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A Phase 1 trial of the histone deacetylase inhibitor AR-42 in patients with neurofibromatosis type 2-associated tumors and advanced solid malignancies

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Conflicts of interest:

Christopher C. Coss, Sophia G. Liva, and Mitch A. Phelps are listed as inventors on a provisional patent for AR-42 for cancer-related cachexia (U.S. Patent Application No. 62/898,992).

The other authors declare no potential conflict of interest.

The Ohio State University (OSU) holds the patent on the investigational drug AR-42 (US 10/597,022). 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 (CTEP) of the National Cancer Institute (NCI) prior to reporting results. Safety issues related to dose increases and attribution of response were monitored by the Ohio State University Data Safety Monitoring Committee and the OSU Cancer Center Institutional Review Board (IRB).

Availability of data and materials: The authors confirm that the data supporting the findings of this study are available within the article and/or supplementary materials. Further data are available on request from the corresponding author, Amir Mortazavi. The data are not publicly available to prevent compromise of the privacy of the research participants.

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Abstract

Purpose: Given clinical activity of AR-42, an oral histone deacetylase inhibitor, in hematologic malignancies and preclinical activity in solid tumors, this phase 1 trial investigated the safety and tolerability of AR-42 in patients with advanced solid tumors, including neurofibromatosis type 2 associated meningiomas and schwannomas (NF2). The primary objective was to define the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs). Secondary objectives included determining pharmacokinetics and clinical activity.

Methods: This phase I trial was an open-label, single-center, dose-escalation study of single-agent AR-42 in primary central nervous system and advanced solid tumors. The study followed a 3+3 design with an expansion cohort at the MTD.

Results: Seventeen patients were enrolled with NF2 (n=5), urothelial carcinoma (n=3), breast cancer (n=2), non-NF2-related meningioma (n=2), carcinoma of unknown primary (n=2), small cell lung cancer (n=1), Sertoli cell carcinoma (n=1), and uveal melanoma (n=1). The recommended phase II dose is 60 mg three times weekly, for three weeks of a 28-day cycle. DLTs included grade 3 thrombocytopenia and grade 4 psychosis. The most common treatment-related adverse events were cytopenias, fatigue, and nausea. The best response was stable disease in 53% of patients (95% CI:26.6–78.7). Median progression-free survival (PFS) was 3.6 months (95% CI:1.2–9.1). Among evaluable patients with NF2 or meningioma (n=5), median PFS was 9.1 months (95% CI:1.9-not reached).

Conclusion: Single-agent AR-42 is safe and well tolerated. Further studies may consider AR-42 in a larger cohort of patients with NF2 or in combination with other agents in advanced solid tumors.

[NCT01129193](#) , registered 5/24/2010.

Keywords

histone deacetylase inhibitor; neurofibromatosis type 2; phase 1; pharmacokinetics; solid tumor

INTRODUCTION

Histone deacetylase (HDAC) enzymes catalyze removal of acetyl groups from lysine in proteins, including histone proteins, resulting in tight DNA interaction with the nucleosome, which regulates transcription.[1] This epigenetic regulation is performed by 18 HDAC proteins, that fall into four classes (I-IV).[2] HDACs have been implicated in hematologic and solid tumor malignancies through altered acetylation of histones and non-histone proteins involved in cell growth, differentiation, and apoptosis.[3–5] As a result, HDAC inhibitors have been developed as antineoplastic agents and have proven efficacy in some

hematologic malignancies.[6] Vorinostat (suberoylanilide hydroxamic acid, SAHA) and romidepsin (depsipeptide) are approved for relapsed cutaneous T-cell lymphoma (CTCL) by the Food and Drug Administration (FDA).[7,8] Efficacy was also found in phase II trials of vorinostat in indolent non-Hodgkin's lymphoma and diffuse large B cell lymphoma (DLBCL),[9–11] panobinostat in CTCL and Hodgkin's lymphoma,[12,13] and mocetinostat (MGCD0103) in Hodgkin's lymphoma, relapsed follicular lymphoma, and DLBCL.[14,15] However, the role of HDAC inhibitors in the treatment of solid tumors is less clear. There are no FDA approvals for single agent HDAC inhibitors, though phase II trials have shown activity of AN-9 (pivaloyloxymethyl butyrate) in metastatic non-small cell lung cancer (NSCLC)[16] and vorinostat in recurrent glioblastoma multiforme (GBM).[17]

AR-42 (previously licensed by Arno Therapeutics, now licensed by Recursion Pharma, REC-2282) is an orally bioavailable, small molecule pan-HDAC inhibitor containing hydroxamate-tethered phenylbutyrate, promoting histone H3 and H4 lysine acetylation, tubulin acetylation, inhibition of the PI3K/Akt pathway, cell-cycle arrest at G₂, and apoptosis via a caspase-dependent mechanism.[18–22] In preclinical studies, AR-42 has activity *in vitro* in prostate cancer[22,23] and breast cancer[24] cell lines. AR-42 has activity both *in vitro* and *in vivo* in cell lines and animal models of CLL,[20] mantle cell lymphoma, [20] acute lymphoblastic leukemia/lymphoma,[20] multiple myeloma,[25] Burkitt lymphoma,[20] hepatocellular carcinoma,[26] urothelial carcinoma,[27,28] colon cancer, [29] embryonal carcinoma,[30] ovarian cancer,[31,32] pancreatic cancer,[33] vestibular schwannomas,[19] and meningiomas.[34] In fact, the activity of AR-42 was superior to that of vorinostat in Burkitt lymphoma mouse models.[20] Preclinical pharmacology studies in rodents showed that AR-42 penetrates the blood brain barrier, suggesting it may be effective in central nervous system (CNS) tumors.[19,35] A phase 1 trial of AR-42 in patients with multiple myeloma and T- and B-cell lymphomas found a maximum tolerated dose (MTD) of 40 mg three times weekly for three weeks of a 28-day cycle, with durable responses in a patient with multiple myeloma and a patient with mantle cell lymphoma, concluding that AR-42 is safe and that further investigation of combination regimens of AR-42 should be performed in lymphoma and multiple myeloma.[36]

In this phase 1 study in patients with primary CNS and advanced solid tumors, the primary objectives were to investigate the safety and tolerability of AR-42 given as a single agent by defining the MTD and describing dose-limiting toxicities (DLTs). The secondary objectives included description of preliminary clinical activity in patients with CNS and solid tumors, and determination of the pharmacokinetics (PK) of AR-42.

MATERIALS AND METHODS

Patients

This phase I trial ([NCT01129193](#)) was approved by The Ohio State University Cancer Institutional Review Board and written informed consent was obtained from all patients. The study was performed in accordance with the Declaration of Helsinki. Eligible patients included adults with histologically or cytologically confirmed advanced or recurrent solid tumors for which no standard therapy was available, or who declined available standard treatment. Effort was made to enroll patients with tumor types for which there was

preclinical data supporting the use of AR-42. Up to three prior cytotoxic chemotherapy treatments in the metastatic setting and prior hormonal, biologic or targeted therapy were allowed. Patients were required to have adequate renal (creatinine $1.5 \times$ upper limit of normal (ULN) or creatinine clearance ≥ 50 mL/min), hepatic (total bilirubin < 1.5 mg/dL, AST/ALT $2.5 \times$ ULN or $5 \times$ ULN with liver metastasis), and bone marrow function (absolute neutrophil count $\geq 1500/\mu\text{L}$ and platelets $\geq 100,000/\mu\text{L}$). Patients were excluded if they had a prolonged QTc >450 ms in males and >470 ms in females, or symptomatic CNS metastases. Asymptomatic, treated brain metastases were allowed.

Study Design

This was a phase I, open-label, single-center, first-in-human, dose-escalation study of single-agent oral AR-42, which followed a 3+3 cohort design and included an expansion cohort at the MTD. In the dose escalation phase, the starting dose was 30 mg/day, one dose level below the MTD of 40 mg/day found in a previous phase I trial of this agent in patients with hematologic malignancies.[36] During stage A of the dose escalation, the dose for successive cohorts was increased by 100% until the first grade 2 drug-related (definite, probable, or possible) toxicity was observed in one patient, which initiated stage B. In stage B, three more patients were enrolled at the last dose level of stage A, then subsequent dose increases were by 33%, rounded to the nearest 10 mg. Intra-patient dose escalation was not allowed. The MTD was planned to be the highest dose at which no more than 1 of 6 patients experienced a DLT. Once the MTD was determined, up to an additional 10 patients could be enrolled at the MTD dose level to investigate the activity of AR-42.

AR-42 was obtained from Arno Therapeutics (Parsippany, NJ). The drug was administered orally on an empty stomach three times weekly, every other day, for three consecutive weeks of a 28-day cycle. Premedication included at least one antiemetic. Upfront prophylactic growth factors were not allowed, but could be used for neutropenic fever. Concurrent radiation was only allowed for palliation of pain from bone metastasis, and the irradiated area could not be used for response assessment. Treatment was continued until disease progression, unacceptable toxicity, or patient withdrawal from study.

Safety and Tolerability

In addition to close monitoring of physical exam, vital signs, performance status, and labs, electrocardiograms (EKGs) were performed frequently to monitor the QTc. Specifically, twelve-lead EKGs were obtained in triplicate pre-dose and 2, 4, 8, and 24 hours post-dose on cycle 1 days 1 and 19. EKGs were also collected prior to AR-42 administration on cycle 1 days 5 and 8, cycle 2 day 1, day 1 of every other subsequent cycle (i.e. C4D1, C6D1, etc.), and at the end of the study. Toxicities were graded based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.0. A DLT was defined as any grade 3–4 non-hematologic adverse event during cycle 1, excluding clinically insignificant lab abnormalities that resolved within 24 hours, nausea/vomiting that resolved to grade 2 or less within 24 hours, or liver function test abnormalities that resolved to less than grade 1 within 7 days. Hematologic DLTs included grade 4 neutropenia for >7 days, febrile neutropenia, grade 3 neutropenia with infection, grade 3 thrombocytopenia, or grade 2 thrombocytopenia with clinically meaningful bleeding that occurred during cycle 1. Dose

delays and dose reductions due to drug-related toxicities were performed per study protocol (Supplemental Table 1). Dose delays longer than 2 weeks for toxicity, or more than 4 weeks for any reason, resulted in removal from the study. Safety was evaluated in all patients who received at least one dose of AR-42. All patients were followed after the end of active study participation for toxicity evaluation for at least 30 days, or longer until resolution of treatment related adverse events.

Response Evaluation

Disease assessment was performed by computed tomography (CT) or magnetic resonance imaging (MRI) at baseline, and after every two cycles, approximately every 6–8 weeks. Response, progression, and stable disease were defined by the NCI Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, v1.1.[37] Patients were evaluable for response if they had measurable disease at baseline and completed at least one cycle of treatment prior to repeat imaging, or had objective disease progression prior to the end of cycle 1. Patients were contacted monthly after the end of study participation to assess survival.

Statistical Methods

Patient characteristics, type and frequency of adverse events, as well as dose and toxicity characteristics were summarized through descriptive statistics. The proportion of patients with stable disease by dose level was summarized, and includes the exact 95% confidence interval for the overall proportion. For evaluable participants, progression free survival (PFS) was defined as the time from enrollment to the date of progression or to the date last known to have stable disease. Patients who began other treatments prior to disease progression were censored on the date of initiation of alternative therapy. The method of Kaplan and Meier summarized PFS time, with confidence intervals calculated based on the log-log transformation. The median PFS of the subsets of patients with CNS and non-CNS tumors were calculated post hoc. All reported p-values and confidence intervals were two-sided, and reported at the nominal level; all analyses were performed using Stata 15.0.

Pharmacokinetics

Plasma pharmacokinetic (PK) samples were collected to investigate AR-42 PK following the first dose (Day 1) and last dose (Day 19) of cycle 1. Plasma sampling time points included pre-dose and post-dose at 0.25, 0.5, 1, 1.5, 2, 4, 8, 10, 24 hours on cycle 1 days 1 and 19. An additional 48-hour sample was obtained post-dose on cycle 1, day 1. Plasma concentrations of AR-42 were measured using a validated LC-MS/MS method as previously described. [38,36]

Non-compartmental analysis (NCA) of plasma AR-42 concentration-time data was completed in WinNonlin (v.6.3, Pharsight, St. Louis, MO) to estimate PK parameters using trapezoidal linear interpolation with extravascular dosing. Uniform weighting and the BestFit method were used for terminal phase regression. Estimation of the elimination rate constant (λ_z) was estimated using regression of the last three or four measurable time points when an adjusted R^2 value >0.85 could be obtained. Area under the curve from zero to 24 hours (AUC_{0-24h}), terminal Half-life ($t_{1/2}$), terminal volume of distribution (V_z/F), systemic

plasma clearance (CL/F) and area under the curve from zero to infinity ($AUC_{0-\infty}$) were estimated and summarized using geometric mean and geometric standard deviation unless otherwise noted. To evaluate for association of cycle 1 pharmacokinetic parameters and incidence of grade 3 or 4 toxicities during cycle 1 or 2, the Mann Whitney *U* test was used to compare the C_{max} , AUC_{0-24h} , and CL/F between patients that experienced a grade 3 or 4 toxicity and patients that did not have a grade 3 or 4 toxicity.

RESULTS

Patients

Seventeen patients were enrolled between June 2012 and November 2013. Baseline demographics are shown in Table 1. The most common disease was NF2-associated schwannoma and meningioma (n=5), followed by urothelial carcinoma (n=3), breast cancer (n=2), non-NF2-related meningioma (n=2), carcinoma of unknown primary (n=2), small cell lung cancer (n=1), Sertoli cell carcinoma (n=1), and uveal melanoma (n=1). Going forward, NF2-associated schwannoma and meningioma will be referred to as “NF2” and non-NF2-related meningioma will be referred to as “meningioma.” Together NF2 and meningioma will be considered “CNS tumors” and the remaining solid tumors will be considered “non-CNS tumors.” Clinically, the two carcinomas of unknown primary were suspected to be urothelial carcinoma and pancreaticobiliary in origin. Patients were heavily pretreated with a median of 2 (range 0–3) prior systemic therapies.

As shown in Table 2, three patients (cohort 1A) were enrolled at the starting dose of 30 mg with no DLTs. The dose was increased to 60 mg (cohort 2A), where the second patient experienced grade 2 thrombocytopenia, so dose escalation phase B was initiated. Three more patients were enrolled at 60 mg (cohort 1B) with one patient having a DLT of grade 3 thrombocytopenia. Another three patients were initiated at 60 mg (cohort 1B), with a fourth patient enrolled to replace a patient who was non-compliant and not evaluable. There were no DLTs in this group. At the 60 mg dose, there were two patients that required a dose reduction and four that had a dose delay. The dose was then increased by 33% to 80 mg, at which two patients were enrolled (cohort 2B). The first patient withdrew after two doses and was replaced; the second patient experienced grade 3 thrombocytopenia and a grade 4 psychiatric disorder, which was considered a DLT. At this point, although two DLTs were not reached at the 80 mg dose, the decision was made to define the recommended phase II dose (RP2D) as 60 mg once daily, three times weekly, every other day, for three consecutive weeks of a 28-day cycle. The episode of psychosis was only “possibly” related to AR-42 and may have been due to concurrent medications or underlying psychiatric illness, however, given the severity of the grade 4 psychosis in the context of multiple patients with transient, mild episodes of confusion in this study and the phase I study of AR-42 in hematologic malignancies,[36] as well as the frequency of thrombocytopenia, it was felt to be unsafe to enroll further patients at the 80 mg dose. This decision was further supported by the MTD of only 40 mg in the phase I trial of AR-42 in hematologic malignancies.[39] The expansion cohort enrolled three patients at the RP2D, for a total of twelve patients at 60 mg.

The median duration of treatment was 85 days (range 5–838), equivalent to 3.0 cycles (range 0.2–29.9). Most commonly, patients stopped the study drug due to progression of disease

(n=12), followed by patient decision to discontinue treatment resulting in withdrawal from the study (n=3).

Safety and Tolerability

Seventeen patients were evaluable for toxicity. As shown in Table 3, the most common treatment-related adverse events (TRAEs) of any grade were thrombocytopenia (n=13), fatigue (n=11), nausea (n=10), anemia (n=10), elevated creatinine (n=8), leukopenia (n=8), lymphopenia (n=7), hypophosphatemia (n=7), neutropenia (n=7), and diarrhea (n=6). Grade 3 TRAEs included thrombocytopenia (n=5), lymphopenia (n=3), hypophosphatemia (n=3), nausea (n=2), anemia (n=2), anorexia (n=1), weight loss (n=1), hematuria (n=1), and venous thromboembolism (n=1). The only grade 4 TRAE was psychosis at the 80 mg dose. There were no treatment-related deaths. The only cardiac toxicity observed was one patient with a grade 1 QTc prolongation. All treatment-related adverse events are listed in Supplemental Table 2 and all overall adverse events are listed in Supplemental Table 3.

During cycle one, one patient (5.9%) developed a grade 4 toxicity, five patients (29.4%) developed at most a grade 3 toxicity, five patients (29.4%) developed at most a grade 2 toxicity, four patients (23.5%) developed at most a grade 1 toxicity, and the remaining two patients (11.8%) experienced no toxicities. In subsequent cycles, there were no patients with a grade 4 toxicity, two of the patients with grade 2 toxicity during cycle one developed a grade 3 toxicity, and three of the patients with grade 1 toxicity during cycle one developed a grade 2 toxicity. One patient, who remained on study for 69 days, never experienced any toxicity of any grade.

The only dose delay or reduction during cycle 1 was for a patient on 60 mg, who experienced the DLT of grade 3 thrombocytopenia. In subsequent cycles, there were four patients at the 60 mg dose who required dose delays and one of these four patients required a dose reduction. The dose delays, regardless of attribution, were due to creatinine elevation, thrombocytopenia, upper respiratory infection, and a hospitalization for fatigue and urinary tract infection. The dose reduction for one patient during cycle 2 was due to fatigue, which was ultimately attributed to hypopituitarism from previous cranial irradiation for meningioma.

Response

Fifteen patients were evaluable for response. Two of the seventeen patients did not complete cycle 1, one due to non-compliance and one due to a DLT (grade 4 psychosis) without evidence of progression, and thus were not evaluable. The best overall response was stable disease, seen in 1 of 3 patients who received 30 mg and 7 of 11 patients who received 60 mg, resulting in 8 of 15 patients, or 53% (95% CI: 26.6–78.7) of patients experiencing stable disease. Among the patients with stable disease, three patients had a 5–18% decrease in the sum of the diameters of the target lesions from baseline. These patients had NF2, Sertoli cell carcinoma, and carcinoma of unknown primary, suspected to be urothelial carcinoma.

As shown in Figure 1, the median PFS time was 3.6 months (95% CI 1.2–9.1). In patients with non-CNS solid tumors (n=10), median PFS was 1.7 months (95% CI: 0.1 – 5.0). The two patients that were non-evaluable for response had CNS tumors, but among the

remaining evaluable patients with NF2 or meningioma (n=5), median PFS was 9.1 months (95% CI: 1.9-not reached (NR)). All evaluable patients with NF2 or meningioma received 60 mg.

Pharmacokinetics

Analysis was performed using data from 3, 12, and 2 patients at the 30 mg, 60 mg, 80 mg dose levels, respectively. Mean plasma concentration and time profiles of AR-42 in patients on day 1 and day 19 after an oral administration of AR-42 are shown in Figure 2. Individual plasma AR-42 peak plasma concentration (C_{max}) and AUC_{0-24hr} values ranged from 0.54 to 3.25 μM and 4.93 to 27.6 $\mu M \cdot Hr$ across the dose range, respectively. The elimination rate constant λ_z was not estimable on day 19 for two patients taking 60 mg daily. Elimination $t_{1/2}$ ranged from approximately 8 to 13 hours. AR-42 did not accumulate in these patients when given 3 times weekly for three weeks of a four-week cycle. C_{max} , AUC_{0-24} and $AUC_{0-\infty}$ remained consistent between Day 1 and Day 19 (Supplemental Table 4). AUC_{0-24} and $AUC_{0-\infty}$ were roughly 50% higher in the 80 mg dose group as compared to the 60 mg dose group, which suggests a potential loss of dose proportionality. However, only two patients were treated at the 80 mg dose level, and a conclusion cannot be drawn.

During cycle 1 and 2, patients that experienced a grade 3 or 4 toxicity had significantly higher median C_{max} (1.54 μM vs. 1.04 μM , $p=0.0016$) and median AUC (16.45 $\mu M \cdot hr$ vs 11.3 $\mu M \cdot hr$, $p=0.036$), but not median CL/F (10.48 L/hr vs 15.8 L/hr, $p=0.0745$), compared to patients that did not experience a grade 3 or 4 toxicity. These data support an observable plasma exposure-toxicity relationship, which may be useful for management of grade 3–4 toxicities in future studies.

DISCUSSION

This phase I study demonstrates that AR-42 is safe and tolerable in patients with primary CNS and advanced solid tumors. The phase II recommended dose is 60 mg orally once daily, three times weekly, every other day, for three consecutive weeks of a 28-day cycle.

The most common toxicities were cytopenias, fatigue, and nausea. The toxicities seen with AR-42 are consistent with the reported side effect profile of other HDAC inhibitors. Previously reported side effects of AR-42 and other HDAC inhibitors include cytopenias, fatigue, nausea, vomiting, diarrhea, anorexia, weight loss, asthenia, dehydration, and non-specific GI symptoms.[17,9,10,40,41,36,42–44] The most common toxicity of any grade was thrombocytopenia, but there were no clinically significant bleeding events, and platelet count improved with dose hold and reduction, if necessary. Thrombocytopenia was also common in the phase I trial of AR-42 in hematologic malignancies (16 of 27 patients), but the degree was less severe here, with no grade 4 thrombocytopenia. Historically, there has been concern for cardiac toxicity with HDAC inhibitors, particularly QTc prolongation, non-specific ST and T wave changes, and arrhythmias.[45–47] In this study there was only one patient who experienced a grade 1 QTc prolongation at 30 mg and there were no other cardiac toxicities observed at any dose. This is consistent with the clinically insignificant QT prolongation seen in the phase I study of AR-42 in hematologic malignancies, where 8 of 27 patients had a grade 1 QTc prolongation, with a mean QTc change of 27.4 ms, which all

spontaneously resolved without dose hold or adjustment.[36] Overall, AR-42 is safe and tolerable.

Although this was only a phase I study, not designed to evaluate efficacy, the anti-tumor activity of this small, heterogeneous cohort of patients is reported. The best response to AR-42 was stable disease in 53% (95% CI: 26.6–78.7) of patients. The overall cohort median PFS was 3.6 months (95% CI 1.2–9.1) with the wide confidence interval likely due to the inclusion of patients with NF2, which has a more indolent natural history than the non-CNS malignant solid tumors included in this study. In patients with non-CNS solid tumors, median PFS was 1.7 months (95% CI: 0.1 – 5.0). The seeming lack of activity of single-agent AR-42 seen here in advanced non-CNS solid tumors is consistent with phase II studies of other single agent HDAC inhibitors in solid tumors including romidepsin in metastatic castrate resistant prostate cancer,[42] GBM,[48] and colorectal cancer,[49] panobinostat in metastatic renal cell carcinoma,[43] and vorinostat in recurrent GBM,[17] ovarian carcinoma, primary peritoneal carcinoma,[50] head and neck cancer,[41] NSCLC, colorectal cancer, and metastatic breast cancer.[51,40] Overall, the lack of single-agent activity of AR-42 in non-CNS solid tumors is consistent with the limited activity of other single agent HDAC inhibitors in solid tumors, and does not warrant further investigation.

NF2 is a rare disease of multiple, slow-growing tumors for which the standard of care is surgery and radiation, with no effective systemic therapies.[52,53] In this study, the median PFS was 9.1 months (95% CI: 1.9-NR) in patients with NF2 or meningioma, with 1 of these 5 patients having longer than 27 months of follow up without progression. Based on preclinical studies, the mechanism of action of AR-42 in NF2 involves deacetylation and deactivation of AKT. The *NF2* gene encodes the tumor suppressor protein merlin and loss of merlin in NF2 results in proliferation of Schwann and leptomeningeal cells, in part through activation of the PI3K/AKT pathway.[54] AR-42 dephosphorylates and deactivates AKT. [55] In preclinical studies *in vitro*, AR-42 decreased AKT phosphorylation and suppressed proliferation of schwannoma and meningioma cell lines by cell cycle arrest at G₂ and apoptosis.[18] *In vivo*, AR-42 crossed the blood brain barrier in a mouse model and suppressed peripherally implanted xenograft and allograft schwannoma growth.[19] Based on the current preclinical and clinical data for AR-42 in NF2, and given the lack of effective systemic therapies for this disease, further investigation of the clinical activity of AR-42 in a larger cohort of patients with NF2 may be considered. For future clinical studies in NF2, in accordance with published suggested response criteria in NF2, use of volumetric radiographic measurements and validated hearing response assessments with word recognition scores should be considered.[53] Also, notably, one of the five patients with NF2 in this study eventually chose to discontinue treatment after many months on therapy due to persistent mild side effects, highlighting that it may be difficult for young patients to adhere to a long-term treatment with even low-grade, symptomatic toxicities. This population may benefit from the minimum effective dose, rather than the maximum tolerated dose.

Future studies should explore AR-42 in combination with other cancer-directed therapies for advanced solid tumors. There have been attempts to combine HDAC inhibitors with DNA damaging agents including platinum-based chemotherapy,[56] PARP inhibitors,[57] topoisomerase inhibitors,[58] and radiation,[59] as well as other cytotoxic chemotherapy

agents,[60] proteasome inhibitors,[61] hormonal therapy,[62] tyrosine kinase inhibitors,[63] hypomethylating agents,[64] rituximab,[65] bevacizumab,[66] and immune checkpoint inhibitors.[67–69] The only FDA approved HDAC inhibitor in combination is panobinostat with bortezomib and dexamethasone for relapsed or refractory multiple myeloma.[70] Specifically AR-42 has shown preclinical activity when combined with decitabine for AML, doxorubicin for osteosarcoma, 5-FU for breast cancer, cisplatin for urothelial carcinoma, and pazopanib for melanoma cells resistant to trametinib plus dabrafenib.[71–75] A recent preclinical study also demonstrated potential for AR-42 to overcome anabolic resistance in cancer-associated cachexia.[76] AR-42 and other HDAC inhibitors downregulate thymidylate synthetase, so AR-42 may be able to overcome resistance to pemetrexed or 5-FU, and AR-42 has also been shown to modulate ERBB2 receptor phosphorylation.[77,74,78–80] Both of these effects may be exploited with combination therapies. A phase I study of AR-42 combined with decitabine in thirteen patients with acute myeloid leukemia (AML) demonstrated similar safety and toxicities to those reported here, though the biological endpoint of increased *miR-29b* expression was not reached, and AR-42 will not be explored further in AML.[39] Unfortunately, the only phase I study of AR-42 in combination for a solid tumor to date, which used AR-42 with pazopanib in sarcoma and kidney cancer, was terminated due to two DLTs at the first dose level (NCT02795819), highlighting the risk for higher toxicity with combination regimens.

Finally, patient selection for genetic or molecular markers may identify a subset of patients most likely to respond to AR-42 and other HDAC inhibitors. Unfortunately, despite encouraging preclinical studies, in a phase II study that enrolled patients with urothelial carcinoma with mutations or deletions in CREB binding protein (*CREBBP*) and/or E1A binding protein p300 (*EP300*), mocetinostat failed to show efficacy.[81] However, for example, mutations in BRCA1-associated protein (*BAP1*), which predispose to mesotheliomas, uveal melanomas, cutaneous melanoma, and renal cell carcinoma, dysregulate HDAC proteins and sensitize cells to HDAC inhibitors.[82–84] Targeting solid tumors with *BAP1* mutations may reveal a subset of patients that respond to AR-42.

In summary, AR-42 is safe and tolerable in primary CNS and advanced solid tumors. A larger study is needed to evaluate efficacy in NF2. Consideration may be given to studies of AR-42 in combination with other agents for solid tumors and in subsets of patients with sensitizing mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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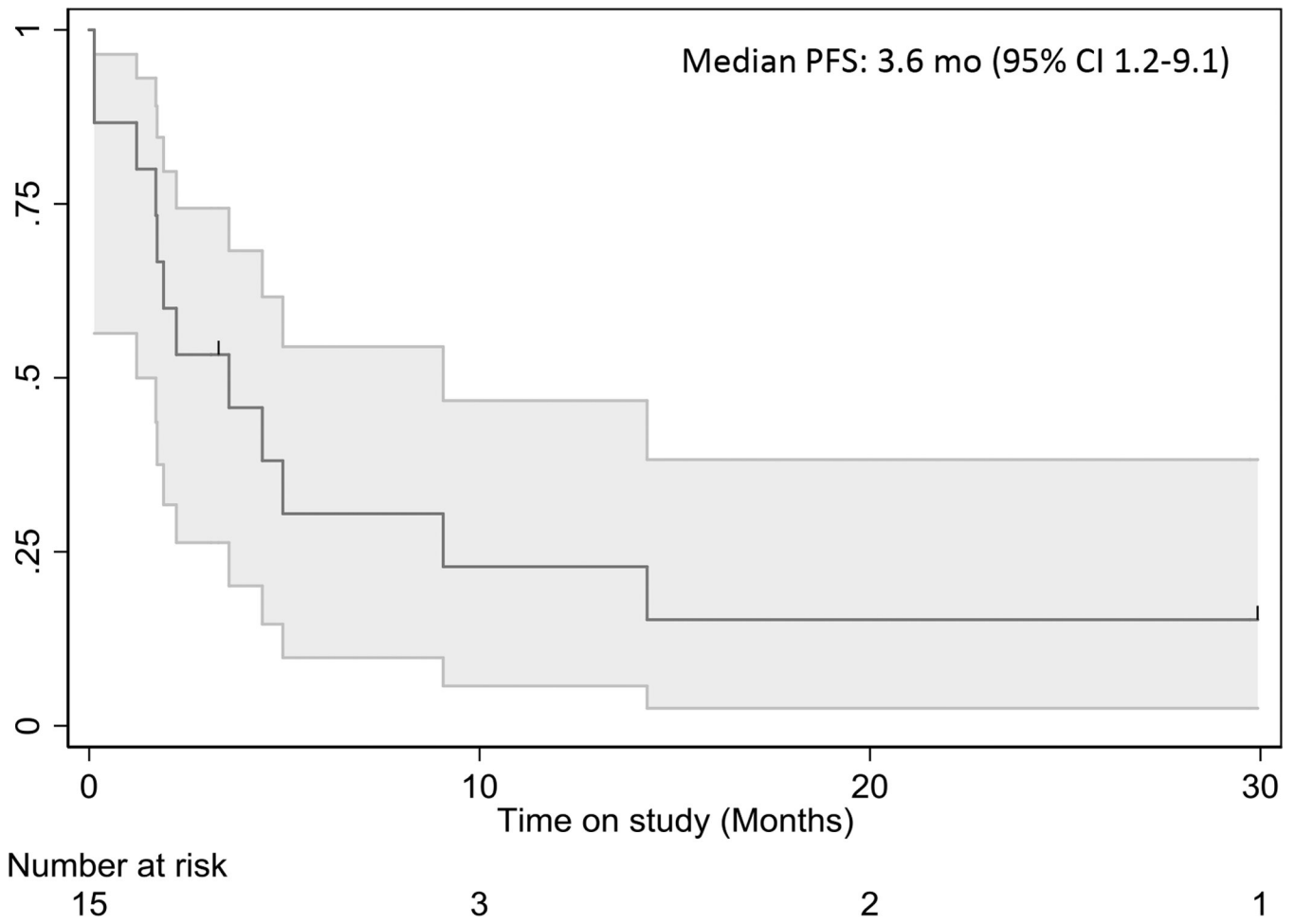


Figure 1. Median PFS of primary CNS and advanced solid tumors treated with AR-42.

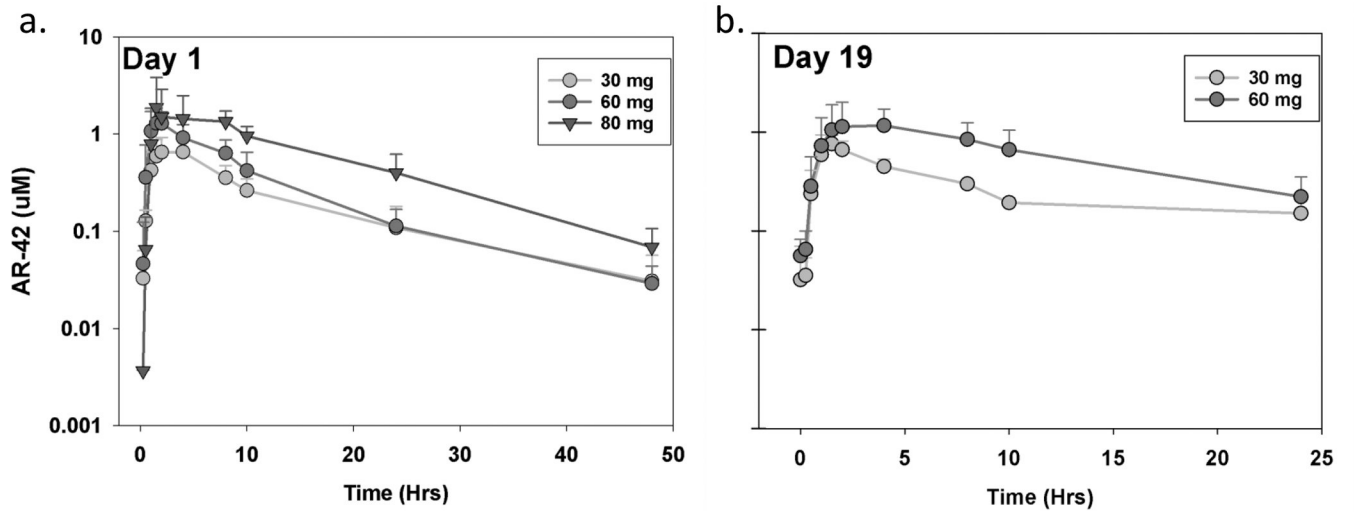


Figure 2.
Pharmacokinetics by dose on a) Day 1 and b) Day 19.

Table 1.

Patient demographics and characteristics at baseline

| Characteristic | N = 17 |
|---|------------|
| Median age (range) – year | 49 (20–80) |
| Gender - no. (%) | |
| Male | 6 (35.3) |
| Female | 11 (64.7) |
| Race - no. (%) | |
| White non-Hispanic | 15 (88.2) |
| Other | 2 (11.8) |
| ECOG performance status - no. (%) | |
| 0 | 10 (58.8) |
| 1 | 7 (41.2) |
| Primary tumor - no. (%) | |
| Neurofibromatosis type 2 | 5 (29.4) |
| Urothelial carcinoma | 3 (17.6) |
| Carcinoma of unknown primary ^a | 2 (11.8) |
| Breast | 2 (11.8) |
| Meningioma | 2 (11.8) |
| Small cell lung cancer | 1 (5.9) |
| Sertoli cell carcinoma of testis | 1 (5.9) |
| Uveal melanoma | 1 (5.9) |
| Prior lines of systemic therapy - no. (%) | |
| 0 | 5 (29.4) |
| 1–2 | 5 (29.4) |
| 3 | 7 (41.2) |

^aOne likely urothelial carcinoma, one likely pancreaticobiliary

Dose levels with number of patients, dose delays, dose reductions and dose limiting toxicities (DLTs)

Table 2.

| Dose level | AR-42 dose (mg) | No. of patients enrolled (N=17) | No. of dose delays | No. of dose reductions | No. of patients with a DLT | Description of DLT |
|------------------|-----------------|---------------------------------|--------------------|------------------------|----------------------------|--|
| 1A | 30 | 3 | 0 | 0 | 0 | --- |
| 2A | 60 | 2 ^a | 0 | 0 | 0 | --- |
| 1B | 60 | 7 ^b | 4 | 2 | 1 | Thrombocytopenia (grade 3) |
| 2B | 80 | 2 ^c | 0 | 0 | 1 | Thrombocytopenia (grade 3) ^d Psychiatric disorder (grade 4) ^d |
| Expansion cohort | 60 | 3 | 0 | 0 | --- | |

^aGrade 2 thrombocytopenia experienced by second patient, so advanced to stage B.

^bIncludes 2 cohorts of 3 patients, plus one patient to replace a patient who was non-compliant and not evaluable.

^cFirst patient withdrew after 2 doses due to progression and was replaced. Second patient experienced a DLT.

^dGrade 3 thrombocytopenia and grade 4 psychiatric disorder at dose level 2B occurred in the same patient, counting as one DLT.

Table 3.

All grade 3–4 and most common grade 1–2 treatment-related adverse events

| | Grade 1–2, n (%) | Grade 3–4, n (%) | All grades, n (%) |
|---|------------------|------------------|-------------------|
| Thrombocytopenia | 8 (47.1%) | 5 (29.4%) | 13 (76.5%) |
| Fatigue | 11 (64.7%) | --- | 11 (64.7%) |
| Nausea | 8 (47.1%) | 2 (11.8%) | 10 (58.8%) |
| Anemia | 8 (47.1%) | 2 (11.8%) | 10 (58.8%) |
| Elevated creatinine | 8 (47.1%) | --- | 8 (47.1%) |
| Leukopenia | 8 (47.1%) | --- | 8 (47.1%) |
| Lymphopenia | 4 (23.5%) | 3 (17.6%) | 7 (41.2%) |
| Hypophosphatemia | 4 (23.5%) | 3 (17.6%) | 7 (41.2%) |
| Neutropenia | 7 (41.2%) | --- | 7 (41.2%) |
| Diarrhea | 6 (35.3%) | --- | 6 (35.3%) |
| Anorexia | 4 (23.5%) | 1 (5.9%) | 5 (29.4%) |
| Hypoalbuminemia | 5 (29.4%) | --- | 5 (29.4%) |
| Vomiting | 4 (23.5%) | --- | 4 (23.5%) |
| Dizziness | 4 (23.5%) | --- | 4 (23.5%) |
| Myalgia | 4 (23.5%) | --- | 4 (23.5%) |
| Constipation | 4 (23.5%) | --- | 4 (23.5%) |
| Elevated alanine aminotransferase (ALT) | 4 (23.5%) | --- | 4 (23.5%) |
| Elevated alkaline phosphatase | 4 (23.5%) | --- | 4 (23.5%) |
| Weight loss | 2 (11.8%) | 1 (5.9%) | 3 (17.6%) |
| Elevated aspartate aminotransferase (AST) | 3 (17.6%) | --- | 3 (17.6%) |
| Impaired concentration | 3 (17.6%) | --- | 3 (17.6%) |
| Dry mouth | 3 (17.6%) | --- | 3 (17.6%) |
| Dysgeusia | 3 (17.6%) | --- | 3 (17.6%) |
| Headache | 3 (17.6%) | --- | 3 (17.6%) |
| Hyponatremia | 3 (17.6%) | --- | 3 (17.6%) |
| Cough | 2 (11.8%) | --- | 2 (11.8%) |
| Dehydration | 2 (11.8%) | --- | 2 (11.8%) |
| Limb edema | 2 (11.8%) | --- | 2 (11.8%) |

| | Grade 1-2, n (%) | Grade 3-4, n (%) | All grades, n (%) |
|---|------------------|------------------|-------------------|
| Hypermagnesemia | 2 (11.8%) | --- | 2 (11.8%) |
| Hypematremia | 2 (11.8%) | --- | 2 (11.8%) |
| Hypersomnia | 2 (11.8%) | --- | 2 (11.8%) |
| Elevated international normalized ratio (INR) | 2 (11.8%) | --- | 2 (11.8%) |
| Psychiatric disorder | --- | 1 (5.9%) | 1 (5.9%) |
| Thromboembolic event | --- | 1 (5.9%) | 1 (5.9%) |
| Hematuria | --- | 1 (5.9%) | 1 (5.9%) |

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