotic event			1		1					
Leukocytosis						2				2
Non-cardiac chest pain						2				2
CSF leak							2			2
Lymphocytosis							1			1
Tremor						1				1
Scleral disorder						1				1

3.3 | Pilot 2

3.3.1 | Patients and tolerability

Six patients with sporadic VS and one patient with sporadic meningioma enrolled between 2014 and 2017. Two patients with VS (2.4 and 2.6) withdrew prior to beginning AR-42 treatment. Demographics are shown in Table 1, and TRAEs are illustrated in Table 2. Two patients (2.5 and 2.7) had thrombocytopenia (<100K platelets) preoperatively, but withholding the last dose of AR-42 prior to surgery resulted in rapid recovery to a normal platelet count, and surgery proceeded as scheduled. Two subjects had postoperative CSF leaks. There were no arrhythmias or QT changes.
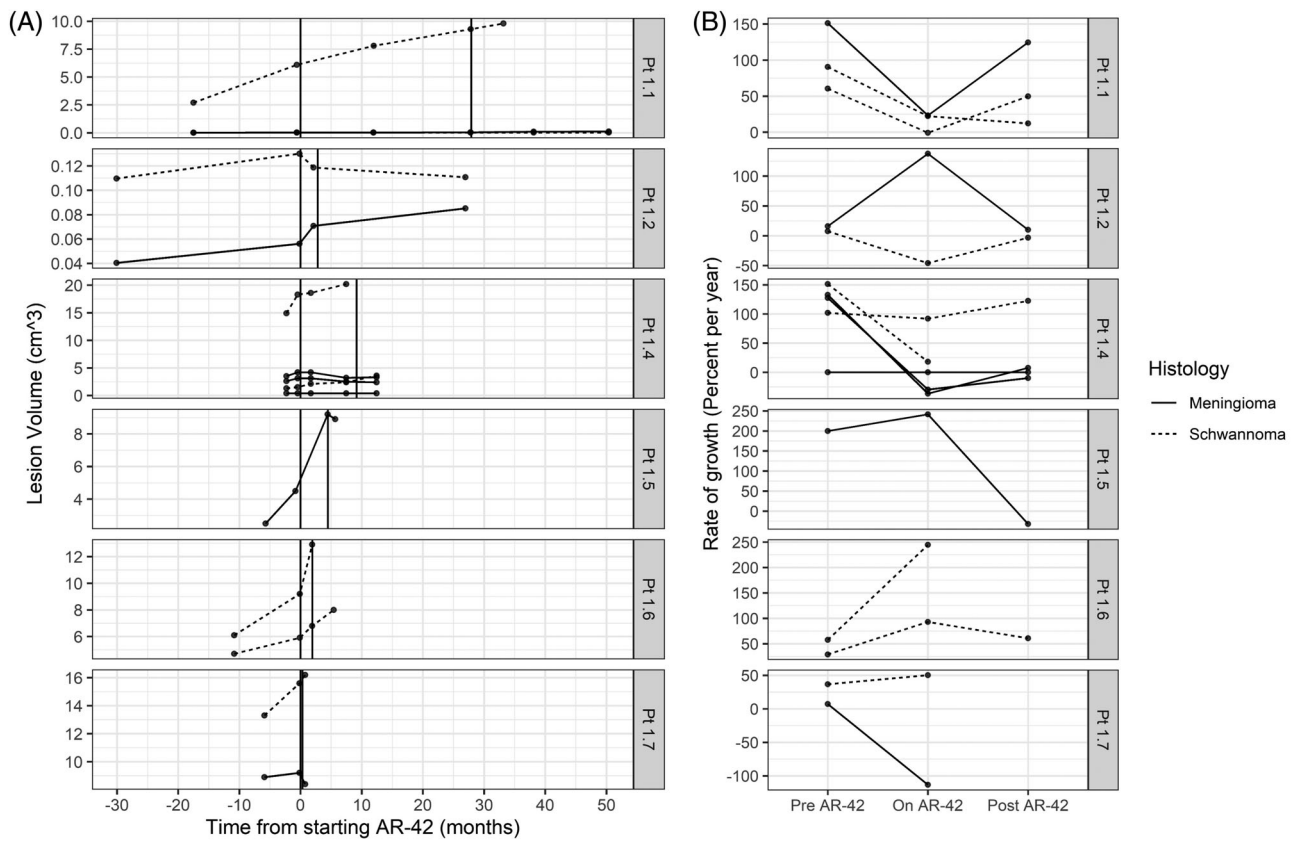
FIGURE 1 Pilot 1—Change in tumor volumes and annual rate of tumor growth. A, Change in 3-D tumor volume in cm³ over time. Vertical black lines indicate initiation and discontinuation of AR-42. B, Annual estimated rate of growth, in percent per year, pre-treatment, on AR-42, and post-treatment

TABLE 3 Pilot 1—Summary of volumetric tumor growth and annual estimated growth rates

Patient	Days on AR-42	Tumor location	Histology	% Volume growth on Rx	On Rx response	Est. annual growth rate Pre Rx (%)	On Rx (%)	Post Rx	Change in GR Pre to On Rx (%)	Change in GR Pre to post Rx
1.1	837	Right IAC	Schwannoma	−2	SD	60	−1	50%	−61	−10%
		Left parieto-occipital	Meningioma	54	PD	151	23	125%	−128	−26%
		Right neck	Schwannoma	52	PD	91	22	12%	−69	−79%
1.2	84	Left trigeminal	Schwannoma	−9	SD	8	−46	−3%	−54	−11%
		Right intradural C1	Meningioma	26	PD	16	138	10%	122	−6%
1.4	275	Left IAC	Schwannoma	60	PD	102	92	122%	−10	20%
		Left posterior fossa	Meningioma	−24	PR	133	−37	8%	−170	−125%
		Let foramen magnum	Meningioma	−19	SD	128	−30	−10%	−158	−138%
		Right IAC	Schwannoma	9	SD	151	18	Surgery	−133	N/A
		Right anterior falx	Meningioma	0	SD	0	0	0%	0	0%
1.5	134	Right petroclinoid	Meningioma	104	PD	200	241	−32%	41	−232%
1.6	58	Left IAC	Schwannoma	15	SD	29	93	61%	64	32%
		Right IAC	Schwannoma	40	PD	58	245	Surgery	187	N/A
1.7	10	Left IAC	Schwannoma	4	SD	37	50	N/A	13	N/A
		Left prepontine cistern	Meningioma	−9	SD	7	−113	N/A	−120	N/A

Abbreviations: C1, cervical level 1; Est., estimated; GR, growth rate; IAC, internal auditory canal; NA, not applicable; PD, progressive disease (>20% increase in volume); PR, partial response (>20% decrease in volume); Rx, AR-42; SD, stable disease.

TABLE 4 Pilot 2—Plasma and tumor concentrations of AR-42

AR-42	Concentration in plasma (nM)				
Patient #	2.1	2.2	2.4	2.6	2.7
Before surgery	362.3	224.2	61.8	3.5	99.1
At tumor removal	303.7	121.1	36.8	3.4	72.9
Concentration in tumors (nM)					
Capsule	624.2	429.4	230.6	16.2	5296.6
Center	577.0	586.6	289.0	7.1	1766.2
Capsule/plasma	1.72	1.87	3.73	4.67	53.4
Center/plasma	1.59	2.62	4.68	2.05	17.8

Note: Nanomolar (nM) drug concentrations were measured in plasma pre-operatively before anesthesia and at tumor removal. AR-42 concentration was measured in the peripheral capsule and center of each tumor. The tumor to plasma ratios of AR-42 were calculated by dividing the drug concentration for the tumor capsule or center by the corresponding pre-anesthesia plasma concentration. Plasma and tumor concentrations listed are the mean of three trials. The VS and meningioma exhibited higher concentrations of AR-42 than did the plasma samples.
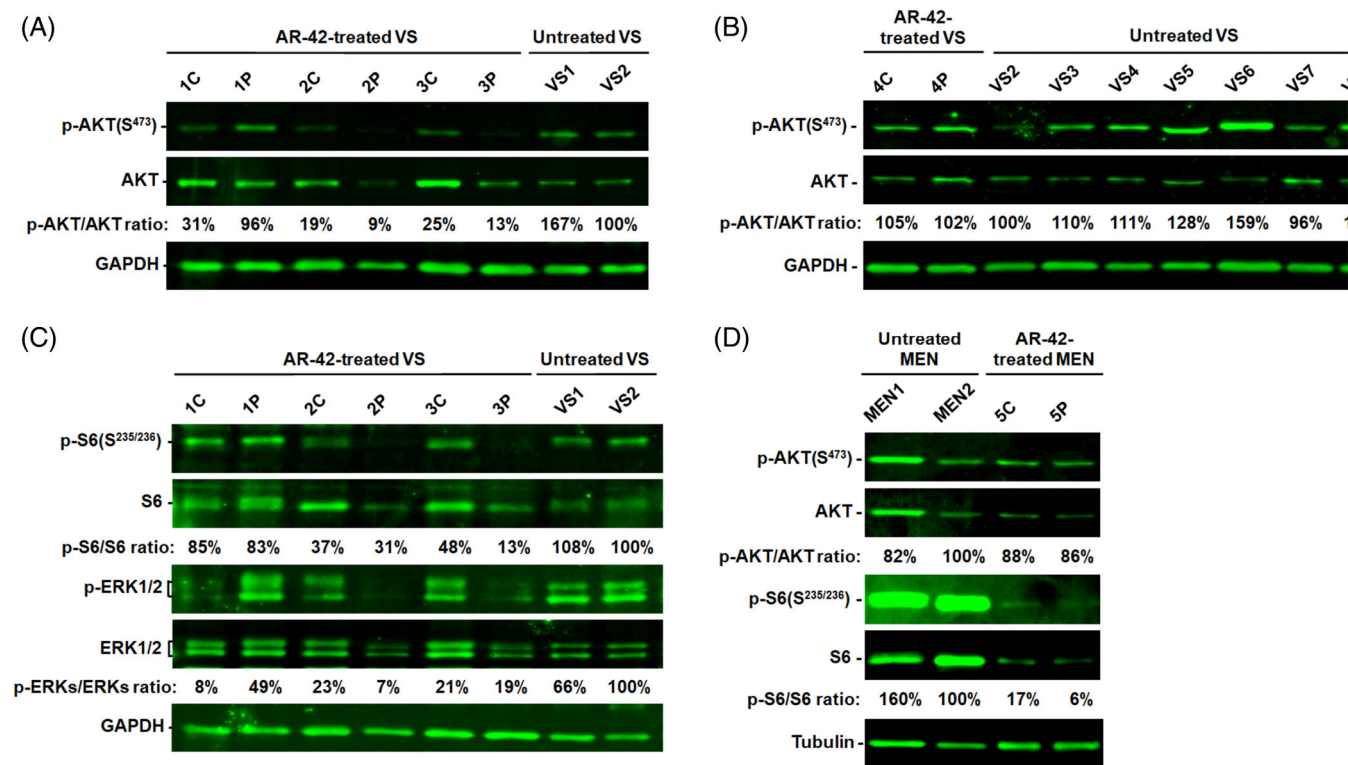

FIGURE 2 Pilot 2—AR-42 suppressed the AKT and ERK pathways in VS. A, AR-42 (or REC-2282) decreased p-AKT levels in three of four treated VS compared to untreated tumors VS1 and VS2. B, Patient 2.4 did not have reduced p-AKT in the tumor, compared with seven untreated VS controls. Quantitation of the normalized p-AKT/AKT ratio is depicted as percentage relative to the untreated VS2 set as 100%. C, AR-42-treated VS also showed reduced levels of p-S6, downstream of the AKT/mTOR pathway, as well as p-ERKs. Quantitation shown is a percentage of p-S6/S6 or p-ERKs/ERKs relative to VS2 set as 100%. D, Suppression of p-S6 was also observed in an AR-42-treated meningioma compared to untreated tumors (MEN1 and MEN2); however, reduction of p-AKT was not detected. Shown below the blot is the relative percentage of p-AKT/AKT or p-S6/S6 relative to the untreated MEN2 set as 100%. C, core of tumor; MEN, meningioma; P, periphery of tumor; VS, vestibular schwannoma

3.3.2 | Plasma and tumor PK/PD

Table 4 shows plasma drug concentrations before and at the time of tumor removal. For PK analysis, AR-42 was stable in blood kept on ice for more than 4 hours (Table S3). In the first three patients with VS, the plasma concentration of AR-42 ranged from 0.062 to 0.36 μM and remained stable before and at the time of tumor removal. There was no statistically significant difference between the preoperative serum levels and serum levels at the time of tumor removal by paired *t* test with $P = .07$. The concentrations of AR-42 in VS ranged from

0.23 to 0.62 μM. AR-42 appeared to concentrate in three of the four VS tumors, with similar drug levels observed in the center and capsule of each tumor. A fourth patient with VS exhibited very low plasma levels of AR-42. This patient had withheld the final dose of AR-42 prior to surgery to allow recovery of thrombocytopenia. The plasma levels of AR-42 in the patient with meningioma were 0.099 and 0.073 μM before and after anesthesia, respectively. Interestingly, the concentrations of AR-42 in the meningioma reached high concentrations in the tumor center (1.8 μM) and capsule (5.3 μM). This analysis was repeated in triplicate to verify the unexpectedly high AR-42 levels compared to VS tumors. The results indicate that AR-42 may concentrate in VS and meningioma tumors. In our previous in vitro studies, we had found an IC_{50} of ~0.5 μM for schwannomas and ~1 μM for meningiomas.[39,40] Thus, the tumor concentrations of AR-42 reached likely effective doses in three of four VS and the meningioma.

In line with these results, we observed decreased p-AKT levels in three of four AR-42-treated VS tumors, particularly in the center of tumors (Figure 2A,B). The levels of p-ERKs and the downstream target of the AKT/mTOR pathway, p-S6, were also substantially reduced in three of four AR-42-treated VS (Figure 2C). However, the VS in the fourth AR-42-treated patient did not show reduction in p-AKT, which is consistent with the observed low concentrations of drug in the plasma. Drug logs indicate that this patient took 8 of 10 planned doses. Similarly, the meningioma from Patient 2.7 also showed reduction in p-S6 (Figure 2D). Surprisingly, p-AKT was not reduced in this tumor compared with untreated meningioma tumors, despite high tumor drug concentrations. AR-42 also reduced AKT phosphorylation in primary human VS cells (Figure S2). Taken together with the PK data, these results suggest that AR-42 decreases p-AKT, p-ERK, and p-S6 in the majority of treated tumors.

4 | DISCUSSION

These pilot studies found AR-42 had a tolerable profile for patients with NF2 and NF2-related tumors. Overall toxicity was not different from other patients with solid tumors in the parent study. The most common TRAEs of any grade in the two pilot studies combined were thrombocytopenia, fatigue, and nausea. This is consistent with the phase I study of AR-42 in hematologic malignancies, where the most common adverse events were cytopenias.[41] Thrombocytopenia occurred in all Pilot 1 subjects and 2 of our 5 Pilot 2 subjects. Only one patient (1.7) reached grade 3 thrombocytopenia and no bleeding episodes occurred. Two patients in Pilot 2 had mild pre-operative thrombocytopenia that improved with dose hold. One patient in Pilot 1 required a dose reduction for fatigue, ultimately not attributed to AR-42. The only adverse event leading to discontinuation of treatment was grade 4 psychosis in one patient in Pilot 1, which may have been attributable to AR-42, steroids, anti-emetics, or an underlying mood disorder. Notably, this patient received the highest dose of 80 mg, and also had the smallest body weight. Collier et al noted that higher maximum serum concentrations of AR-42 and median area-

under-the-curve (AUC) were associated with an increase in grade 3 and 4 toxicities.[1] The mean maximal plasma concentration of AR-42 for patients who received the 60 mg dose on day one was 1.48 (±0.38) μM, but for Patient 1.7, who received the 80 mg dose on day one, the maximum plasma concentration was 3.25 μM.[1] However, since only two patients in the phase I study received 80 mg, no conclusions can be drawn. Notably, in Pilot 2 where patients only received 3 weeks of AR-42 at 40 mg, there were no grade 3 or 4 toxicities.

In Pilot 2, two subjects had postoperative CSF leaks, which is higher than the usual rate of 5%. This is of uncertain significance and was not thought to be related to AR-42. If a phase 2 study is initiated, consideration should be given for a pre-operative washout period. There were no other surgical side effects seen; in particular, no intra- or post-operative bleeding was noted. No QT prolongation or cardiac toxicity occurred in any patient in either pilot. Early concerns of QT prolongation and cardiac toxicity from HDACis[43] have failed to show clinically relevant drug-induced QTc changes.[44,45] Nonetheless, further due diligence with QT monitoring is warranted with AR-42.

Overall, there was variable volumetric response of tumors, even within patients. Of note, the volumetric measurements are from a post-hoc, subset analysis of a phase I trial, which was not designed or powered to show efficacy. Although tumor volumes showed that only one tumor (7%) had a partial response, eight others (53%) remained stable. The partial response, which was the posterior fossa meningioma of Patient 1.4, was durable, lasting 3 years. Looking at estimated percent annual growth rates, eight tumors (53%) slowed their growth and three (20%) had stable growth. Overall, this suggests possible anti-tumor activity. There was no appreciable difference between the response of VS and meningiomas. A larger phase II trial of AR-42 for sporadic or NF2-associated VS and meningiomas is needed to adequately evaluate efficacy. In Pilot 1, three patients had initially stable disease, then had progression months later, suggesting that they developed resistance to AR-42. The mechanism of resistance to AR-42 in NF2 or sporadic meningioma is not known. The variability of response and developed resistance to AR-42 may occur at a genomic, expression, epigenetic, or microenvironment level, or may be due to variability of drug delivery, and needs further exploration.

In Pilot 2, AR-42 given at 40 mg for 3 weeks pre-operatively appeared to concentrate in tumors at levels sufficient to suppress the AKT/PI3K pathway in most tumors. Intra-tumoral drug concentrations at or about the IC_{50} were reached. Subjects 2.6 and 2.7 are noteworthy, both who held the last dose for thrombocytopenia. Subject 2.6 had plasma and tumor levels of AR-42 which were 10 to 100-fold less than tumor levels in the other four subjects; however, the ratios of drug in tumor to plasma were similar to the other subjects with VS. Interestingly, the WHO grade I meningioma in subject 2.7 concentrated AR-42 at levels 10-fold higher than the VS. Although AR-42-treated VS exhibited reduced levels of p-AKT and p-S6, the treated meningioma only showed decreased p-S6. One possible explanation could be feedback signaling, resulting in the reactivation

of p-AKT.[46-48] Alternatively, p-AKT may be more liable to dephosphorylation in meningioma than VS tissues during biopsy specimen storage. Although *NF2* mutations disrupt the merlin pathway in both tumor types, the mechanism of tumor growth appears to be different by kinome analysis.[31] Drug combinations will likely be necessary to suppress alternative growth pathways and adequately treat both NF2-associated tumor types. Further, preclinical studies and early phase clinical trials (NCT00731731, NCT00302159) support combination of HDACis with radiation.[49-55] Future studies for NF2 or sporadic meningioma may consider the use of AR-42 as a radio-sensitizing agent.

We acknowledge the limitations of these pilot studies. Pilot 1 is a post-hoc subset analysis of a larger phase I study, which was not designed to evaluate efficacy. For Pilot 2, the supplier of AR-42 was unfortunately unable to continue to supply drugs after 2017. We elected to present the data on five of the originally planned 20 patients hoping to expedite further drug availability. Accrual to Pilot 2 was also limited by patient willingness to accept potential additional perioperative risk without a reasonable likelihood of benefit from only 3 weeks of therapy with AR-42. Other limitations inherent in small pilot studies include possible selection bias, lack of control subjects, and the inability to perform meaningful statistical analysis. Despite these limitations, we feel that the presented pilot studies are valuable to the field.

5 | CONCLUSION

In summary, AR-42 was safe and well tolerated in both pilots, but no grade 3-4 toxicities were seen at the lower dose in Pilot 2: 40 mg three times weekly for 3 weeks of a 28-day cycle. Importantly, this lower dose was sufficient to concentrate in tumors at levels sufficient to suppress the intended AKT/PI3K pathway in most tumors, indicating that the 40 mg regimen may be a biologically active, well-tolerated dose of AR-42 for future clinical trials for NF2-associated tumors. Preliminary evidence of possible anti-tumor activity based on volumetric measurements is suggested. A phase II trial of AR-42 for sporadic or NF2-associated VS and meningiomas is needed to adequately evaluate efficacy. Consideration should be given to combination therapy with other medications or radiation.

CONFLICT OF INTEREST
D. Bradley Welling is a consultant for CereXis, Science 24/7, NFBio, NF2 Biosolutions, and Mulberry Bio. Craig C. Hofmeister has received research grants from Takeda and Oncolytics Biotech; research and personal grants from Janssen, BMS, Sanofi, Nektar, Karyopharm, Imbrium, and Oncopeptides, all outside the submitted work. Amir Mortazavi is on the advisory board for Seattle Genetics and Pfizer and is on the scientific advisory board for Debiopharm Group. His institution (but not him) has received research funding from Acerta Pharma, Genentech, Roche, Merck, Novartis, Seattle Genetics, Astellas Pharma, Mirati Therapeutics, and Bristol-Myers Squibb. The other authors declare no potential conflict of interest. The Ohio State University (OSU) holds the patent on the investigational drug AR-42 (US 10/597022). The Technology Commercialization Office has licensed AR-42 (now called REC-2282) to Recursion Pharmaceuticals using the institution's standard terms, conditions, and approval process, in which no author participated. To assure absence of institutional conflict of interest in assessment of response and attribution of toxicity, both were reviewed by the Cancer Therapy Evaluation Program of the National Cancer Institute prior to reporting results for the phase I study. Safety issues related to dose increases and attribution of response were monitored by The OSU Data Safety Monitoring Committee and The OSU Cancer Center Institutional Review Board for the phase 1 pilot. A separate Data Safety and Monitoring Board of Massachusetts Eye and Ear oversaw pilot study 2.

AUTHOR CONTRIBUTIONS
Conceived the study: D. Bradley Welling, Amir Mortazavi, Craig C. Hofmeister. Performed experiments: Long-Sheng Chang, Janet L. Oblinger, Sarah S. Burns. Provided patient care: D. Bradley Welling, S. Alireza Mansouri, Brian A. Neff, Janet L. Oblinger, Robert K. Jackler, Amir Mortazavi. Analyzed the data: D. Bradley Welling, Katharine A. Collier, Beth A. Miles-Markley, Mina S. Makary, H. Wayne Slone. Prepared the manuscript: D. Bradley Welling, Katharine A. Collier, Long-Sheng Chang, Amir Mortazavi, Edina Shu. Supervised project: D. Bradley Welling, Amir Mortazavi, Long-Sheng Chang. All authors contributed to and approved the manuscript.

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