

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)



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ABSTRACT

Purpose: Adavosertib (AZD1775), an inhibitor of WEE1 kinase, potentiates replicative stress induced by oncogenes or chemotherapy. Antitumor activity of adavosertib has been demonstrated in preclinical models of pediatric cancer. This phase I trial was performed to define dose-limiting toxicities (DLT), recommended phase II dose (RP2D), and pharmacokinetics of adavosertib in combination with irinotecan in children and adolescents with relapsed or refractory solid tumors or primary central nervous system tumors.

Patients and Methods: Using a 3+3 escalation design, five dose cohorts of the combination of adavosertib and irinotecan (50/70; 65/70; 65/90; 85/90; 110/90 mg/m²/day) delivered on days 1–5 of a 21-day cycle were studied. Pharmacokinetics and analysis of peripheral blood γ H2AX was performed.

Results: Thirty-seven patients were enrolled; 27 were evaluable. The median (range) age was 14 (2–20) years. Twenty-five (93%) received prior chemotherapy (median, three regimens) and 21 (78%) received prior radiotherapy. Eleven patients had a primary central nervous system (CNS) malignancy. Common toxicities were hematologic and gastrointestinal. Two patients receiving adavosertib (110 mg/m²) in combination with irinotecan (90 mg/m²) experienced dose-limiting grade 3 dehydration. A patient with Ewing sarcoma had a confirmed partial response and 2 patients (ependymoma and neuroblastoma) had prolonged stable disease (≥ 6 cycles). Pharmacokinetics of adavosertib were variable but generally dose proportional and clearance was lower in younger patients.

Conclusions: Adavosertib (85 mg/m²) in combination with irinotecan (90 mg/m²) administered orally for 5 days was the MTD in children and adolescents with solid and CNS tumors.

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 (1, 2). These checkpoints allow for repair of DNA prior to mitosis and tolerance of replication stress, thereby maintaining tumor cell viability. Inhibition of CHK1 or WEE1 leads to replication fork collapse or mitotic catastrophe, generation of single, then double-strand DNA breaks and ultimately cellular death. In the presence of single-stranded DNA or DNA damage, there is induction of phos-

phorylated H2A (γ H2AX), a histone family member. Adavosertib (AZD1775) is a highly selective, ATP competitive, small-molecule inhibitor of WEE1 kinase and sensitizes tumor cells to cytotoxic agents and replication stress, including tumors with high levels of the MYCN oncogene (3, 4). Preclinical studies have shown antitumor activity of adavosertib as a single agent and in combination therapy in several carcinomas and the pediatric solid tumors including neuroblastoma (4), rhabdomyosarcoma (5), diffuse intrinsic pontine glioma (6), and medulloblastoma (7).

Adavosertib has been evaluated in adult solid tumor phase I studies as monotherapy and in combination with gemcitabine, cisplatin, or carboplatin (8, 9). No MTD was reached with monotherapy adavosertib. In the combination regimens, the most common adverse events included fatigue, nausea and vomiting, diarrhea, and hematologic toxicity. Ten percent of 176 evaluable patients had a partial response (PR; 4% confirmed) and 53% had stable disease (SD) of at least 6 weeks (8). The greatest activity within these studies was shown in BRCA-mutated cancers (9) and those with mutant TP53 (8, 10). A phase II study of adavosertib in combination with carboplatin confirmed that Wee1 inhibition enhanced efficacy in patients with TP53-mutant ovarian cancers refractory to prior platinum therapy (11). A glioblastoma multiforme (GBM) phase 0 study demonstrated the central nervous system tumor penetration of adavosertib (12). Therefore, adavosertib is being developed for the treatment of patients with advanced solid tumors and CNS malignancies with genetic deficiencies in DNA repair mechanisms.

The rationale for combining adavosertib with irinotecan for children with solid tumors was multifactorial. Irinotecan induces replication stress and adavosertib's mechanism of action involves

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Translational Relevance

Wee1 tyrosine kinase phosphorylates and inactivates cyclin-dependent kinases (CDK) in response to DNA damage or replication stress. Adavosertib (AZD1775) is a first-in-class inhibitor of Wee1 with promising antitumor activity as monotherapy and in combination with chemotherapy in several adult solid tumors. This study describes the first clinical experience of adavosertib in children and is the first clinical trial to evaluate adavosertib in combination with a topoisomerase inhibitor. Determination of the recommended phase II dose supports the ongoing phase II clinical trial in children with neuroblastoma, rhabdomyosarcoma, medulloblastoma, and other central nervous system embryonal tumors.

replication arrest override providing mechanistic support for this combination (2). Adavosertib in combination with irinotecan demonstrates synergistic growth inhibition in preclinical models (4, 5). The 5-day dosing regimen of irinotecan delivered every 21 days used in childhood cancer allows for serial dosing and exposure of adavosertib. In addition, irinotecan induces downregulation of another major cell-cycle checkpoint kinase, CHK1 (13), whose dual inhibition with WEE1 is synergistic in preclinical models (4, 14).

We report the phase I results of ADVL1312, a Children's Oncology Group multi-institutional dose escalation study of adavosertib in combination with irinotecan for children with relapsed or refractory solid and CNS tumors. The primary objectives were to estimate the MTD or recommended phase II dose (RP2D) of adavosertib administered on days 1 through 5 every 21 days in combination with oral irinotecan, to define and describe the toxicities of adavosertib in combination with oral irinotecan administered on this schedule and to characterize the pharmacokinetics of adavosertib. Secondary objectives included assessment of objective antitumor activity within the confines of a phase I trial, utilization of phosphorylation of histone family protein H2AX as a biomarker of induction of DNA damage and checkpoint over-ride in peripheral blood cells and assessment of MYCN by IHC in archival tumor samples from enrolled patients. This is the first report of adavosertib in children and in combination with a topoisomerase inhibitor in cancer.

Patients and Methods

Patient eligibility

Patients age 1 to 21 years with measurable or evaluable solid tumors, including central nervous system (CNS) tumors, refractory to standard treatment and for whom no known curative therapy existed, were eligible. Histologic verification of malignancy, at diagnosis or recurrence, was required with the exception of diffuse intrinsic brainstem tumors (DIPG). Other eligibility criteria included a Lansky or Karnofsky performance score of ≥ 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 (ANC) $\geq 1,000/\text{mm}^3$ and platelet count $\geq 100,000/\text{mm}^3$], renal function (normal serum creatinine for age and gender, or creatinine clearance $\geq 70 \text{ mL/minute}/1.73 \text{ m}^2$), liver function [bilirubin 1.5 times upper limit of normal for age, ALT $< 110 \text{ U/L}$, serum albumin $\geq 2 \text{ g/dL}$], QTc $\leq 480 \text{ msec}$, 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.

The protocol was reviewed and approved by the Cancer Therapeutics Evaluation Program (CTEP) 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

Adavosertib (10, 25, or 100 mg capsules) was supplied by Astra Zeneca and distributed by CTEP, NCI. Adavosertib was administered orally one 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, met discontinuation of protocol therapy criteria or a maximum of 18 cycles. Dose escalation proceeded step-wise for both adavosertib and irinotecan using a 3 + 3 design. Dose levels (DL) were adavosertib 50 mg/m²/day (DL1), 65 mg/m²/day (DL 2 and 3), 85 mg/m²/day (DL4), and 110 mg/m²/day (DL5) in combination with irinotecan 70 mg/m²/day (DL 1 and 2), or 90 mg/m²/day (DL3, 4, and 5). Inpatient dose escalation was not permitted. Once the MTD or RP2D was defined, up to 6 additional patients with relapsed/refractory solid tumors were enrolled to acquire toxicity and pharmacokinetic data in at least 6 patients less than or equal to 12 years old.

Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4. The DLT observation period for dose escalation and determination of MTD/RP2D was the first cycle of protocol therapy and included toxicity that was possibly, probably or definitely attributable adavosertib in combination with irinotecan. Hematologic dose-limiting toxicity (DLT) was defined as grade 4 neutropenia that persisted for greater than 7 days, platelet counts less than 20,000/mm³ on two separate measurements at least 48 hours apart or requiring two or more platelet transfusions within 7 days. Nonhematologic DLTs were defined as any grade 3 or 4 nonhematologic toxicity possibly, probably or definitely attributable to protocol therapy, with the specific exclusion of grade 3 nausea and vomiting of < 3 days in duration; grade 3 liver enzyme elevation < 7 days duration; grade 3 fever; grade 3 infection; grade 3 hypophosphatemia, hypokalemia, or hypomagnesemia responsive to oral supplementation; grade 3 diarrhea ≤ 3 days duration despite maximal supportive care; grade 3 mucositis or stomatitis ≤ 3 days duration; allergic reactions; any toxicity that required treatment interruption for > 14 days was considered dose limiting. Subjects who experienced a DLT during cycle 1 as well as subjects without DLT who received 100% of the prescribed protocol therapy and completed toxicity monitoring, were evaluable for determination of the MTD. The MTD was defined as the dose level in which fewer than one-third of patients experienced a DLT.

Pretreatment evaluations included a history and a physical exam; routine laboratory evaluations, (complete blood count, urinalysis, electrolytes, renal, and liver function) and electrocardiogram (EKG). A history, physical examination, and laboratory evaluations were obtained weekly during the first cycle, then before each subsequent cycle. Disease evaluations were obtained at baseline, the end of the first cycle, every other cycle $\times 2$ and then every 3 cycles. Disease response for solid tumors was assessed according to the revised Response Evaluation Criteria in Solid Tumors (RECIST v1.1) and neuroblastoma was assessed using bone marrow biopsies, anatomic imaging for measurable disease, and MIBG scintigraphy for evaluable MIBG-avid tumors. Curie scoring was used to assess response in patients with neuroblastoma who had evaluable disease by MIBG scintigraphy

without measurable disease. In patients with primary CNS, disease tumor response was evaluated by MRI; complete response (CR) was complete resolution of all lesions, partial response (PR) for CNS tumor was $\geq 50\%$ decrease in the sum of the products of the two perpendicular diameters of all target lesions. SD was neither sufficient decrease in the sum of the products of the two perpendicular diameters of all target lesions to qualify for PR, 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 performed at least 3 weeks after initial response was recorded. Central imaging review was completed for all participants who had institutional assigned objective response or had stable disease for six or more cycles. Best overall response is reported for each patient who had at least one radiographic assessment after initiation of protocol therapy or clinically progressive disease.

Pharmacokinetics

Blood samples (2–3 mL) were collected in dipotassium EDTA tubes during cycle 1: Day 1 (predose, 1, 2, 4, 6, 8, and 24 hours after dose), day 4 (predose), day 5 (predose, 1, 2, 4, 6, and 8 hours after dose) of cycle 1. Plasma was isolated by centrifugation (10 minutes \times 1,500 \times g) in a refrigerated centrifuge and immediately frozen. Adavosertib plasma concentrations were measured by hydrophilic interaction LC/MS-MS, as described previously (8, 15). Pharmacokinetic parameters were calculated using noncompartmental analysis (Phoenix WinNonlin 6.4; Pharsight Corporation; ref. 16).

Pharmacodynamics and correlative biology

For γ H2AX analysis, blood (1 mL) was processed using Smart Tubes (Thermo Fisher Scientific) at baseline, on day 1 immediately prior to adavosertib (1 hour after irinotecan) and 4 hours after adavosertib, as well as 24 hours after chemotherapy, prior to irinotecan on day 2. Percent positive γ H2AX peripheral blood cells was determined by flow cytometry and described as fold change over baseline for each patient (17). In brief, processed cells were fixed in 90% methanol, washed, then resuspended in staining buffer (0.5% BSA in PBS) with TruStain FcX and FITC-conjugated anti- γ H2AX Ser-139 antibody (1:100; clone JBW301, EMD Millipore). After 1 hour, cells were washed with PBS and resuspended in staining buffer with 2.5 μ g/mL propidium iodide (PI) and data were acquired on CytoflexS. FlowJo software was used to analyze data and identify dual PI/FITC-positive cells.

MYCN IHC was performed on archival tumor and control formalin-fixed paraffin-embedded tissue slides using the N-Myc antibody (Santa Cruz Biotechnology, SC-53993) according to protocol at a 1:100 dilution for 1 hour at room temperature. Antigen retrieval was performed with E1 retrieval solution for 20 minutes and staining was performed on a Bond RXm automated staining system using Bond Refine polymer staining kit (Leica Biosystems). Corresponding hematoxylin and eosin (H&E) and MYCN IHC slides were scored by a pediatric solid tumor pathologist for intensity and percent positivity.

Results

Dose escalation and determination of MTD

Between March 2014 and October 2016, 37 subjects enrolled, all of whom were eligible. Ten subjects were not evaluable for determination of the MTD for the following reasons: 2 subjects developed rapid clinical progression of disease, 6 did not receive 100% of protocol therapy (due to vomiting any amount of the prescribed chemotherapy, maximum two doses), and 2 subjects refused completion of all

Table 1. Characteristics of subjects evaluable for toxicity ($n = 27$).

Characteristic	Number (%)
Age (years)	
Median	14
Range	2–20
Sex	
Male	14 (52)
Female	13 (48)
Race	
White	19 (70.3)
Asian	2 (7.4)
Black or African American	3 (11.1)
Unknown	3 (11.1)
Ethnicity	
Non-Hispanic	24 (89)
Hispanic	3 (11)
Diagnosis	
Carcinoma	4 (14.8)
Ependymoma	4 (14.8)
Ewing sarcoma	4 (14.8)
Malignant glioma	7 (25.9)
Neuroblastoma	2 (7.4)
Osteosarcoma	3 (11.1)
Soft tissue sarcoma	2 (7.4)
Wilms tumor	1 (3.7)
Prior therapy	
Chemotherapy regimens	($n = 25$)
Median	3
Range	1–11
Radiotherapy	($n = 21$)
Median	1
Range	1–3

prescribed adavosertib and irinotecan during cycle 1 for reasons other than tolerability. Because no patient in this group experienced DLT, these patients were replaced per protocol. The characteristics of the 27 patients evaluable for determination of the MTD/RP2D are presented in **Table 1** and of all 37 enrolled (Supplementary Table S1).

Dose escalation and DLTs are presented in **Table 2**. At DL5, a patient with a disseminated epithelial–myoepithelial carcinoma had dose-limiting grade 3 dehydration. Dose level 5 expanded to 3 additional patients, and a patient with a synovial cell sarcoma experienced DLT (grade 3 dehydration, hypotension, and diarrhea). At DL4, there were no DLTs in 12 evaluable patients (6 in dose escalation and 6 in pharmacokinetic expansion), establishing irinotecan 90 mg/m² and adavosertib 85 mg/m², administered orally daily for 5 days as the pediatric MTD and RP2D.

Cycle 1 hematologic (all) and nonhematologic toxicities (in $\geq 10\%$ of all evaluable patients) are shown in **Table 3**, if there was at least one grade 3 event (Supplementary Table S2 for all grades). Of note, while dehydration was the DLT, this AE was not included in **Table 3** because it occurred in 2 of 27 participants, and did not meet the threshold criteria of $\geq 10\%$. The most common toxicity was diarrhea in 89% of subjects (11% grade ≥ 3), despite cefixime prophylaxis for irinotecan-related diarrhea.

Pharmacokinetics

Adavosertib pharmacokinetics, presented in **Table 4**, demonstrated substantial interpatient variability. Maximum concentration (C_{max}) and area under the concentration \times time curve (AUC) appeared to

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Table 2. Dose escalation and DLT summary.

Dose level	Irinotecan mg/m ²	Adavosertib mg/m ²	Number of patients enrolled	Number of patients evaluable for DLT	Number of patients with DLT	Type of DLT (n)
1	70	50	4	3	0	
2	70	65	5	3	0	
3	90	65	4	3	0	
4	90	85	10	6	0	
4 (PK)	90	85	6	6	0	
5	90	110	8	6	2	Dehydration (2), Diarrhea (1), Hypotension (1)

increase with dose. Plasma concentration–time curves for patients treated at the RP2D are shown in Fig. 1. The overall median oral clearance (Cl/F) was 32.5 (range 14.2–1,130 L/hour/m²) and was similar in females and males (33.0 L/hour/m² vs. 27.8 L/hour/m²; $P = 0.22$). However, children younger than age 12 had lower median Cl/F compared with children age 12 or older (21.4 L/hour/m² vs. 35.6 L/hour/m²; $P = 0.03$). Following 5 days of daily administration, modest accumulation of adavosertib was observed based on comparison of the day 1 versus day 5 AUC_{0–8h}. The median (range) accumulation ratio was 1.40 (0.62–5.79).

Antitumor activity

Any patient who received at least one dose of irinotecan and adavosertib and was observed for at least one cycle of therapy, or had progressive disease, was evaluable for response. Thirty-six subjects were evaluable for response, completing an average of 3 (range 1–18) cycles of protocol therapy. One subject with Ewing sarcoma had a confirmed partial response for 5 cycles. A patient with relapsed ependymoma completed the maximum allowable therapy (18 cycles) with a best response of SD; a patient with high-risk neuroblastoma with measurable, MIBG avid and marrow disease at baseline completed 11 cycles with a best response of stable disease.

Pharmacodynamics and correlative biology

Fold change of PBC γ H2AX protein expression relative to baseline was determined in 14 patients and descriptively presented in Table 5, with γ H2AX induction defined as a fold change greater than 1 (bold). It was hypothesized that γ H2AX induction after irinotecan and adavosertib would increase with dose level. There was induction of γ H2AX after treatment with irinotecan alone in 9 of 14 (64%) and adavosertib in combination with irinotecan in 10 of 14 (71%) of patients. At DL5, the mean fold change in induction of γ H2AX after the combination of irinotecan and adavosertib compared with baseline was 4.7 (95% CI: 1.1–19.1; $P = 0.03$).

Archival tissue sections were available for 23 patients. Protein expression of MYCN by IHC was present in an anaplastic astrocytoma and embryonal rhabdomyosarcoma tumor sample. Neither patient had a response or prolonged SD.

Discussion

This is the first clinical trial of adavosertib in pediatric patients, and the first report of adavosertib in combination with a topoisomerase inhibitor in cancer. The MTD and RP2D of this oral regimen was adavosertib (85 mg/m²/day) in combination with irinotecan

Table 3. Most common ($\geq 10\%$ in nonhematologic) toxicities summary (maximum grade shown), in cycle 1.

Toxicity type	Dose level and toxicity grade, n (%)											
	All dose levels (n = 27)		Dose level 1 (n = 3)		Dose level 2 (n = 3)		Dose level 3 (n = 3)		Dose level 4 (n = 12)		Dose level 5 (n = 6)	
	All grades	\geq Grade 3	All grades	\geq Grade 3	All grades	\geq Grade 3	All grades	\geq Grade 3	All grades	\geq Grade 3	All grades	\geq Grade 3
GI Toxicity												
Diarrhea	24 (89)	3 (11)	3 (100)	1 (33)	3 (100)		3 (100)		9 (75)		6 (100)	2 (33)
Vomiting	13 (48)	1 (4)	1 (33)		2 (67)		2 (67)		4 (33)		4 (67)	1 (17)
Nausea	12 (44)	1 (4)	1 (33)		2 (67)		2 (67)		4 (33)		3 (50)	1 (17)
Bone marrow toxicity												
Lymphocyte count decreased	18 (67)	6 (22)	2 (67)		1 (33)		3 (100)	1 (33)	9 (75)	5 (42)	3 (50)	
Anemia	15 (56)	1 (4)	1 (33)		1 (33)		2 (67)		8 (67)	1 (8)	3 (50)	
White blood cell decreased	12 (44)	3 (11)	2 (67)				2 (67)	1 (33)	5 (42)	2 (17)	3 (50)	
Neutrophil count decreased	9 (33)	6 (22)					2 (67)	2 (67)	5 (42)	2 (17)	2 (33)	2 (33)
Other												
Hypotension	1 (4)	1 (4)									1 (17)	1 (17)
Investigations												
Hypophosphatemia	7 (26)	1 (4)			2 (67)				4 (33)		1 (17)	1 (17)
Hypokalemia	6 (22)	2 (7)							3 (25)	1 (8)	3 (50)	1 (17)

Table 4. Adavosertib pharmacokinetic parameters, median (range).

Pharmacokinetic parameter	Dose level 1, 50 mg/m ²		Dose level 2, 65 mg/m ²		Dose level 3, 85 mg/m ²		Dose level 4, 110 mg/m ²	
	Day 1 (n = 4)	Day 5 (n = 4)	Day 1 (n = 4)	Day 5 (n = 4)	Day 1 (n = 4)	Day 5 (n = 3)	Day 1 (n = 12)	Day 5 (n = 2)
T _{max} (hour)	2.0 (2.0-8.0)	2.0 (2.0-2.1)	1.5 (1.0-3.8)	3.9 (2.0-4.0)	3.0 (2.0-4.0)	2.0 (1.0-4.0)	2.1 (2.0-8.0)	2.0 (2.0-4.0)
C _{max} nM	500 (208-567)	485 (280-809)	584 (61-815)	687 (360-747)	471 (312-905)	1,288 (779-1,430)	524 (328-1,151)	901 (54-1,069)
Half-life (hour)	4.6 (3.6-6.0)	5.8 (3.9-6.5)	5.8 (3.9-6.5)	5.4 (4.5-7.5)	5.4 (4.5-7.5)	5.4 (4.5-7.5)	4.7 (4.0-7.1)	4.4 (1.3-6.9)
AUC _{0-8h} h*nM	2,100 (776-2,181)	2,185 (1,312-3,112)	2,338 (329-3,511)	3,004 (1,905-4,064)	2,382 (1,281-4,605)	5,321 (3,798-6,301)	2,745 (1,381-5,050)	3,743 (266-5,236)
Accumulation (AUC day 5/day 1)	1.4 (0.6-1.7)	1.4 ^a (1.0-5.8)	1.4 ^a (1.0-5.8)	1.8 (1.4-2.0)	1.8 (1.4-2.0)	1.4 (1.2-2.0)	1.4 (1.2-2.0)	1.1 (0.9-1.3)
Cl/F (L/hour)	38 (31-170)	49 (27-247)	49 (27-247)	39 (26-76)	39 (26-76)	45 (23-114)	45 (23-114)	62 (38-1,198)
Cl/m ² (L/hour/m ²)	32 (17-80)	42 (21-129)	42 (21-129)	29 (17-59)	29 (17-59)	33 (14-63)	33 (14-63)	36 (21-1,130)
V/F (L)	271 (174-1,145)	348 (211-2,205)	348 (211-2,205)	298 (171-822)	298 (171-822)	320 (153-673)	320 (153-673)	386 (130-4,770)
V/m ² (L/m ²)	185 (150-540)	269 (181-1,148)	269 (181-1,148)	222 (112-642)	222 (112-642)	209 (95-645)	209 (95-645)	251 (70-4,500)

^aAUC ratio at dose level 2, n=4.

(90 mg/m²/day) daily for 5 days every 21 days. The DLTs, which occurred at DL5 (adavosertib 110 mg/m²/day in combination with irinotecan 90 mg/m²/day) included grade 3 dehydration (n = 2), diarrhea (n = 1), and hypotension (n = 1). Adavosertib (85 mg/m²/day) is equivalent to an adult fixed dose of 147 mg/day (735 mg/cycle). In adults receiving adavosertib twice daily × 2.5 days (5 doses/cycle) in combination with cisplatin or carboplatin, the MTD of adavosertib was 200 mg/dose (1,000 mg/cycle) and 225 mg/dose (1,125 mg/cycle), respectively. Differences in chemotherapy regimens including known nausea and vomiting associated with using the intravenous formulation of irinotecan orally in children, schedule and population may account for the lower MTD observed in children and adolescents compared with adult studies.

In our study, the plasma maximum concentration and exposure of adavosertib had moderate to high interpatient variability and generally increased in proportion to dose (Table 4) that also occurred in the adult studies (8, 9). As noted above, the lower pediatric MTD is likely multifactorial, however, comparison of the C_{max} and AUC_{0-8h} at the adult dose level of 225 mg, equivalent to 132 mg/m² in an individual with BSA of 1.7 m², suggest adavosertib might have lower oral clearance (Cl/F) in children. The high median C_{max} and AUC_{0-8h} values of 900 nM and 3,743 nM*hr at the 110 mg/m² dose level in our study compared with median C_{max} and AUC_{0-8h} values of 667 nM and 3,980 nM*hr at the 225 mg dose level in adults are consistent with a lower apparent clearance in children or coadministration of irinotecan with adavosertib. However, since oral clearance was not determined in the published adult phase I study (8), and adavosertib was administered with irinotecan in this study, it is difficult to directly compare this pharmacokinetic parameter. The maximum (Table 4) and median plasma concentrations of adavosertib at DL4 (Fig. 1) met the preclinical target of 240 nM also achieved in the adult clinical trials (8).

Prior pharmacodynamics studies to evaluate on-target inhibition of Wee1 by adavosertib and chemotherapy involved pre- and posttreatment tumor core or skin punch biopsies for inhibition of CDK1 phosphorylation or induction of γH2AX signifying enhanced DNA damage (8-10). For this study in children and adolescents, we examined peripheral blood mononuclear cell γH2AX induction by flow cytometry prior to irinotecan, prior to adavosertib (after irinotecan) and at 2 time points after the combination. There was induction of γH2AX after irinotecan alone or in combination with adavosertib in 10 of the 14 of patients (71%), and of those with induction, 80% had greater induction with the combination of irinotecan and adavosertib than with irinotecan alone (Table 5). A limitation of this analysis is that we do not have a time course of irinotecan PBC γH2AX induction alone to determine whether the increased induction of γH2AX is due to the addition of adavosertib. Of interest, DL2 patient 3 (DL2-3) received irinotecan on day 1 but had emesis of the adavosertib, and no induction of γH2AX in PBCs was observed at either time point. For patient 1 of DL5 (DL5-1), γH2AX induction in PBCs was observed after irinotecan only, but not after the combination. This patient had an adavosertib C_{max} of 54 nM, which is below the goal 240 nM for Wee1 target engagement concentration.

A secondary objective of this study was to assess objective antitumor activity within the confines of a phase I trial. There was one confirmed PR observed in a patient with Ewing sarcoma. In addition, 2 patients received ≥ 6 cycles of therapy, including one patient with ependymoma and another with neuroblastoma who completed 18 cycles and 11 cycles, respectively. None of these patient's archival tumor samples had high levels of MYCN, and genomic analysis was not available to determine whether they have a defective DNA damage and repair

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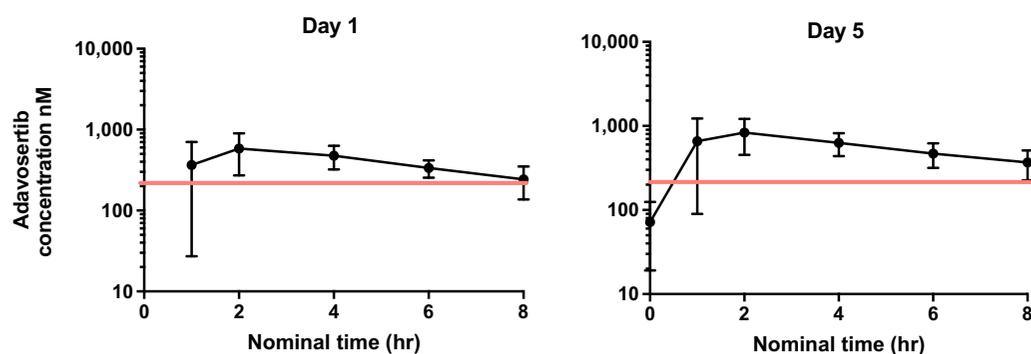


Figure 1.

Median plasma concentration curves of adavosertib on day 1 and 5 ($N = 12$) at the RP2D of irinotecan 90 mg/m² and adavosertib 85 mg/m². Blood samples were collected pre-dose and during cycle 1 at 1, 2, 4, 6, and 8 hours after the first dose on day 1 and last dose on day 5. The adavosertib concentrations met the goal Wee1 target engagement concentration of 240 nM (red line).

Table 5. Peripheral blood cell γ H2AX fold induction on day 1, 1 hour after irinotecan (column 4), and 4 hours after irinotecan and adavosertib (column 5); and day 2, approximately 24 hours after chemotherapy (column 6).

DL Patient ID	Adavosertib day 1 C_{max} (nM)	Day 1 Prechemo baseline	Day 1 fold change IRN alone	Day 1 fold change IRN and adavosertib	Day 2 fold change IRN and adavosertib
DL1-1	439	1.0	0.9	3.0	1.3
DL1-2	567	1.0	1.8	1.6	2.5
DL1-3	208	1.0	1.2	9.3	ND
DL2-1	435	1.0	0.7	0.7	0.4
DL2-2	733	1.0	2.0	0.3	2.0
DL2-3 ^a	ND	1.0	0.5	0.3	0.7
DL3-1	380	1.0	1.4	3.3	3.2
DL3-2	ND	1.0	32.0	4.3	4.7
DL4-1	1050	1.0	1.8	4.5	2.7
DL4-2	681	1.0	0.7	0.9	0.3
DL4-3	433	1.0	1.0	0.6	1.2
DL5-1 ^b	54	1.0	1.8	0.7	0.6
DL5-2	737	1.0	1.3	5.8	7.6
DL5-3	1069	1.0	3.0	23.4	4.7

Note: Bolded numbers indicate fold induction > 1 over baseline.

Abbreviation: IRN, irinotecan.

^aPatient vomited adavosertib.

^bAdavosertib maximum plasma concentration is below goal target concentration of 240 nM.

pathway, which may be of interest in future studies given the prevalence of these conditions in pediatric cancer (18). The previously published Children's Oncology Group solid tumor phase II trial of single-agent irinotecan-treated 161 patients with a 5% overall objective response rate (19).

In conclusion, the RP2D in children and adolescents with relapsed and/or refractory solid and CNS tumors is adavosertib 85 mg/m²/day in combination with irinotecan (90 mg/m²/day) daily for 5 days every 21 days. The regimen was tolerable with the proportion of grade 3 and grade 4 hematologic and nonhematologic adverse events and DLT consistent with those that could be expected from an irinotecan-containing regimen (19, 20). There was suggestion of clinical activity in this heavily pretreated relapsed and recurrent population of patients. The phase II expansion cohorts of adavosertib in combination with irinotecan in patients with relapsed and refractory neuroblastoma, medulloblastoma, and

CNS embryonal tumors or rhabdomyosarcoma are actively accruing further evaluating the potential of this combination in the treatment of children and adolescents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Pediatric Phase 1 Trial of Adavosertib/AZD1775 and Irinotecan

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