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ORIGINAL ARTICLE

Pediatric phase 2 trial of a WEE1 inhibitor, adavosertib (AZD1775), and irinotecan for relapsed neuroblastoma, medulloblastoma, and rhabdomyosarcoma

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Abstract

Background: Inhibition of the WEE1 kinase by adavosertib (AZD1775) potentiates replicative stress from genomic instability or chemotherapy. This study reports the pediatric solid tumor phase 2 results of the ADVL1312 trial combining irinotecan and adavosertib.

Methods: Pediatric patients with recurrent neuroblastoma (part B), medulloblastoma/central nervous system embryonal tumors (part C), or rhabdomyosarcoma (part D) were treated with irinotecan and adavosertib orally for 5 days every 21 days. The combination was considered effective if there were at least three of 20 responses in parts B and D or six of 19 responses in part C. Tumor tissue was analyzed for alternative lengthening of telomeres and ATRX. Patient's prior tumor genomic analyses were provided.

Results: The 20 patients with neuroblastoma (part B) had a median of three prior regimens and 95% had a history of prior irinotecan. There were three objective responses (9, 11, and 18 cycles) meeting the protocol defined efficacy end point. Two of the three patients with objective responses had tumors with alternative lengthening of telomeres. One patient with pineoblastoma had a partial response (11 cycles), but parts C and D did not meet the protocol defined efficacy end point. The combination was well tolerated and there were no dose limiting toxicities at cycle 1 or beyond in any parts of ADVL1312 at the recommended phase 2 dose.

Conclusion: This is first phase 2 clinical trial of adavosertib in pediatrics and the first with irinotecan. The combination may be of sufficient activity to consider further study of adavosertib in neuroblastoma.

See editorial on pages 000-000, this issue.

KEYWORDS
adavosertib, alternative lengthening of telomeres (ALT), AZD1775, irinotecan, pediatric cancer, WEE1

INTRODUCTION

WEE1 is a tyrosine kinase that phosphorylates and inhibits CDK1, affecting proper coordination of DNA replication as well as entry into mitosis. In the presence of a DNA damage repair deficiency (such as *TP53* or *BRCA* mutation) or replication stress (by chemotherapy, radiation, oncogenes), CDK1 activity is restrained by the checkpoint kinases CHK1 and WEE1. These checkpoints allow for repair of DNA before mitosis and tolerance of replication stress, thereby maintaining tumor cell viability. Inhibition of the checkpoint kinases leads to replication fork collapse or mitotic catastrophe, generation of single-, then double-strand DNA breaks and ultimately cellular death.

Adavosertib (AZD1775) is a highly selective, ATP competitive, small-molecule inhibitor of the WEE1 kinase and sensitizes tumor cells to cytotoxic agents and replication stress. Several adult cancer phase 2 clinical trials examining adavosertib as monotherapy or in combination with radiation or chemotherapy have been completed.[1] For example, adavosertib monotherapy resulted in a 29% objective response rate and 47% 6-month progression-free survival in recurrent uterine serous carcinoma; carboplatin chemo-potentiation in *TP53*-mutant ovarian cancers refractory to prior platinum therapy[2,3]; and prolonged survival in combination with gemcitabine in serous ovarian cancer.[4] Potential biomarkers of clinical response include *TP53* mutation, replication stress, *BRCA* mutation, and *CCNE1* amplification.[2,4] A pediatric efficacy study has not yet been reported to date. Preclinical work has shown antitumor activity of adavosertib in pediatric solid tumors including neuroblastoma, rhabdomyosarcoma, diffuse intrinsic pontine glioma, and medulloblastoma.[5–9]

ADVL1312 is a Children's Oncology Group multi-institutional phase 1/2 study of adavosertib in combination with irinotecan for children with relapsed or refractory solid and central nervous system (CNS) tumors. The rationale for combining adavosertib with irinotecan was multifactorial. Irinotecan induces replication arrest for repair of DNA damage that can be overridden by adavosertib. Irinotecan can also downregulate protein expression of CHK1. Preclinical data support synergistic growth inhibition of adavosertib in combination with irinotecan in neuroblastoma. Finally, the 5-day dosing regimen of irinotecan used clinically allows for serial dosing of adavosertib and a more prolonged exposure.[5] The phase 1 component of ADVL1312 was previously reported and (1) established the recommended phase 2 dose as irinotecan (90 mg/m^2/dose) and adavosertib (85 mg/m^2/dose) orally for 5 days every 21 days; (2) described the toxicities of the combination, which were mainly gastrointestinal; and (3) described the pediatric pharmacokinetics and determined that the peak plasma concentration of adavosertib at the recommended phase 2 dose met the preclinical target concentration.[10] To look for potential early signals of activity, and to obtain more information regarding toxicity at the recommended phase 2 dose, the ADVL1312 trial proceeded with a phase 2 expansion in three pediatric disease-specific cohorts. In this report, we present the phase 2 expansion results of ADVL1312, describing the initial efficacy of adavosertib and irinotecan in children with relapsed and refractory neuroblastoma, rhabdomyosarcoma, medulloblastoma, and other CNS embryonal tumors.

METHODS

Patient eligibility

Patients enrolled on the phase 2 expansion of ADVL1312 (ClinicalTrials.gov ID: NCT02095132) from October 2016 through May 2020 at 24 participating institutions. Patients (aged <21 years) with measurable or metaiodobenzylguanidine (MIBG)-evaluable neuroblastoma (part B), medulloblastoma/CNS embryonal tumors (part C), or rhabdomyosarcoma (part D) refractory to standard treatment and for whom no known curative therapy existed, were eligible. Patients treated at the recommended phase 2 dose from part A (phase 1) were lent to part B (n = 1), part C (n = 2), and part D (n = 1). Histologic verification of malignancy, at diagnosis or recurrence, was required. Other eligibility criteria included a Lansky or Karnofsky performance score >50; recovery from the acute toxic effects of prior therapy including resolution of therapy-related neurologic effects resolved to grade 2; adequate bone marrow function (absolute neutrophil count >1000/mm^3 and platelet count >100,000/mm^3), renal function (normal serum creatinine for age and sex, or creatinine clearance >70 mL/minute/1.73 m^2), liver function (bilirubin 1.5 times upper limit of normal for age, alanine aminotransferase < 110 U/L, serum albumin > 2 g/dL), QTc ≤ 480 ms, and ability to swallow capsules. Patients receiving drugs known to be moderate or strong inhibitors or inducers of CYP3A4 or CYP3A4 substrates with a narrow therapeutic range were not eligible. Patients previously treated with irinotecan were eligible even if they had progressed on a prior irinotecan-containing regimen.

The protocol was reviewed and approved by the Cancer Therapeutics Evaluation Program of the National Cancer Institute (NCI) and institutional review boards of all participating institutions. The study was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. Informed consent and

child assent, when appropriate, were obtained from all participants and/or parents or legal guardians.

Protocol therapy administration and study design

Eligible patients were treated with irinotecan (90 mg/m^2/dose) and adavosertib (85 mg/m^2/dose) orally for 5 days every 21 days. Adavosertib (10-, 25-, or 100-mg capsules) was supplied by Astra Zeneca and distributed by the Cancer Therapeutics Evaluation Program, NCI. The appropriate volume of irinotecan solution (20 mg/mL) was mixed with cranberry juice immediately before administration. Adavosertib was administered orally 1 hour after oral irinotecan for 5 days every 21 days. Cefixime prophylaxis for irinotecan-related diarrhea was required. Protocol therapy could continue until participants experienced disease progression or met discontinuation of protocol therapy criteria or a maximum of 18 cycles. Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5, with dose-limiting toxicities defined as previously described.[10]

Disease evaluations were obtained at baseline, the end of the first cycle, every other cycle twice, and then every three cycles. Disease response for solid tumors was assessed according to the revised Response Evaluation Criteria in Solid Tumors (v1.1) and neuroblastoma response was assessed using bone marrow biopsies, anatomic imaging for measurable disease, and MIBG scintigraphy for evaluable MIBG-avid tumors (Figure S1).[11] Curie scoring was used to assess response in patients with neuroblastoma who had evaluable disease by MIBG scintigraphy without measurable disease.[12] The evaluations were as per the Revised International Neuroblastoma Response Criteria[13] with overall responses as summarized in Table S1. In patients with primary CNS disease, tumor response was evaluated by magnetic resonance imaging using a modified RANO criteria; complete response was complete resolution of all lesions, partial response for CNS tumors was 50% decrease in the sum of the products of the two perpendicular diameters of all target lesions. Stable disease (SD) was neither sufficient decrease in the sum of the products of the two perpendicular diameters of all target lesions to qualify for partial response, nor sufficient increase in a single target lesion to qualify for PD. Progressive disease (PD) was defined as 25% or more increase in the sum of the products of the perpendicular diameters of the target lesions. For patients with objective response, confirmatory imaging was repeated after the next cycle. Central imaging review was completed for all participants who had institutional assigned objective response or had SD for six or more cycles.

Statistical analysis

Any patient who enrolled, met the eligibility criteria, and received at least one dose of protocol therapy was considered evaluable for response. Parts B/D each used a 10 + 10 Simon two-stage design. At least one response was required among 10 evaluable patients in stage 1 to open stage 2. Overall, at least three responses were required among 20 evaluable patients to conclude evidence of possible efficacy. Because of the single-agent activity of irinotecan in medulloblastoma,[14] part C used a 9 + 10 Simon two-stage design. At least two responses were required among nine evaluable patients in stage 1 to open stage 2. Overall, at least six responses were required among 19 evaluable patients to conclude evidence of possible efficacy.

The objective response rate was calculated using two-stage inference methods, which returns the uniformly minimum-variance unbiased estimator, *p* value, and CI using the clinfun R package. Because of the two-stage design, the simple proportion (3/20) is biased. The two-stage inference methods correct this bias.[15,16] All results of the correlative biology are descriptive.

Correlative biology

Tissue immunofluorescence–fluorescence in situ hybridization

Ultrabright telomeric foci (UBTF), a marker of alternative lengthening of telomeres (ALT), was first validated on a pediatric brain tumor tissue microarray (TMA) with 88 tumors punched in duplicate from 82 patients with known *ATRX* mutation and c-circle assay status, a measurement of ALT, including 61 cases of pediatric high-grade glioma and then applied to 12 ADVL1312 neuroblastoma archived formalin-fixed paraffin-embedded (FFPE) tissues.[17] The tissue immunofluorescence–fluorescence in situ hybridization protocol was according to Cesare et al.[18] with modifications adapted from Lin et al.[19] for tissue cyclic immunofluorescence to colocalize the UBTF with the neuroblastoma-specific marker Phox2b. High-resolution images were acquired and processed as detailed in the supplementary methods.

ATRX immunohistochemistry

ATRX antibody (Sigma Aldrich HPA001906) was used to stain a formalin fixed paraffin embedded TMA and ADVL1312 neuroblastoma slides. Staining was performed on a Bond Max automated staining system using the Bond Refine polymer staining kit (Leica Biosystems). The standard protocol was followed with the exception that the primary antibody incubation was extended to 1 hour at room temperature. The ATRX antibody was used at 1:1000 dilution, and antigen retrieval was performed with E2 (Leica Microsystems) retrieval solution for 20 minutes. Slides were rinsed, dehydrated through a series of ascending concentrations of ethanol and xylene, and then coverslip applied. Stained slides were then digitally scanned at 20× magnification on an Aperio CS-O slide scanner (Leica Biosystems).

RESULTS

Clinical response results

Twenty eligible patients with neuroblastoma, with a median age of 9 years (6–19 years) were included in part B. They had a median of three prior chemotherapy regimens (range, 1–7); 19 patients (95%) had prior irinotecan and 18 (90%) received prior radiation therapy including radiolabeled therapy (MIBG) (Table 1). The combination therapy resulted in three patients (15%) with objective responses confirmed by central radiology review among 20 evaluable patients, meeting the protocol-defined efficacy end point for part B. The estimated objective response rate using inference for two-stage study designs is 16.7% (one-sided 93% CI, 5.1–100; $p = .069$). The responses included a measurable partial response (11 cycles) (Figures 1 and 2), MIBG evaluable complete response (9 cycles) and MIBG and marrow evaluable partial response (18 cycles). In addition, there were three patients with centrally reviewed prolonged stable disease of 8, 11, and 13 cycles.

Nine patients with medulloblastoma/CNS embryonal tumors enrolled in part C. They had a median of three prior chemotherapy regimens (range, 1–6); four patients (44%) had prior irinotecan and nine (100%) received prior radiation therapy as standard of care (Table 1). One patient with pineoblastoma had a centrally reviewed confirmed partial response (nine cycles). One patient with medulloblastoma had prolonged stable disease (11 cycles) (Figure 3). Part C did not meet the required two responders in stage 1 to proceed

TABLE 1 Demographics of the ADVL1312 phase 2 patient cohorts.

Characteristic	NB (B) No. (%) (*N* = 20)	NB RESP No. (%) (*N* = 3)	MB/PBL (C) No. (%) (*N* = 9)	PBL RESP No. (%) (*N* = 1)	RMS (D) No. (%) (*N* = 10)	RMS RESP No. (%) (*N* = 0)	All No. (%) (*N* = 39)	All RESP No. (%) (*N* = 4)
Age (years)								
Median	9	13	13	16	14		10	14
Range	6–19	7–14	9–20		4–19		4–20	7–16
Sex								
Male	13 (65)	3 (100)	4 (44)	0	8 (80)		25 (64)	3 (75)
Female	7 (35)	0	5 (56)	1 (100)	2 (20)		14 (36)	1 (25)
Race								
White	12 (60)	1 (33)	7 (78)	0 (0)	9 (91)		28 (72)	1 (25)
Asian	1 (5)	1 (33)	0 (0)		0 (0)		1 (3)	1 (25)
American Indian/Alaska Native	0 (0)		0 (0)		0 (0)		0 (0)	
Native Hawaiian/Pacific Islander	0 (0)		0 (0)		0 (0)		0 (0)	
Black or African American	4 (20)	1 (33)	2 (22)	1 (100)	0 (0)		6 (15)	2 (50)
Unknown	3 (15)	0 (0)	0 (0)		1 (9)		4 (10)	
Ethnicity								
Non-Hispanic	17 (85)	2 (67)	8 (89)	1 (100)	6 (60)		31 (79)	3 (75)
Hispanic	1 (5)	1 (33)	1 (11)	0 (0)	2 (20)		4 (10)	1 (25)
Unknown	2 (10)	0 (0)	0 (0)		2 (20)		4 (10)	
Prior therapy								
Chemotherapy regimens	(*N* = 20)	(*N* = 3)	(*N* = 9)	(*N* = 1)	(*N* = 10)		(*N* = 39)	(*N* = 4)
Median	3	4	3	1	3		3	4
Range	1–7	3–4	1–6	1	1–7		1–7	1–4
History of irinotecan	19 (95)	3 (100)	4 (44)	0 (0)	9 (90)		32 (82)	3 (75)
Radiation therapy	(*n* = 18)	(*n* = 3)	(*n* = 9)	(*n* = 1)	(*n* = 10)		(*n* = 37)	(*n* = 4)
Median	1	1	1	1	1		1	1
Range	1–3	1–2	1–3		1–6		1–6	1–2

Abbreviations: MB, medulloblastoma; NB, neuroblastoma; PBL, pineoblastoma; RMS, rhabdomyosarcoma.

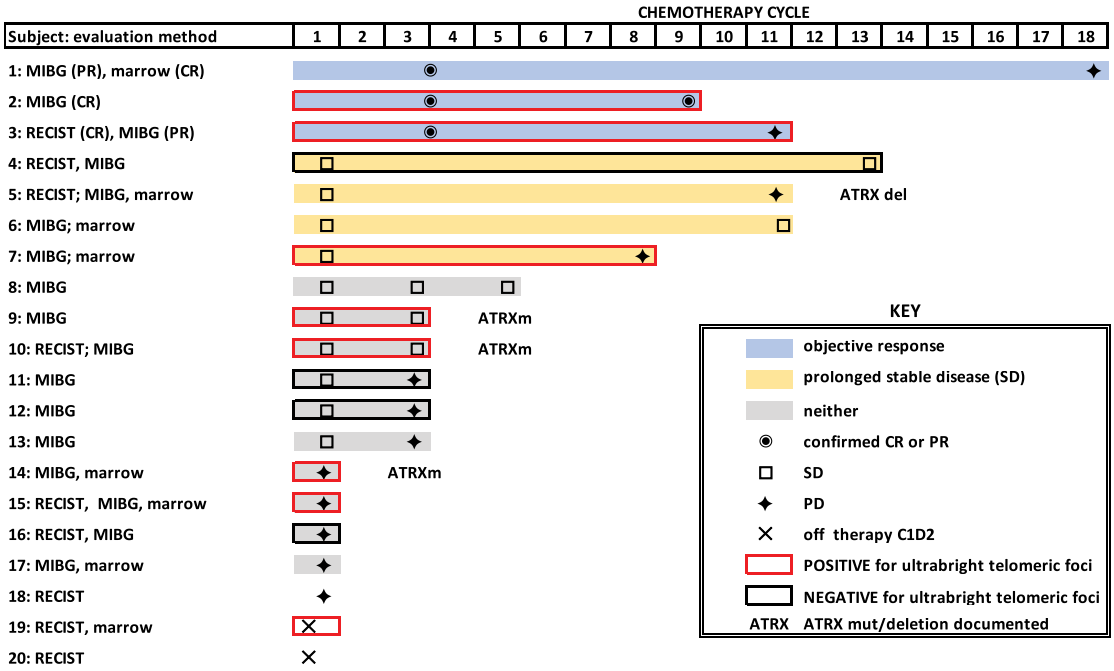

FIGURE 1 Swimmer plot to summarize the patient level methods of evaluation, response, and ALT and ATRX status for neuroblastoma (Part B). The colored bars indicate response or prolonged SD; the gray bars are neither response nor SD. Where there is no bar (subjects 18–20), subjects did not have end of cycle 1 disease evaluations. In the case of responders, the first marker on the bar is placed at the time of the confirmed response. For nonresponders, the first marker will be cycle 1. The final marker is the status at the final study evaluation. Although not marked, routine evaluations occurred after cycle 1 and cycle 2; every other cycle × 2 and then every three cycles until progression or a maximum of 18 cycles. If there is no red or black outline around the bar, this indicates that tissue was not available for correlative biology studies. All patients had *MYCN*-nonamplified disease except for subjects 13 and 18 (*MYCN* amplified) and 3 and 4 (unknown *MYCN* status). All patients had been treated at some point with a prior irinotecan-containing regimen, except subject 4. Subject 3 (PR) enrolled on ADVL1312 after progressing on an irinotecan-containing regimen. PR indicates partial response; SD, stable disease.

to stage 2 of the Simon two-stage design and therefore did not meet the protocol-defined efficacy end point.

Ten patients with rhabdomyosarcoma enrolled on part D. They had a median of three prior chemotherapy regimens (range, 1–7), nine (90%) had prior irinotecan, and 10 (100%) prior had radiation therapy (Table 1). Five patients' tumors were alveolar histology (50%), three embryonal histology (30%), and two other/mixed (20%) (Figure 3). Of the 10 eligible patients, there were no objective responders and therefore part D did not meet the required one responder in stage 1 to proceed to stage 2 with the 10 + 10 Simon two-stage design. However, two patients with rhabdomyosarcoma had stable disease for eight and 10 cycles (Figure 3).

Tolerability

Toxicity of the combination therapy was assessed by standard CTCAEv5 criteria and were mainly hematologic and gastrointestinal related (Table 2). In all parts of ADVL1312, there were no dose-limiting toxicities in cycle 1 or later at the maximum tolerated dose.

Correlative biology

With the early signal of activity in neuroblastoma, we sought to explore potential predictors of response, within the confines of a phase 2 expansion trial. Although patients in this study were not consented for tumor sequencing, for part B, clinical sites provided information about *MYC/N* amplification 11q LOH, *ATRX*, and *ALK* mutation status, if known. Two of 18 patients (11%) had *MYCN*-amplified neuroblastoma (patients 13 and 18; Figure 1), two were unknown (patients 3 and 4), and the remaining 16 were *MYCN* nonamplified. Although we hypothesized that patients with *MYCN*-amplified neuroblastoma may respond to WEE1 inhibition because of oncogene-induced replication stress, neither of the *MYCN*-amplified patients had an objective response. Preclinical studies have recently shown that tumors with the *ATRX* mutation or deletion may have synthetic lethality with adavosertib and irinotecan.[20,21] Given the prevalence of ALT in progressive recurrent neuroblastoma, particularly in association with *ATRX* mutation, we examined whether this could be associated with response.[22–24] We first validated the in situ UBTF assay for ALT in a pediatric high-grade, glioma-enriched TMA patient whose tumors also had known ALT status by c-circle assay (the gold standard measurement), *ATRX* mutation, and

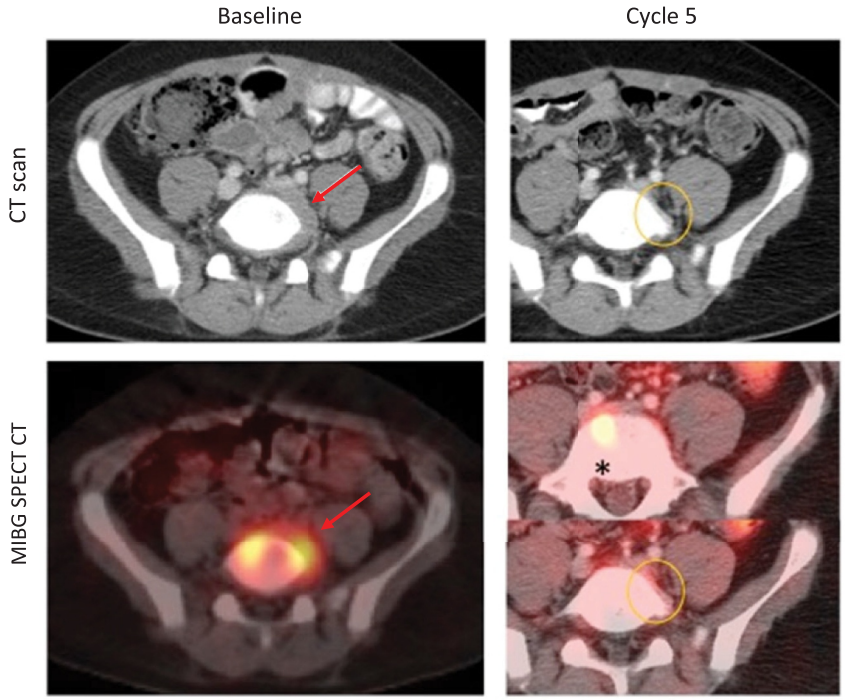

FIGURE 2 Representative images from subject 3 who had both measurable and MIBG-evaluable neuroblastoma and an overall central radiology reviewed PR from cycles 3 through 8, and PD at cycle 11. This patient enrolled with a history of progressive disease on a prior irinotecan-containing regimen. The left top panel shows the baseline 3.5-cm paraspinal mass (denoted by the red arrow), which is absent in cycle 5 in the top right panel. The bottom panels demonstrate the corresponding MIBG imaging. The bottom left panel shows the baseline pretherapy MIBG imaging of the paraspinal mass. Although the cycle 5 MIBG paraspinal mass demonstrated a CR (lower half, bottom right panel), there remained a small focus of residual MIBG-avid disease at the L5 vertebrae at cycle 5 (denoted by the * on the upper half bottom right panel), so the overall response was a PR (Figure S1). CR, complete response; MIBG, metaiodobenzylguanidine; PD, progressive disease; PR, partial response.

immunohistochemistry. In comparison to the c-circle assay results of the TMA, the UBTF assay had a 94.2% accuracy in predicting ALT tumors. With confidence in the assay, we then determined colocalization of UBTF (red) and the neuroblastoma nuclear protein Phox2B (green) on the 12 ADVL1312 neuroblastoma tumors with archival tissue available (Figure 4 and Figure S2). A summary of clinical response and tumor ALT and/or *ATRX* mutation status is shown in Figure 1. The objective response rate was 25% for patients whose tumors were ALT/UBTF positive (two of eight), 0% if ALT/UBTF negative (zero of four), and 12.5% (one of eight) if ALT/UBTF data were not available.

DISCUSSION

The ADVL1312 phase 2 expansion examined the clinical activity of adavosertib in combination with irinotecan in three pediatric cancer cohorts: neuroblastoma (part B), medulloblastoma/CNS embryonal tumors (part C), and rhabdomyosarcoma (part D). Only the neuroblastoma cohort met the protocol-defined efficacy end point and had an estimated 16.7% objective response rate. To put this finding into context, there are two phase 2 single-agent irinotecan monotherapy trials in neuroblastoma, and these informed our trial design. The response rate was 0% in the European (SFOP and UKCCSG) and 5% in the United States (Children's Oncology Group [COG]) recurrent neuroblastoma pediatric phase 2 cooperative group trials.[14,25] The SFOP UKCCSG irinotecan dosing was 600 mg/m^2 intravenously once every 3 weeks and the COG dosing was 50 mg/m^2/day intravenously $\times$ 5 days every 3 weeks. For ADVL1312, we chose to dose irinotecan 90 mg/m^2/day orally $\times$ 5 days every 3 weeks because it had previously been estimated that this dose and schedule has similar pharmacokinetic exposure to the COG phase 2 dose and schedule of 50 mg/m^2/day IV $\times$ 5 days every 3 weeks (in rhabdomyosarcoma with temozolomide, vincristine), enabling us to rationally define an efficacy end point and for convenience to the families.[26,27] The combination of irinotecan and temozolomide in patients with first-relapse neuroblastoma had an objective response rate of 15% and has become a backbone therapy in developmental therapeutic approaches for this disease, some of which are moving into up-front therapy trials.[28,29] To give perspective on neuroblastoma responses in early-phase clinical trials in general, London et al. examined 383 patients with neuroblastoma enrolled in 35 COG trials after first relapse and found that the median time to progression was 58 days (range, 31–183 days).[30] Similarly, among 203 patients with

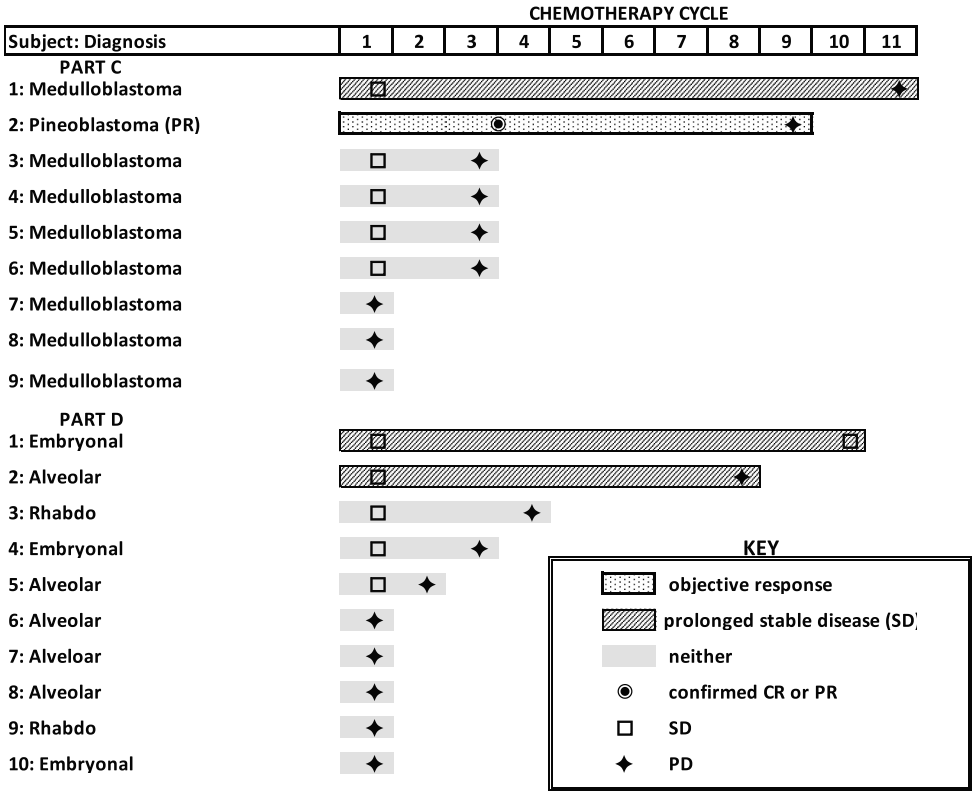

FIGURE 3 Swimmer plot to summarize the patient level diagnosis and response for the medulloblastoma/embryonal tumor (part C) and rhabdomyosarcoma (part D) cohorts. The patterned bars indicate response or prolonged stable disease (≥6 cycles); the gray solid bars are neither response nor SD. In the case of the responder, the first marker on the bar is placed at the time of the confirmed response. For nonresponders, the first marker will be cycle 1. The final marker is the status at the final study evaluation. Although not marked, routine evaluations occurred after cycle 1 and cycle 2; every other cycle × 2 and then every 3 cycles until progression or a maximum of 18 cycles. SD indicates stable disease.

neuroblastoma evaluable for response on New Approaches to Neuroblastoma Therapy consortium early-phase clinical trials, there was a 6.1% complete response/partial response rate.[31]

With the early signal of clinical activity of ADVL1312 therapy in neuroblastoma, we sought to determine factors that could correlate with clinical response within the confines of an early-phase trial. The *ATRX* chromatin remodeler gene, and its resultant phenotype of ALT, was of interest because *ATRX* is (1) mutated in approximately 30% of older pediatric patients with neuroblastoma, particularly those with relapsed/refractory disease; (2) has a role in maintenance of telomeres and replication fork progression, and tumors with *ATRX* mutation have higher levels of replicative stress; and (3) preclinical studies in *ATRX*-deficient models demonstrated synthetic lethality with agents that potentiate replicative stress, including irinotecan and AZD1775 (adavosertib).[20–22,24,32] However, assessing neuroblastoma tumor sections for ATRX protein loss is technically difficult because, even if mutated, the nuclear protein may be present by immunohistochemistry.[33] Moreover, neuroblastoma diagnostic tissues are often small and admixed with normal cells from the biopsy sites, challenging sequencing efforts. Finally, neuroblastoma tumors with ALT define the subset of aggressive, refractory, and poor prognosis disease, but genomic mutation of *ATRX* is identifiable in only half of patient tumors with ALT.[21,22] To overcome these limitations in our study and because we only had FFPE tissues available, we assayed the ADVL1312 tissues for ALT by in situ multiplex immunofluorescence of UBTF with colocalization of the neuroblastoma specific marker Phox2B. Of the three patients who had an objective response, two had ALT-positive tumors. The third responder (subject 1, Figure 1) did not have archival tissue available, but was an adolescent with *MYCN* nonamplified disease.[34–36] *ATRX* mutation/ALT is infrequently found in medulloblastoma[37] or rhabdomyosarcoma,[38] so UBTF were not assessed. We evaluated the pineoblastoma tumor from the patient with a partial response in part C because the frequency of ALT in this histology is unknown, but the tumor did not have ALT UBTF.

ALT is found in tumors from older pediatric and adolescent neuroblastoma patients with indolent, chemo-refractory disease with a dismal prognosis.[21,22] To our knowledge, this is the first trial to assess ALT status in relation to response, and we found that 67% of our neuroblastoma cohort, eight of 12 with tissue available, had tumors that were ALT positive. Although we were not able to demonstrate an association of objective response of ADVL1312 with ALT status because of an incomplete set of tumors, we think that it may be an important potential biomarker to consider in future

TABLE 2 Toxicity of the ADVL1312 combination therapy.

Maximum grade across all cycles				
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Part B neuroblastoma, *n* = 16 evaluable for toxicity				
Neutropenia	2	4	4	1
Thrombocytopenia	4	1	1	2
Anemia	4	3	2	
Leukopenia	5	3	2	
Lymphopenia			3	1
Nausea	6	3	2	
Vomiting	7	1	1	
Diarrhea	9	1		
Part C medulloblastoma, *n* = 7 evaluable for toxicity				
Neutropenia			1	3
Thrombocytopenia	2		1	
Anemia	3	1		
Leukopenia	1	2	2	
Lymphopenia	1	2	2	
Nausea	4			
Vomiting	5			
Diarrhea	4	1		
Part D rhabdomyosarcoma, *n* = 7 evaluable for toxicity				
Neutropenia			3	
Thrombocytopenia	2	2		
Anemia	2	1	1	
Leukopenia	4	1		
Lymphopenia	1			1
Nausea	4	1	1	
Vomiting	3	1	1	
Diarrhea	4	1	1	
Anorexia		2	1	
Dehydration		1	1	
Hypokalemia	1		1	

Note: Adverse events were mainly hematologic and gastrointestinal related. These tables include any toxicity that had at least one patient with grade 3 or greater adverse event OR was present in >50% of cohort.

studies of relapsed refractory neuroblastoma offering the possibility for ALT-directed therapy. We were able to examine for a possible signal of activity for ALT tumors because of the patient enrollment. Our cohort was enriched for older chemo-refractory patients because the ADVL1312 eligibility criteria required patients to swallow adavosertib capsules whole and allowed patients to enroll even if they progressed on a prior irinotecan-containing regimen because it was hypothesized that WEE1 inhibition could reverse chemotherapy resistance.[3,5] Table 1 reflects such a population in which the neuroblastoma cohort had a median age of 9 years (range, 6–19 years) and was heavily pretreated with a median of three prior chemotherapy regimens (range, 1–7), including 95% of the cohort having had a prior irinotecan containing regimen.

ADVL1312 therapy did not meet the efficacy end point in the rhabdomyosarcoma cohort (part D) despite compelling preclinical data of activity of adavosertib, irinotecan, and vincristine in orthotopic patient–derived xenograft models of rhabdomyosarcoma.[6] Preclinically adavosertib and irinotecan had a 39% combined (complete response + partial response) response rate across four orthotopic patient–derived xenograft models of rhabdosarcoma that increased to 70% with addition of vincristine to the irinotecan/adavosertib combination. Considerations for the lack of responses in patients with rhabdomyosarcoma enrolled on ADVL1312 include a predominance of alveolar histology with large bulky or widely metastatic disease, characteristics that are associated with less favorable postrelapse outcome, as may be expected from an early-phase clinical trial[39] (Table 1, Figure 3).

A notable limitation of the trial's correlative biology was tissue availability in only 12 of 20 patients with neuroblastoma, limiting conclusions based on molecular analysis. If a patient had archival tissue available, they were required to submit it. However, the protocol did not block enrollment if tissue was not available. Reasons that patients may not have had tissue include (1) in pediatric clinical trials, procedures (including rebiopsy) are not often done unless there is direct prospect for benefit to the child; (2) diagnostic tissue is often small and may only be a core biopsy; and (3) there was no limit to number of prior therapies at enrollment, so tissue may have been exhausted for prior trials. In addition, during the years of enrollment (2016–2020), tumor sequencing was not yet part of standard clinical care and subjects could not be consented for research sequencing retrospectively.

General limitations of this study include those related to a phase 1/2 design. Patients were eligible for response without completing end of cycle 1 disease evaluations or only receiving 2 of 5 planned days of therapy (subjects 18, 19, and 20). In addition, it is not possible to discern the contribution of each agent to the response, or if a combination is necessary. Of interest is that subject 3 (Figures 2 and 4, partial response, 11 cycles) enrolled on ADVL1312 having progressed on an irinotecan containing regimen, and subject 1 discontinued irinotecan after cycle 5 with a continued partial response on adavosertib monotherapy until cycle 18.

In summary, the ADVL1312 combination of adavosertib and irinotecan was tolerable and demonstrates early signal of activity in relapsed neuroblastoma. This is the first positive signal of clinical activity in pediatric oncology for a small molecule DNA damage repair/cell-cycle checkpoint inhibitor and the ability to target replication stress.[40] The findings are potentially impactful to neuroblastoma and other malignancies of children, adolescents, and young adults. Although adavosertib has not been Food and Drug Administration–approved, it is the first in class and there are novel WEE1 inhibitors in development. Additional clinical studies are needed to determine whether adavosertib, novel WEE1 inhibitors, or

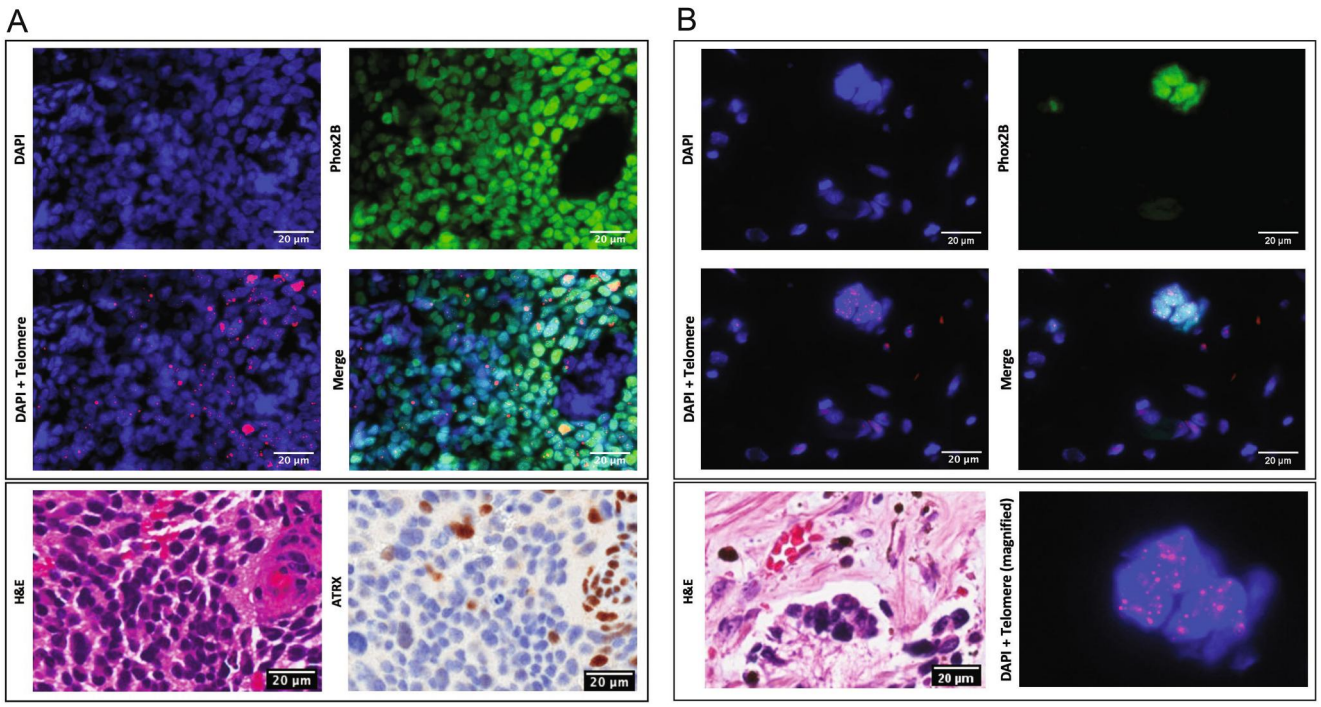

FIGURE 4 Ultrabright telomeric foci by multiplex Tel-FISH and immunofluorescence. (A) Archival tissue obtained at diagnosis from subject 7 demonstrates colocalization in the middle right panel of nuclear Phox2B (green) and ultrabright telomeric foci (red) in the neuroblastoma tumor, which is absent in nonneuroblastoma cells that stain only for DAPI nuclear stain (blue). ATRX IHC in the lower right panel demonstrates loss of nuclear ATRX protein staining (brown) in the neuroblastoma nuclei consistent with an *ATRX*-mutant tumor. (B) Archival tissue from subject 3 similarly demonstrates colocalization of nuclear Phox2B and ultrabright telomeric foci in a scattered clusters of residual neuroblastoma after induction chemotherapy. The lower right panel is a magnified image of the left middle panel to highlight the telomeric foci. The DAPI, Phox2B, and ultrabright telomeric foci for subject 3 are imaged from the same histopathology slide. Although the H&E and ATRX immunohistochemistry images (Figure S2) are from the same tissue block and are from adjacent sections, they are different slides and hence show different cellular clusters of the tumor. H&E, hematoxylin and eosin; IHC, immunohistochemistry.

agents that target replicative stress will have activity in neuroblastoma and other *ATRX* mutant, ALT-activated tumors.

AUTHOR CONTRIBUTIONS

Kristina Cole: Conceptualization, project administration, writing - original draft, and writing - review and editing. **Heba Ijaz:** investigation and visualization. **Lea Surrey:** Validation. **Mariarita Santi:** Resources and validation. **Xiaowei Liu:** Data curation. **Charles Minard:** Methodology, formal analysis, and writing - review and editing. **John M. Maris:** Conceptualization and writing - review and editing. **Stephan Voss:** Validation and visualization. **Joel Reid:** Formal analysis. **Elizabeth Fox:** Conceptualization, supervision, and writing - review and editing. **Brenda Weigel:** Supervision, funding acquisition, and writing - review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

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REFERENCES

1. Leijen S, van Geel RM, Pavlick AC, et al. Phase I study evaluating WEE1 inhibitor AZD1775 as monotherapy and in combination with gemcitabine, cisplatin, or carboplatin in patients with advanced solid tumors. *J Clin Oncol*. 2016;34(36):4371-4380. doi:10.1200/jco.2016.67.5991
2. Liu JF, Xiong N, Campos SM, et al. Phase II study of the WEE1 inhibitor adavosertib in recurrent uterine serous carcinoma. *J Clin Oncol*. 2021;39(14):1531-1539. doi:10.1200/jco.20.03167
3. Leijen S, van Geel RM, Sonke GS, et al. Phase II study of WEE1 inhibitor AZD1775 plus carboplatin in patients with TP53-mutated ovarian cancer refractory or resistant to first-line therapy within 3 months. *J Clin Oncol*. 2016;34(36):4354-4361. doi:10.1200/jco.2016.67.5942
4. Lheureux S, Cristea MC, Bruce JP, et al. Adavosertib plus gemcitabine for platinum-resistant or platinum-refractory recurrent ovarian

cancer: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet*. 2021;397(10271):281-292. doi:10.1016/s0140-6736(20)32554-x
5. Russell MR, Levin K, Rader J, et al. Combination therapy targeting the Chk1 and Wee1 kinases shows therapeutic efficacy in neuroblastoma. *Cancer Res*. 2013;73(2):776-784. doi:10.1158/0008-5472.can-12-2669
6. Stewart E, McEvoy J, Wang H, et al. Identification of therapeutic targets in rhabdomyosarcoma through integrated genomic, epigenomic, and proteomic analyses. *Cancer Cell*. 2018;34(3):411-426.e419. doi:10.1016/j.ccell.2018.07.012
7. Mueller S, Hashizume R, Yang X, et al. Targeting Wee1 for the treatment of pediatric high-grade gliomas. *Neuro Oncol*. 2014;16(3):352-360. doi:10.1093/neuonc/not220
8. Moreira DC, Venkataraman S, Subramanian A, et al. Targeting MYC-driven replication stress in medulloblastoma with AZD1775 and gemcitabine. *J Neuro Oncol*. 2020;147(3):531-545. doi:10.1007/s11060-020-03457-0
9. Kolb EA, Houghton PJ, Kurmasheva RT, et al. Preclinical evaluation of the combination of AZD1775 and irinotecan against selected pediatric solid tumors: a Pediatric Preclinical Testing Consortium report. *Pediatr Blood Cancer*. 2020;67(5):e28098. doi:10.1002/pbc.28098
10. Cole KA, Pal S, Kudgus RA, et al. Phase I clinical trial of the Wee1 inhibitor adavosertib (AZD1775) with irinotecan in children with relapsed solid tumors: a COG Phase I Consortium Report (ADVL1312). *Clin Cancer Res*. 2020;26(6):1213-1219. doi:10.1158/1078-0432.ccr-19-3470
11. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247. doi:10.1016/j.ejca.2008.10.026
12. Ady N, Zucker JM, Asselain B, et al. A new 123I-MIBG whole body scan scoring method--application to the prediction of the response of metastases to induction chemotherapy in stage IV neuroblastoma. *Eur J Cancer*. 1995;31A(2):256-261. doi:10.1016/0959-8049(94)00509-4
13. Park JR, Bagatell R, Cohn SL, et al. Revisions to the International Neuroblastoma Response Criteria: a consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol*. 2017;35(22):2580-2587. doi:10.1200/jco.2016.72.0177
14. Bomgaars LR, Bernstein M, Krailo M, et al. Phase II trial of irinotecan in children with refractory solid tumors: a Children's Oncology Group Study. *J Clin Oncol*. 2007;25(29):4622-4627. doi:10.1200/jco.2007.11.6103
15. Jung SH, Kim KM. On the estimation of the binomial probability in multistage clinical trials. *Stat Med*. 2004;23(6):881-896. doi:10.1002/sim.1653
16. Koyama T, Chen H. Proper inference from Simon's two-stage designs. *Stat Med*. 2008;27(16):3145-3154. doi:10.1002/sim.3123
17. Stundon J, Ijaz H, Gaonkar KS, et al. ALT in pediatric high-grade gliomas can occur without ATRX mutation and is enriched in patients with pathogenic germline MMR variants. *Neuro Oncol*. 2022. doi:10.1093/neuonc/noac278
18. Cesare AJ, Heaphy CM, O'Sullivan RJ. Visualization of telomere integrity and function in vitro and in vivo using immunofluorescence techniques. *Curr Protoc Cytom*. 2015;73(1):12.40.11-12.40.31. doi:10.1002/0471142956.cy1240s73
19. Lin JR, Izar B, Wang S, et al. Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes. *Elife*. 2018;7. doi:10.7554/elife.31657
20. Liang J, Zhao H, Diplas BH, et al. Genome-wide CRISPR-Cas9 screen reveals selective vulnerability of ATRX-mutant cancers to WEE1 inhibition. *Cancer Res*. 2020;80(3):510-523. doi:10.1158/0008-5472.can-18-3374
21. Koschmann C, Calinescu AA, Nunez FJ, et al. ATRX loss promotes tumor growth and impairs nonhomologous end joining DNA repair in glioma. *Sci Transl Med*. 2016;8(328):328ra328. doi:10.1126/scitranslmed.aac8228
22. Hartlieb SA, Sieverling L, Nadler-Holly M, et al. Alternative lengthening of telomeres in childhood neuroblastoma from genome to proteome. *Nat Commun*. 2021;12(1):1269. doi:10.1038/s41467-021-21247-8
23. Koneru B, Lopez G, Farooqi A, et al. Telomere maintenance mechanisms define clinical outcome in high-risk neuroblastoma. *Cancer Res*. 2020;80(12):2663-2675. doi:10.1158/0008-5472.can-19-3068
24. Dyer MA, Qadeer ZA, Valle-Garcia D, Bernstein E. ATRX and DAXX: mechanisms and mutations. *Cold Spring Harb Perspect Med*. 2017;7(3):a026567. doi:10.1101/cshperspect.a026567
25. Vassal G, Giammarile F, Brooks M, et al. A phase II study of irinotecan in children with relapsed or refractory neuroblastoma: a European cooperation of the Societe Francaise d'Oncologie Pediatrique (SFOP) and the United Kingdom Children Cancer Study Group (UKCCSG). *Eur J Cancer*. 2008;44(16):2453-2460. doi:10.1016/j.ejca.2008.08.003
26. Wagner LM, Perentesis JP, Reid JM, et al. Phase I trial of two schedules of vincristine, oral irinotecan, and temozolomide (VOIT) for children with relapsed or refractory solid tumors: a Children's Oncology Group phase I consortium study. *Pediatr Blood Cancer*. 2010;54(4):538-545. doi:10.1002/pbc.22407
27. Wagner LM. Fifteen years of irinotecan therapy for pediatric sarcoma: where to next? *Clin Sarcoma Res*. 2015;5(1):20. doi:10.1186/s13569-015-0035-x
28. Bagatell R, London WB, Wagner LM, et al. Phase II study of irinotecan and temozolomide in children with relapsed or refractory neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*. 2011;29(2):208-213. doi:10.1200/jco.2010.31.7107
29. Mody R, Naranjo A, Van Ryn C, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol*. 2017;18(7):946-957. doi:10.1016/s1470-2045(17)30355-8
30. London WB, Bagatell R, Weigel BJ, et al. Historical time to disease progression and progression-free survival in patients with recurrent/refractory neuroblastoma treated in the modern era on Children's Oncology Group early-phase trials. *Cancer*. 2017;123(24):4914-4923. doi:10.1002/cncr.30934
31. Villablanca JG, Ji L, Shapira-Lewinson A, et al. Predictors of response, progression-free survival, and overall survival using NANT Response Criteria (v1.0) in relapsed and refractory high-risk neuroblastoma. *Pediatr Blood Cancer*. 2018;65(5):e26940. doi:10.1002/pbc.26940
32. George SL, Lorenzi F, King D, et al. Therapeutic vulnerabilities in the DNA damage response for the treatment of ATRX mutant neuroblastoma. *EBioMedicine*. 2020;59:102971. doi:10.1016/j.ebiom.2020.102971
33. Chami R, Marrano P, Teerapakpinyo C, et al. Immunohistochemistry for ATRX can miss ATRX mutations: lessons from neuroblastoma. *Am J Surg Pathol*. 2019;43(9):1203-1211. doi:10.1097/pas.0000000000001322
34. Cheung NK, Zhang J, Lu C, et al. Association of age at diagnosis and genetic mutations in patients with neuroblastoma. *JAMA*. 2012;307(10):1062-1071. doi:10.1001/jama.2012.228
35. Farooqi AS, Dagg RA, Choi LM, Shay JW, Reynolds CP, Lau LM. Alternative lengthening of telomeres in neuroblastoma cell lines is associated with a lack of MYCN genomic amplification and with p53 pathway aberrations. *J Neuro Oncol*. 2014;119(1):17-26. doi:10.1007/s11060-014-1456-8
36. Zeineldin M, Federico S, Chen X, et al. MYCN amplification and ATRX mutations are incompatible in neuroblastoma. *Nat Commun*. 2020;11(1):913. doi:10.1038/s41467-020-14682-6

37. Mangerel J, Price A, Castelo-Branco P, et al. Alternative lengthening of telomeres is enriched in, and impacts survival of TP53 mutant pediatric malignant brain tumors. *Acta Neuropathol*. 2014;128(6):853-862. doi:10.1007/s00401-014-1348-1
38. Henson JD, Hannay JA, McCarthy SW, et al. A robust assay for alternative lengthening of telomeres in tumors shows the significance of alternative lengthening of telomeres in sarcomas and astrocytomas. *Clin Cancer Res*. 2005;11(1):217-225. doi:10.1158/1078-0432.217.11.1
39. Heske CM, Mascarenhas L. Relapsed rhabdomyosarcoma. *J Clin Med*. 2021;10(4):804. doi:10.3390/jcm10040804
40. Groelly FJ, Fawkes M, Dagg RA, Blackford AN, Tarsounas M. Targeting DNA damage response pathways in cancer. *Nat Rev Cancer*. 2022;23(2):78-94. doi:10.1038/s41568-022-00535-5

SUPPORTING INFORMATION

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