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Preclinical evaluation of the combination of AZD1775 and irinotecan against selected pediatric solid tumors: A Pediatric Preclinical Testing Consortium report

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Abstract

Introduction: WEE1 is a serine kinase central to the G₂ checkpoint. Inhibition of WEE1 can lead to cell death by permitting cell-cycle progression despite unrepaired DNA damage. AZD1775 is a WEE1 inhibitor that is in clinical development for children and adults with cancer.

Methods: AZD1775 was tested using a dose of 120 mg/kg administered orally for days 1 to 5. Irinotecan was administered intraperitoneally at a dose of 2.5 mg/kg for days 1 to 5 (one hour after AZD1775 when used in combination). AZD1775 and irinotecan were studied alone and in combination in neuroblastoma ($n = 3$), osteosarcoma ($n = 4$), and Wilms tumor ($n = 3$) xenografts.

Results: AZD1775 as a single agent showed little activity. Irinotecan induced objective responses in two neuroblastoma lines (PRs), and two Wilms tumor models (CR and PR). The combination of AZD1775 + irinotecan-induced objective responses in two neuroblastoma lines (PR and CR) and all three Wilms tumor lines (CR and 2 PRs). The objective response measure improved compared with single-agent treatment for one neuroblastoma (PR to CR), two osteosarcoma (PD1 to PD2), and one Wilms tumor (PD2 to PR) xenograft lines. Of note, the combination yielded CR ($n = 1$) and PR ($n = 2$) in all the Wilms tumor lines. The event-free survival was significantly longer for the combination compared with single-agent irinotecan in

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CONFLICTS OF INTEREST

The authors consider that there are no actual or perceived conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

all models tested. The magnitude of the increase was greatest in osteosarcoma and Wilms tumor xenografts.

Conclusions: AZD1775 potentiates the effects of irinotecan across most of the xenograft lines tested, with effect size appearing to vary across tumor panels.

Keywords

AZD1775; irinotecan; neuroblastoma; osteosarcoma; preclinical testing; Wilms tumor

1 | INTRODUCTION

Reproducible and precise progression through the cell cycle is dependent on the activation of the cyclin-dependent kinases (CDKs). There are nine CDKs involved in cell-cycle regulation. Inhibition of CDK function may result in cell-cycle arrest and apoptosis. CDK1 is critical for progression from G₂ to the M phase of the cell cycle. Through phosphorylation of CDK1 at tyrosine 15, the WEE1 kinase halts cell-cycle progression in response to DNA damage.¹ WEE1 is also involved in stabilizing DNA replication forks and homologous recombination repair.² The ataxia-telangiectasia-related (ATR) kinase is a key mediator for the repair of DNA single-strand breaks through phosphorylation of the checkpoint kinase CHK1.³ CHK1 in turn phosphorylates and activates WEE1. Phosphorylated WEE1 inhibits CDK1/cyclin B function, thus inducing cell-cycle arrest at G₂ and permitting DNA-damage repair. Conversely, inhibition of WEE1 will permit premature entry into the M phase leading to mitotic catastrophe and apoptosis.¹ WEE1 kinase also plays a central role in the G₂-M cell-cycle checkpoint and is also required during S phase. WEE1 phosphorylation of CDK2 during S phase allows the maintenance of stalled replication forks.

AZD1775 is a potent and selective ATP-competitive inhibitor of WEE1, with promising activity potentiating the genotoxic activity of anticancer agents. Preclinical studies demonstrate AZD1775 potentiation of topoisomerase-I inhibitors, antimetabolites, DNA cross-linking agents and radiation. In osteosarcoma xenografts, a 50% reduction in tumor growth is reported with single-agent AZD1775 and a 70% reduction in combination with gemcitabine. In these models, AZD1775 predictably induced G₂-M escape and apoptosis. AZD1775 enhanced the effect of gamma radiation in orthotopic glioma xenograft tumors.⁵ The combination of AZD1775 with irinotecan and vincristine improved response for orthotopic xenograft models of high-risk rhabdomyosarcoma relative to irinotecan and vincristine alone.⁶ In acute myeloid leukemia, AZD1775 enhanced the antitumor effect of the histone deacetylase inhibitor panobinostat,⁷ the CDK inhibitor roscovitine,⁸ and cytarabine.⁹ It is possible that G₁ checkpoint control is deficient in tumors with *TP53* mutations. Tumors harboring such mutations may therefore be more dependent on the S and G₂ phase checkpoints, suggesting that S and G₂ checkpoint abrogation by WEE1 inhibition may selectively sensitize TP53-deficient tumors.¹⁰ However, others have demonstrated that AZD1775 effect is independent of TP53.^{11,12} It is likely that the relationship between AZD1775 activity and TP53 status is context dependent and may not be the critical determinant of activity.

Two pediatric phase 1 trials that are assessing the safety and feasibility of combining of AZD1775 either with local radiation therapy in treating newly diagnosed DIPG (NCT01922076) or with irinotecan hydrochloride in advanced solid tumors (NCT02095132). These data will inform the pediatric development of AZD1775 in combination with conventional therapies for pediatric malignancies. The PPTC sought to supplement these data by evaluating the ability of AZD1775 to potentiate the in vivo activity of irinotecan across a range of pediatric solid tumors, given the broad use of irinotecan in the relapse setting for pediatric patients with solid tumors.

2 | METHODS

2.1 | In vivo tumor growth inhibition studies

CB17SC *scid^{-/-}* female mice (Taconic Farms, Germantown NY) were used to propagate subcutaneously implanted tumors as previously described.¹³ All mice were maintained under barrier conditions, and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Female mice were used irrespective of the gender from which the tumor was derived. Ten mice were used per group.

2.2 | Statistical analysis

A tumor event is defined as a quadrupling of tumor volume from the day of treatment initiation, where tumor volume is estimated from caliper measurements as $(4/3) \times \pi \times [(length + width)/4]^3$.³ The exact time to event is estimated by interpolating between the measurements directly preceding and following the event, assuming log-linear growth. Differences in event-free survival (EFS) between experimental groups are tested using the Gehan-Wilcoxon test. Relative tumor volume (RTV) is defined for each mouse as the ratio of its current tumor volume divided by baseline tumor volume. Comparisons between treatments groups of the minimum attained RTV are performed using the Wilcoxon rank sum test.

The objective response categories are progressive disease (PD, which is subdivided among treated mice into PD without and with growth delay, PD1 and PD2, respectively), stable disease (SD), partial response (PR), complete response (CR), and maintained complete response (MCR), defined below.

PD	< 50% tumor regression throughout study and > 25% tumor growth at the end of the study.
PD1	PD and the mouse's time to event \geq 200% the Kaplan-Meier (KM) median time to event in the control group.
PD2	PD and the mouse's time to event > 200% of the KM median time to event in the control group.
SD	< 50% tumor regression throughout study and \leq 25% tumor growth at the end of the study.
PR	\geq 50% tumor regression at any point during study, but measurable tumor throughout the study period.
CR	Disappearance of measurable tumor mass during the study period.
MCR	No measurable tumor mass for at least three consecutive weekly readings at any time after treatment has been completed.

Overall group response is determined by the median response among evaluable mice. Each individual mouse is assigned a score from 0 to 10 based on its response: PD1 = 0, PD2 = 2, SD = 4, PR = 6, CR = 8, and MCR = 10, with the median for each group determining overall response. If the median score is half-way between response categories, the objective response is assigned to the lower response category (e.g., a median response score of 9 is scored CR).

Mice experiencing a possibly treatment-related death (i.e., drug toxicity), mice with failed engraftment, and mice which unexpectedly die for reasons unrelated to treatment are excluded from statistical analyses of time to event, minimum tumor volume, and objective response.

For combination testing projects, one objective was to demonstrate that the combination is significantly more effective than either agent utilized at their optimal/standard single-agent dose/schedule. This condition is termed therapeutic enhancement, which represents a therapeutic effect for which a tolerated regimen of a combination treatment exceeds the optimal effect achieved at any tolerated dose of monotherapy associated with the same drugs used in the combination.^{14,15} This definition was operationalized as follows: therapeutic enhancement was considered present when the tumor growth delay (T-C) for a combination was greater than the tumor growth delay for each of the single agents tested at their maximum tolerated dose (MTD) and when the EFS distribution for the combination treatment was significantly better than the EFS distributions for both of the single agents tested at their MTD. In order to control experiment-wise Type I error at 5%, statistical tests were evaluated at the Bonferroni-corrected significance level $\alpha = 0.01$, due to the five comparisons being made (combination vs agent 1 alone, combination vs agent 2 alone, agent 1 vs control, agent 2 vs control, and combination vs control). Testing was considered not evaluable for therapeutic enhancement if either single agent used alone produced median EFS beyond the observation period.

Combination testing results were also analyzed for evidence of supra-additivity for the combination, in comparison with the expected treatment effect assuming additivity. As described previously,¹⁵ a linear regression model for time to event (T) was employed, with testing to determine whether the treatment interaction of the two-drug combination is significantly different from zero. The interaction model was as follows: $T = a_0 + a_1 \times I_1 + a_2 \times I_2 + a_3 \times I_1 \times I_2$, in which I_1 and I_2 are indicator functions for drugs 1 and 2, respectively. The coefficients are for the no-treatment control (a_0), the two-drug effects relative to control (a_1 and a_2), and the treatment interaction effect of the two drugs relative to control (a_3). Whether the value of a_3 is significantly greater or less than zero ($P < 0.01$) indicates supra-additivity or sub-additivity of the drug combination, respectively; otherwise, the drug combination is considered additive. In addition, the estimated values for these coefficients are the estimated time to event (or additional time to event) associated with the corresponding treatment effects. To allow comparison of the a_3 values across xenografts and across doses of the standard agents, values were normalized by computing the ratio of a_3 to the expected effect of the combination under the assumption of additivity (i.e., $a_1 + a_2$). If any animals were censored in any treatment group, the combination was not considered evaluable unless (1) the censoring occurred exclusively in the combination group, and the

combination effect was supra-additive, or (2) the censoring occurred exclusively in one or both single-agent groups, and the combination effect was sub-additive.

2.3 | Drugs and formulation

AZD1775 was provided to the PPTC by AstraZeneca. It was administered by oral gavage as a suspension in a vehicle of 0.5% w/v methyl cellulose in deionized water on days 1 to 5 at a dose of 120 mg/kg. Irinotecan was purchased from the University Hospital Pharmacy (camptosar) and was administered intraperitoneally at a dose of 2.5 mg/kg on days 1 to 5. The combination of AZD1775 and irinotecan used the same doses and schedules of agents as applied for single-agent testing.

3 | RESULTS

AZD1775 was tested alone and in combination with irinotecan. Detailed testing results are provided in Supporting Information Table S1. As a single agent, the average maximum weight loss across all lines tested was $1.4\% \pm 0.6\%$ for AZD1775, whereas irinotecan produced an average weight loss of $0.9\% \pm 0.4\%$. In combination, the average maximum weight loss was $6.7\% \pm 1.8\%$, which was greater than the weight loss observed with either AZD1775 or irinotecan when administered as a single agent. However, no mortality occurred following the administration of AZD1775 alone or in combination with irinotecan.

3.1 | Neuroblastoma

Three neuroblastoma lines (NB-1643, NB-SD, and NB-EBc-1) were evaluated (Table 1 and Figure 1). Single-agent AZD1775 showed minimal activity, with only one of three lines showing a significant prolongation in time to event for the treated versus control groups. All three lines showed PD1 responses with EFS T/C values ranging from 1.12 to 1.42. Irinotecan as a single agent caused significant differences in EFS distribution for all three lines evaluated. Two of three lines showed objective responses (both PRs) and EFS T/C values ranged from 2.58 to 5.37. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with control in all lines tested and produced objective responses in two lines (CR and PR) and a PD2 response in the remaining line. EFS T/C values ranged from 3.13 to 6.7. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with single-agent irinotecan for all three neuroblastoma lines, although the prolongation in time to event was only two to eight days. The combination also produced for each of the neuroblastoma models minimum tumor volumes that were significantly smaller than those produced by single-agent irinotecan.

3.2 | Osteosarcoma

Four osteosarcoma lines (OS-1, OS-2, OS-31, and OS-33) were evaluated (Table 1 and Figure 1). Single-agent AZD1775 showed limited activity, with three of the four lines showing a significant prolongation in time to event for the treated versus control groups. However, all four lines showed only PD1 responses with EFS T/C values ranging from 1.06 to 1.66. Irinotecan as a single agent caused significant differences in EFS distribution for all four lines evaluated. Three of four lines showed PD1 responses and a PD2 response in the remaining line. The EFS T/C values for single-agent irinotecan ranged from 1.23

to 2.12. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with control in all lines tested and produced a PD2 response in three of the lines. EFS T/C values ranged from 1.69 to 3.48. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with single-agent irinotecan for all osteosarcoma lines, with prolongation in time to event of 17 to 23 days. The combination also produced for each of the osteosarcoma models minimum tumor volumes that were significantly smaller than those produced by single-agent irinotecan.

3.3 | Wilms tumor

Three tumor lines (KT10, KT-11, and KT-13) were evaluated (Table 1 and Figure 1). Single-agent AZD1775 showed limited activity. All three lines showing a significant prolongation in time to event for the treated versus control groups, but each showed only PD1 responses with EFS T/C values ranging from 1.21 to 1.62. Irinotecan as a single agent caused significant differences in EFS distribution for all three lines evaluated. Irinotecan showed objective responses in two lines (CR and PR) and a PD2 response in the remaining line. The EFS T/C values ranged from 2.72 to 3.64. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with control in each of the three tumor lines tested and produced objective responses in all the lines (CR and two PRs). EFS T/C values ranged from 3.23 to 4.71. The combination of AZD1775 and irinotecan significantly prolonged time to event compared with single-agent irinotecan for two tumor lines with prolongation in time to event of 8.1 and 17 days. The combination significantly reduced minimum tumor volume in only one tumor model when compared with irinotecan alone.

3.4 | Evaluation for therapeutic enhancement and for supra-additivity for the AZD1775 and irinotecan combination

Therapeutic enhancement requires that the activity of the combination under evaluation be superior to either of the single agents given at tolerable preclinical doses. Therapeutic enhancement was observed for the AZD1775 plus irinotecan combination for each of the neuroblastoma and osteosarcoma models tested, and it was observed for two of three Wilms tumor models evaluated (Table 2).

A model-based analysis for supra-additivity was applied using analysis methods previously applied to rapamycin plus chemotherapy combinations.¹⁵ Supra-additivity was identified for one of three neuroblastoma lines (NB-1643), two of four osteosarcoma lines (OS-31 and OS-33), and zero of three Wilms tumor lines (Table 3). The combination effect for the remaining lines was consistent with additivity. The “normalized interaction term” shown in Table 3 can be conceptualized as representing the percentage gain or loss of the expected treatment effect observed for the combination under additivity. The normalized interaction term was greatest for the OS-31 followed by OS-33 and NB-1643. The greater magnitude for the combination effect for OS-31 compared with OS-33 and NB-1643 can be visually appreciated in Figure 1.

4 | DISCUSSION

Evaluation of the WEE1 kinase inhibitor AZD1775, alone and in combination against xenograft models of neuroblastoma ($n = 3$), osteosarcoma ($n = 4$), and Wilms tumor ($n = 3$), demonstrated that all treatment regimens were relatively well tolerated with no toxic mortality observed. Murine dosing of irinotecan is well described.^{16,17} The dose of irinotecan used (2.5 mg/kg/day for five days) was intended to provide an SN-38 concentration to approximate the 50mg/m²/day for five days dosing in children. The maximally tolerated dose for irinotecan in the mouse is upward of 50 mg/kg for five days (approximately 20 times the dose used in the current experiment). Toxicity mortality was not observed, nor would it be expected at the administered dose or irinotecan. Extrapolation of murine to human dosing for AZD1775 is not as well established. For the experiments described herein, AZD1775 dosing was 120 mg/kg administered orally for days 1 to 5. At a dose of 50 mg/kg in athymic mice, Pokorny et al. reported peak whole blood AZD1755 concentration of 7.78 μ M (\pm 2.15 μ M) 1 to 2 hours after dosing.¹⁸ In a phase 1 pharmacokinetic analysis, Do et al. reported peak AZD1775 serum concentrations of 1.65 μ M after five doses at 225 mg twice daily.¹⁹ Stewart et al. performed a detailed pharmacokinetic analysis of AZD1755 in mice and found that a dose of 60 mg/kg twice daily best approximated the achievable human clinical exposure based on a comparison of murine plasma AUC_{inf} and C_{avg} with reported human plasma pharmacokinetic values.⁶ Hence, it is likely that the murine dose of AZD1775 that we used produces peak concentrations beyond those achievable in humans, but the dosing used in these experiments is within the range of other preclinical evaluations of AZD1775. The fact that weight loss for the combination was more than additive (1.4% and 0.9% in single-agent groups vs 6.7% in the combination) suggests that there may be increased toxicity with the addition of AZD1775 to irinotecan.

As a single-agent AZD1775 shows little activity with all groups demonstrating progressive disease with some tumor growth delay. Irinotecan as a single agent yielded objective responses in the neuroblastoma and Wilms tumor lines. In all lines tested except one (KT-10), the AZD1775 with irinotecan combination demonstrated significantly improved EFS and induced smaller minimum relative tumor volumes when compared with irinotecan alone. The impact on survival was modest, likely because the predominant response was a delay in tumor regrowth following single cycle of therapy. These results suggest that this rationally designed combination of a cell-cycle checkpoint inhibitor with a DNA-damaging agent may have clinical utility in pediatric cancers.

Although AZD1775 potentiated the effects of irinotecan across most of the xenograft lines tested, the magnitude of potentiation of irinotecan by AZD1775 appeared greater for the osteosarcoma and Wilms tumor xenograft lines compared with the neuroblastoma lines. Others have previously reported similarly favorable combination strategies with AZD1775, both in osteosarcoma cell lines and in vitro and in vivo for neuroblastoma and in vivo for rhabdomyosarcoma.^{4,6,20,21} This is the first report of combination activity in Wilms tumor. Though the in vivo combination effect in the current study is significant, we did not observe complete, sustained responses. Russell et al. report improved activity with combination CHK1 and WEE1 inhibition that may provide more potent means for cells to escape G₂-M

with subsequent apoptosis.¹⁶ Additionally, other DNA-damaging agents (e.g., cisplatin) or antimetabolites (e.g., gemcitabine) may enhance cell dependence on cell-cycle checkpoint control for survival. Nonetheless, together these data demonstrate the role of WEE1 in limiting anticancer activity following irinotecan-induced DNA damage in osteosarcoma, neuroblastoma, and Wilms tumor.

The relationship between TP53 status and AZD1775-mediated chemopotential remains poorly understood. There is evidence in some tumor types that AZD1775-mediated chemopotential is greatest when TP53 is defective.^{10,22,23} Others have found no association with TP53 status and AZD1775 effect.^{12,24,25} In the current study, the osteosarcoma lines lack TP53 demonstrated through absent expression. KT-13 and NB-SD have p.C176Y and p.C176FTP53 mutations, respectively. The degree of chemopotential among the xenograft lines tested in this study was greatest for lines with defective TP53, although there was variability in the magnitude of potentiation, suggesting that there are additional unknown mediators adding to the role of WEE1 in cell-cycle regulation in these malignant tumor lines.

When considered with recently reported AZD1775 in vivo combination testing results for rhabdomyosarcoma,⁶ these results support pediatric clinical evaluations of AZD1775 in combination with irinotecan, as is ongoing in [NCT02095132](#). The European Proof-of-Concept Therapeutic Stratification Trial of Molecular Anomalies in Relapse or Refractory Tumors (ESMART, [NCT2813135](#)) includes an arm combining AZD1775 with carboplatin. Future studies should focus on combination strategies to enhance G₂-M escape as well as additional genotoxic therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

Abbreviations:

CDKs	cyclin-dependent kinases
CR	complete response
MCR	maintained complete response
PD	progressive disease

PR	partial response
SD	stable disease

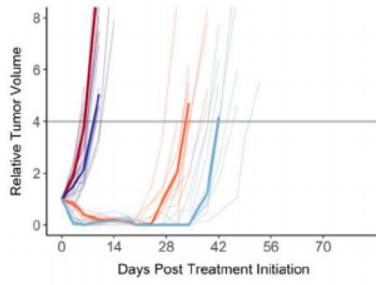
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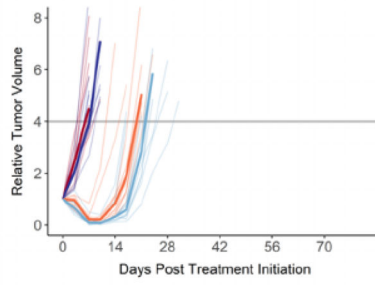
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A: Relative Tumor volume

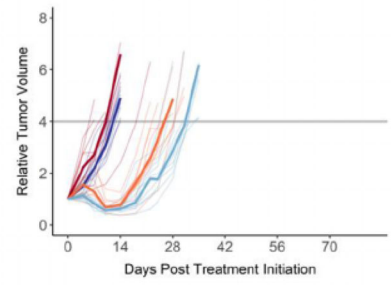
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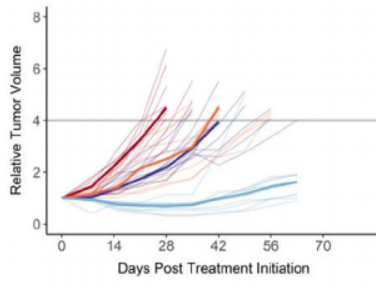
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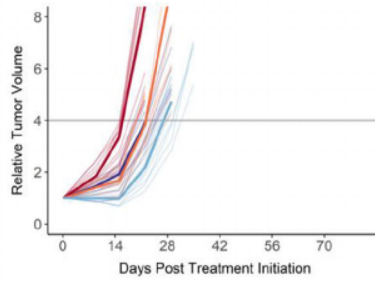
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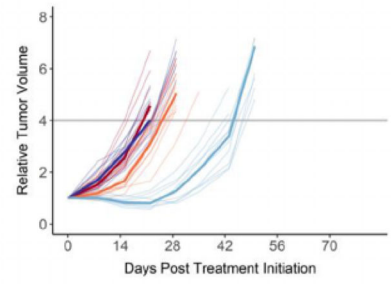
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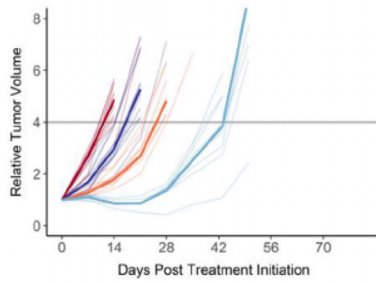
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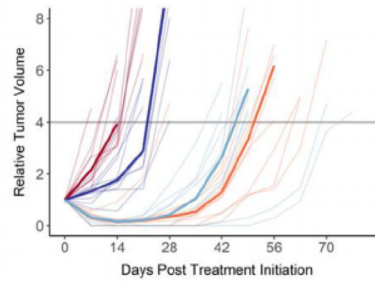
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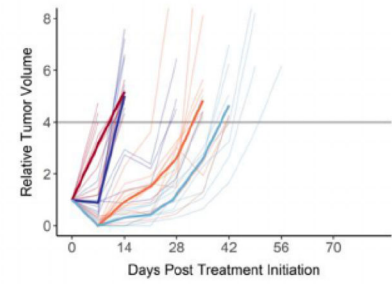
OS-33



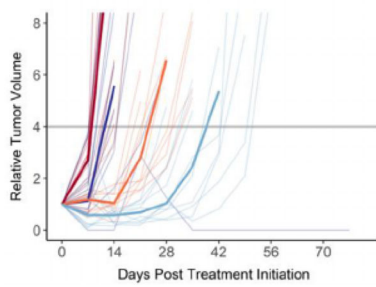
KT-10



KT-11



KT-13



— Control
— AZD1775
— Irinotecan
— AZD1775 + Irinotecan

B: Kaplan-Meier Event-Free Survival

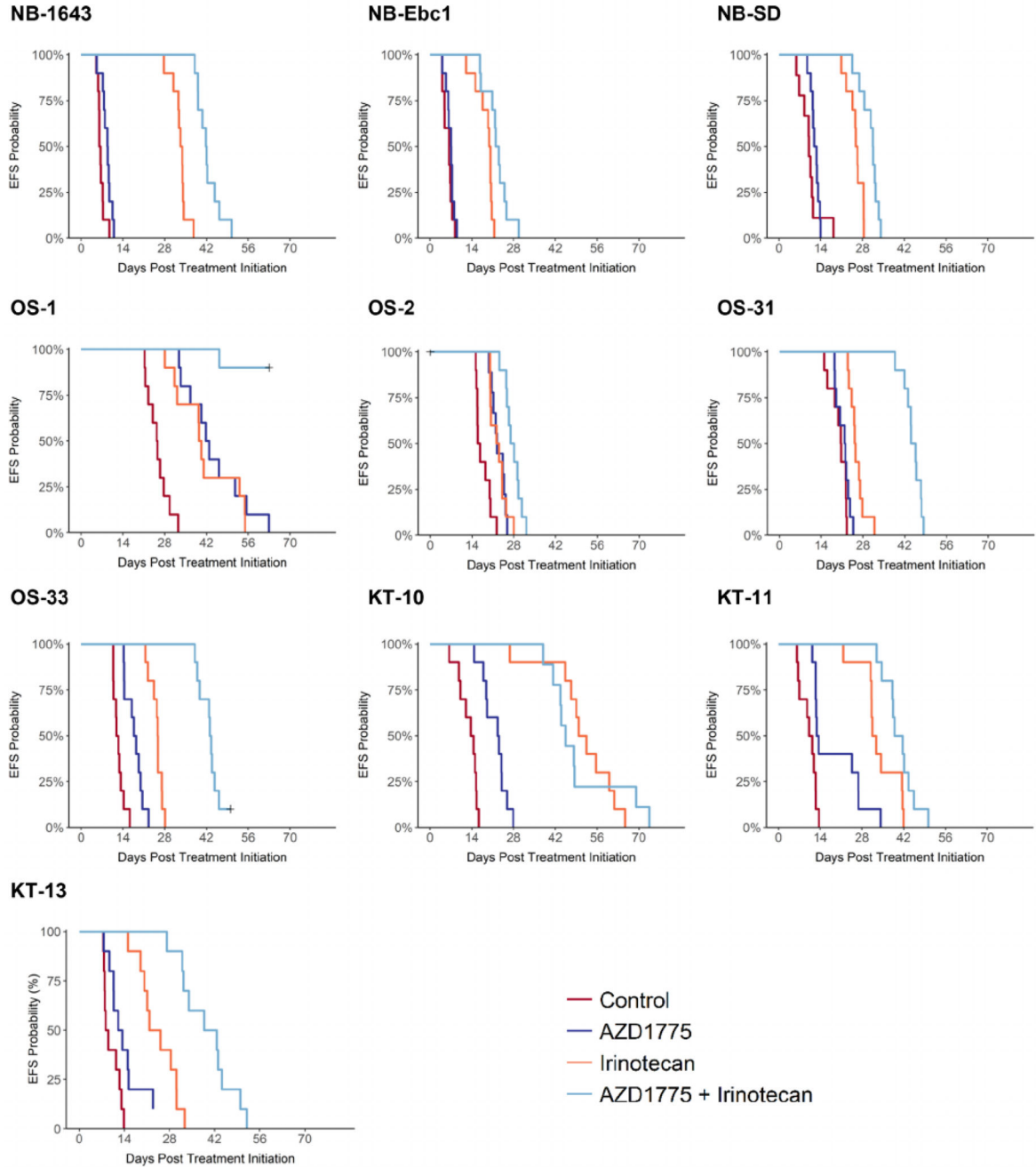


FIGURE 1.

Four treatment groups were employed: A, control; B, single-agent AZD1775; C, single-agent irinotecan; D, combination of AZD1775 and irinotecan. Doses and schedules are provided in the Methods section. Results are shown for neuroblastoma, osteosarcoma, and Wilms tumor xenograft lines

TABLE 1
Summary of the in vivo activity of AZD1775 and irinotecan as single agents and in combination

Line	Histology	Treatment	EFS (d)	P	EFS T-C	EFS T/C	MinRTV	Response
NB-1643	Neuroblastoma	AZD1775	8.9	$P=0.009$	2.6	1.42	1.455	PDI
NB-1643	Neuroblastoma	IR	33.6	$P<0.001$	27.3	5.37	0.032	PR
NB-1643	Neuroblastoma	AZD1775 + IR	41.9	$P<0.001$	35.6	6.70	0.000	CR
NB-SD	Neuroblastoma	AZD1775	12.2	$P=0.021$	2.1	1.21	1.544	PDI
NB-SD	Neuroblastoma	IR	26	$P<0.01$	15.9	2.58	0.730	PD2
NB-SD	Neuroblastoma	AZD1775 + IR	31.6	$P<0.01$	21.5	3.13	0.532	PD2
NB-Ebc1	Neuroblastoma	AZD1775	7.1	$P=0.257$	0.8	1.12	1.997	PDI
NB-Ebc1	Neuroblastoma	IR	20.1	$P<0.001$	13.7	3.18	0.232	PR
NB-Ebc1	Neuroblastoma	AZD1775 + IR	22.5	$P<0.001$	16.2	3.56	0.091	PR
OS1	Osteosarcoma	AZD1775	12.7	$P<0.001$	16.9	1.66	1.063	PDI
OS1	Osteosarcoma	IR	23.5	$P<0.001$	14.4	1.57	1.127	PDI
OS1	Osteosarcoma	AZD1775 + IR	40.6	$P<0.001$	>37.6	>2.48	0.579	PD2
OS2	Osteosarcoma	AZD1775	42.4	$P<0.001$	6.0	1.37	1.403	PDI
OS2	Osteosarcoma	IR	39.9	$P<0.001$	6.2	1.38	1.385	PDI
OS2	Osteosarcoma	AZD1775 + IR	>63	$P<0.001$	11.2	1.69	0.957	PDI
OS31	Osteosarcoma	AZD1775	21.9	$P=0.327$	1.3	1.06	1.765	PDI
OS31	Osteosarcoma	IR	25.3	$P<0.001$	4.7	1.23	1.349	PDI
OS31	Osteosarcoma	AZD1775 + IR	44.9	$P<0.001$	24.3	2.18	0.789	PD2
OS33	Osteosarcoma	AZD1775	18.1	$P<0.001$	5.9	1.49	1.714	PDI
OS33	Osteosarcoma	IR	25.7	$P<0.001$	13.6	2.12	1.365	PD2
OS33	Osteosarcoma	AZD1775 + IR	43.4	$P<0.001$	31.3	3.58	0.837	PD2
KT-10	Wilms	AZD1775	22.8	$P<0.001$	8.8	1.62	1.278	PDI
KT-10	Wilms	IR	51.1	$P<0.001$	37.1	3.64	0.149	PR
KT-10	Wilms	AZD1775 + IR	45.4	$P<0.001$	31.4	3.23	0.090	PR
KT-11	Wilms	AZD1775	13.1	$P=0.003$	2.3	1.21	0.832	PDI
KT-11	Wilms	IR	32.2	$P<0.001$	21.3	2.97	0.107	CR
KT-11	Wilms	AZD1775 + IR	40.3	$P<0.001$	29.4	3.72	0.050	CR
KT-13	Wilms	AZD1775	12.7	$P=0.055$	4.1	1.48	1.356	PDI
KT-13	Wilms	IR	23.5	$P<0.001$	14.9	2.71	0.973	PD2

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Line	Histology	Treatment	EFS (d)	P	EFS T-C	EFS T/C	MinRIV	Response
KT-13	Wilms	AZD1775 + IR	40.6	$P < 0.001$	21.0	4.71	0.311	PR

TABLE 2

Evaluation for therapeutic enhancement

Disease	Xenograft	P (combination vs AZD1775)	P (combination vsirinotecan)	Therapeutic enhancement
Neuroblastoma	NB-1643	<0.001	<0.001	Yes
Neuroblastoma	NB-Ebc1	<0.001	0.009	Yes
Neuroblastoma	NB-SD	<0.001	0.002	Yes
Osteosarcoma	OS1	<0.001	<0.001	Yes
Osteosarcoma	OS2	<0.001	<0.001	Yes
Osteosarcoma	OS31	<0.001	<0.001	Yes
Osteosarcoma	OS33	<0.001	<0.001	Yes
Wilms tumor	KT-10	<0.001	0.281	No
Wilms tumor	KT-11	<0.001	0.010	Yes
Wilms tumor	KT-13	0.001	>0.001	Yes

P values reflect differences in EFS between experimental groups and are tested using the Gehan-Wilcoxon test.

TABLE 3

Evaluation for supra-additivity

Disease	Xenograft	Normalized interaction term	Total effect expected	Interaction parameter estimate	P	R ²	Interpretation
Neuroblastoma	NB-1643	0.26	28.56	7.32	<0.001	0.98	Supra-additive
Neuroblastoma	NB-Ebc1	0.22	13.54	2.97	0.085	0.89	Additive
Neuroblastoma	NB-SD	0.18	17.22	3.11	0.083	0.92	Additive
Osteosarcoma	OS1	0.03	34.53	1.16	0.812	0.75	Additive
Osteosarcoma	OS2	0.24	8.16	1.98	0.481	0.44	Additive
Osteosarcoma	OS31	2.34	7.39	17.30	<0.001	0.94	Supra-additive
Osteosarcoma	OS33	0.69	18.17	12.47	<0.001	0.95	Supra-additive
Wilms tumor	KT-10	-0.19	47.55	-8.80	0.117	0.82	Additive
Wilms tumor	KT-11	-0.06	32.31	-1.87	0.626	0.82	Additive
Wilms tumor	KT-13	0.17	25.36	4.35	0.615	0.41	Additive