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ORIGINAL REPORT

Phase I Study of the Aurora A Kinase Inhibitor Alisertib in Combination With Irinotecan and Temozolomide for Patients With Relapsed or Refractory Neuroblastoma: A NANT (New Approaches to Neuroblastoma Therapy) Trial

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ABSTRACT

Purpose
Alisertib is an oral Aurora A kinase inhibitor with preclinical activity in neuroblastoma. Irinotecan and temozolomide have activity in patients with advanced neuroblastoma. The goal of this phase I study was to determine the maximum tolerated dose (MTD) of alisertib with irinotecan and temozolomide in this population.

Patients and Methods
Patients age 1 to 30 years with relapsed or refractory neuroblastoma were eligible. Patients received alisertib tablets at dose levels of 45, 60, and 80 mg/m^2 per day on days 1 to 7 along with irinotecan 50 mg/m^2 intravenously and temozolomide 100 mg/m^2 orally on days 1 to 5. Dose escalation of alisertib followed the rolling six design. Samples for pharmacokinetic and pharmacogenomic testing were obtained.

Results
Twenty-three patients enrolled, and 22 were eligible and evaluable for dose escalation. A total of 244 courses were administered. The MTD for alisertib was 60 mg/m^2, with mandatory myeloid growth factor support and cephalosporin prophylaxis for diarrhea. Thrombocytopenia and neutropenia of any grade were seen in the majority of courses (84% and 69%, respectively). Diarrhea in 55% of courses and nausea in 54% of courses were the most common nonhematologic toxicities. The overall response rate was 31.8%, with a 50% response rate observed at the MTD. The median number of courses per patient was eight (range, two to 32). Progression-free survival rate at 2 years was 52.4%. Pharmacokinetic testing did not show evidence of drug-drug interaction between irinotecan and alisertib.

Conclusion
Alisertib 60 mg/m^2 per dose for 7 days is tolerable with a standard irinotecan and temozolomide backbone and has promising response and progression-free survival rates. A phase II trial of this regimen is ongoing.

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INTRODUCTION

Patients with relapsed or refractory neuroblastoma have poor outcomes.[1] The combination of irinotecan and temozolomide is an established salvage regimen for these patients.[2,3] Although response rates are modest (16% in one trial[2]), the regimen is well tolerated and may provide a backbone upon which to add promising novel agents.

Alisertib (formerly known as MLN8237) is an investigational selective inhibitor of Aurora A kinase metabolized in part via glucuronidation.[4,5] Key toxicities in early-phase adult trials included myelosuppression, mucositis, and mood alterations.[6,7] Clinical experience with alisertib in combination with standard chemotherapy agents, to date, is limited and exclusive to adult malignancies. Preliminary reports have shown that alisertib can be combined with docetaxel, gemcitabine, or

paclitaxel.[8-10] An adult trial of alisertib together with irinotecan is ongoing (NCT01923337). In a pediatric phase I trial of alisertib monotherapy, myelosuppression and mucositis were commonly observed, and the recommended single-agent phase II dose was 80 mg/m^2 once per day for 7 days in 21-day cycles.[11] Two patients with neuroblastoma had prolonged stable disease (SD).

Emerging data suggest that inhibiting Aurora A kinase may be a novel strategy for reducing the stability of Mycn protein, a key driver of aggressive disease in neuroblastoma that has not been previously targetable in the clinic.[12-14] Additional lines of evidence support further evaluation of Aurora A kinase inhibition in neuroblastoma. Increased expression of Aurora A kinase correlates with inferior outcomes in neuroblastoma.[15,16] Knockdown of Aurora A kinase mRNA expression or pharmacologic inhibition with Aurora A kinase small-molecule inhibitors such as alisertib is cytotoxic to neuroblastoma cells in vitro and in vivo.[12,14,16,17] Combination approaches may be particularly compelling because knockdown of Aurora A kinase sensitizes neuroblastoma cells to the cytotoxic effects of doxorubicin.[16] The addition of alisertib to irinotecan and temozolomide yielded additive effects in preclinical models of neuroblastoma.[18]

In this article, we describe the first pediatric evaluation of alisertib in combination with cytotoxic chemotherapy. The primary objective of this phase I trial was to define the maximum tolerated dose (MTD) of alisertib when administered with fixed doses of irinotecan and temozolomide in patients with advanced neuroblastoma. Preclinical data indicate a shared metabolic pathway dependent upon glucuronidation for both irinotecan and alisertib. We therefore included detailed pharmacokinetic and *UGT1A1* pharmacogenomic analyses, and we assessed the preliminary antitumor activity of this combination.

PATIENTS AND METHODS

Patients

Patients were eligible if they were age 1 to 30 years at the time of enrollment, had high-risk neuroblastoma, and had evaluable disease by bone marrow (BM) morphology, computed tomography, magnetic resonance imaging, and/or metaiodobenzylguanidine (MIBG) scans obtained within 4 weeks of enrollment. Patients were required to be classified in one of the following disease categories: relapse or progression, refractory to initial therapy (less than a partial response [PR] by International Neuroblastoma Response Criteria [INRC][19] after at least four cycles of chemotherapy), or persistent biopsy-proven disease after initial therapy (an INRC PR after at least four cycles of chemotherapy).

Patients had adequate performance scores (Lansky or Karnofsky performance score $\geq$ 50) and were a minimum of 3 weeks from last systemic therapy, 12 weeks from previous myeloablative therapy, 2 weeks from previous small-port radiation, 6 weeks from previous iodine-131 [^{131}I]-MIBG therapy, and 3 months from large-field radiation. Patients previously treated with alisertib were excluded. Patients previously treated with irinotecan and/or temozolomide were eligible if they did not have prior disease progression while being treated with a regimen containing those agents.

All patients were required to meet standard organ function criteria before enrolling: absolute neutrophil count $\geq$ 1,000/μL, unsupported platelet count $\geq$ 100,000/μL, serum creatinine $\leq$ 1.5 times the upper limit of age-adjusted normal value, total bilirubin $\leq$ 1.5 times upper limit of normal, and ALT $<$ 135 U/L.

Patients were excluded if they were pregnant, breastfeeding, unable to swallow intact pills, had a body surface area less than 0.38 m^2, had undergone previous allogeneic stem-cell transplantation, required hemodialysis, had active infection, had known history of HIV or hepatitis B or C infection, or had known active intraparenchymal brain metastasis. Patients who required scheduled benzodiazepines, scheduled antacid medications, specific phosphoglycoprotein substrates, or specific strong inducers of hepatic cytochrome enzymes were excluded.

Patients and/or legal guardians provided written informed consent, with assent obtained as appropriate. The institutional review board of each NANT (New Approaches to Neuroblastoma Therapy) site approved the study.

Protocol Therapy

Patients received fixed doses of irinotecan and temozolomide. Doses of irinotecan 50 mg/m^2 were administered intravenously over 60 minutes on days 1 to 5. Doses of temozolomide 100 mg/m^2 were administered orally 1 hour before irinotecan on days 1 to 5. Alisertib, as intact enteric-coated tablets, was administered orally once per day on days 1 to 7 after fasting. On days 1 to 5, alisertib was administered at the same time as temozolomide according to the dose level assigned at study entry. Cycles repeated every 21 days. In the absence of disease progression, patients were eligible to receive up to 34 courses of therapy for approximately 2 years.

Patients with dose-limiting toxicities (DLTs) were allowed to receive subsequent courses of therapy with dose modifications. For the first episode of hematologic DLT, temozolomide was reduced by 25%. If hematologic toxicity recurred, alisertib was reduced by 25%.

After the protocol was amended (see description of dose escalation in Results), all patients in dose levels marked with a "B" also received mandatory myeloid growth factor support (short- or long-acting at the discretion of the treating investigator) starting on day 8 and oral cefixime or cefpodoxime prophylaxis for diarrhea for a minimum of 10 days starting 2 days before each course.[20]

Toxicity Assessment

Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 4. Hematologic DLT was defined as grade 4 neutropenia for more than 7 days, need for platelet transfusion for a platelet count of less than 20,000/μL twice within a 7-day period, or greater than 14-day delay in the start of a subsequent course because of neutropenia or thrombocytopenia. Nonhematologic DLT was defined as any nonhematologic toxicity that delayed the start of a subsequent cycle by more than 14 days or any grade $\geq$ 3 toxicity with the exception of the following grade 3 toxicities: nausea, vomiting, anorexia, or dehydration resolving to grade $\leq$ 2 within 72 hours; increase in hepatic transaminase or electrolyte abnormality resolving to grade $\leq$ 1 within 7 days; diarrhea persisting for less than 72 hours; fever; infection; or febrile neutropenia. DLT definitions included only toxicities deemed at least possibly related to therapy.

Response Evaluation

Patients underwent disease staging at baseline, after courses 2 and 4, and then every four courses. Response was graded according to the NANT modification from the INRC[19] (complete response [CR], PR, mixed response, SD, and progressive disease [PD]). These criteria use Response Evaluation Criteria in Solid Tumors (RECIST) criteria for measurable tumors,[21] Curie score for MIBG scan response,[22] and BM morphology. BM response was graded as CR (requiring two time points to confirm), CR unconfirmed (requiring one time point only), SD, or PD. BM biopsies were not required at disease evaluations if they were negative at enrollment. Patients with at least SD or better underwent central review of MIBG scans, computed tomography scans, and BM pathology slides (all sites reviewed if the patient achieved PR or CR; involved sites were reviewed if the patient achieved mixed response or SD). Patients with overall response of PD reported by the treating site did not undergo central review and were

graded as PD. Overall responses of CR or PR were considered objective responses (see Appendix, online only).

Pharmacokinetic and Pharmacogenomic Studies

All patients were required to submit serial plasma samples during course 1 for both alisertib and irinotecan pharmacokinetic studies. For alisertib, 3 mL of blood was drawn into EDTA tubes before the first dose on day 1, before the dose on day 4, and then at 0.5, 1, 2, 3, 4, 7, and 24 hours after the dose on day 4. Samples were centrifuged within 48 hours, and plasma was extracted and frozen at –80°C. Samples were then batch analyzed for alisertib concentrations, and pharmacokinetic parameters were estimated as previously described.[11,23]

For irinotecan, 2 mL of blood was drawn into sodium heparin tubes before the dose of alisertib on days 1, 4, and 5. Samples were also obtained on day 4 at the end of the irinotecan infusion and at 1, 2, and 5 hours after completion of the infusion on day 4. Samples were immediately centrifuged, and plasma was extracted and frozen at –80°C. Samples were then batch analyzed for irinotecan, APC (7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin), SN-38, and SN-38G concentrations.[24] Pharmacokinetic parameters were calculated by using standard noncompartmental analysis.[25]

The study included an optional pharmacogenomics aim to correlate *UGT1A1* polymorphisms with toxicity and response to protocol therapy. Consenting patients provided whole blood in EDTA tubes before day 7 of the first course. After extracting DNA with a QIAamp DNA Blood Mini Kit (Qiagen, Santa Clarita, CA) per manufacturer instructions, *UGT1A1**28 (rs8175347) genotyping was performed as described previously[26] by using a modified method from Akaba et al.[27]

Statistical Methods

Evaluation of alisertib dose levels followed the rolling six dose escalation design.[28] Only DLTs in the first course of therapy had an impact on decisions regarding dose escalation. Patients were evaluable for DLT if they had a DLT during the first course or if they completed the first course of therapy without DLT and had received a minimum of five doses of alisertib, four doses of irinotecan, and four doses of temozolomide. The MTD was the highest dose level tested at which fewer than two of six patients had first-course DLT. Progression-free survival (PFS) was estimated by using Kaplan-Meier methods as time from the start of treatment to first episode of disease progression or death; patients who were alive and without progression were censored at last follow-up. Differences in the proportion of responders on the basis of *MYCN* status or previous exposure to irinotecan were compared by using Fisher's exact tests with two-sided *P* values. Analyses were performed with STATA version 11 (STATA, College Station, TX).

RESULTS

Patient Characteristics

Twenty-three patients were enrolled from May 2012 to December 2013. One patient was unable to swallow intact tablets and was deemed ineligible. The remaining 22 patients received 100% of the prescribed therapy in the first course and were evaluable for toxicity. Characteristics of these 22 patients are provided in Table 1. Six (30%) of 20 patients with available data had *MYCN*-amplified tumors. Five patients had received prior irinotecan.

Table 1. Characteristics of Enrolled and Eligible Patients (N = 22)

Characteristic	Value
Median age at study entry, No. (range), years	7.7 (4.3-22.7)
Median time from diagnosis to entry, No. (range), months	25 (4.5-91.1)
Sex, No.	
Male	18
Female	4
Stage 4 at initial diagnosis	20
Disease status, No.	
Relapsed disease	20
Primary refractory disease	1
PR to induction chemotherapy	1
MYCN-amplified tumor	6/20 with known status
Prior therapies, No.	
Myeloablative therapy	19
Irinotecan	5
Temozolomide	4
Disease involvement at study entry, No.	
BM involvement	10
MIBG-avid disease	17
Measurable disease by anatomic imaging	14

Abbreviations: BM, bone marrow; MIBG, metaiodobenzylguanidine; PR, partial response.

Dose Escalation and Toxicity

Details of the dose escalation are provided in Table 2. Six patients were treated at dose level 1. Two patients developed first-course DLT (neutropenia that delayed the second course in one patient; grade 3 anorexia, oropharyngeal mucositis, oral pain, prolonged grade 4 neutropenia, and need for more than two platelet transfusions in 7 days, all occurring in one patient). In addition, two patients had first-course grade 3 diarrhea that did not meet the protocol definition of a DLT. The protocol was amended, and all subsequent patients were mandated to receive myeloid growth factor support and cephalosporin prophylaxis for diarrhea (see Patients and Methods). Dose escalation resumed with dose level 1B, which used the same dose of alisertib as dose level 1 along with supportive care measures. Six patients were treated, none of whom had first-course DLT. At dose level 2B (alisertib 60 mg/m^2 per dose), six patients were treated, and one had first-course DLT (prolonged grade 4 neutropenia). At dose level 3B (alisertib 80 mg/m^2 per dose; single-agent pediatric phase II dose), four patients were treated and two had first-course DLT (both with dose-limiting neutropenia and thrombocytopenia). Dose level 2B was therefore declared the MTD.

The 22 eligible patients received a total of 244 courses of treatment. Hematologic toxicity was common, with thrombocytopenia and neutropenia of any grade reported in 84% and 69% of courses, respectively (Table 3). The incidence of grade 4 thrombocytopenia was dose related, increasing from 3% to 20% to 35% from dose level 1B to 2B to 3B, respectively. A similar pattern was observed for grade 4 neutropenia, increasing from 9% to 14% to 35% across dose levels. Despite this degree of myelosuppression, infectious complications were uncommon (nine [3.7%] of 244 courses with febrile neutropenia).

The most common nonhematologic toxicities were diarrhea, nausea and/or vomiting, and increased hepatic transaminases (Table 3). Before mandatory diarrhea prophylaxis was implemented, diarrhea occurred in 76% of courses (7% grade 3). After prophylaxis was implemented, diarrhea occurred in 48% of courses (2% grade 3). No patients experienced grade 4 diarrhea. Mood alterations (adverse events) were a composite of agitation, anxiety, depression, or somnolence, and were reported in 17% of courses, all grade 1 or 2. Mucositis was reported in 9% of courses.

Table 2. Dose Escalation of Alisertib in Combination With Fixed Doses of Irinotecan and Temozolomide

Dose Level	Alisertib (mg/m^2 per dose)	No. of Patients Entered	No. Eligible and Evaluable for DLT	No. Evaluable With First-Course DLT
1	45	6	6	2*
1B	45	6	6	0
2B	60	6	6	1†
3B	80	5	4	2‡

NOTE. Dose levels designated with a "B" included required myeloid growth factor support and cephalosporin prophylaxis for diarrhea.
Abbreviation: DLT, dose-limiting toxicity.
*Neutropenia delaying start of second course (n = 1); grade 3 anorexia, oropharyngeal mucositis, oral pain, prolonged grade 4 neutropenia, and need for at least two platelet transfusions in 7 days (n = 1).
†Prolonged grade 4 neutropenia.
‡Neutropenia and thrombocytopenia delaying start of second course.

After course 1, six (35%) of 17 patients without first-course DLT required dose modifications for DLTs occurring in later courses. Among the five patients at dose level 2B who did not have first-course DLT, three patients required subsequent dose modifications (two with single reductions in both temozolomide and alisertib, and one with a single reduction in irinotecan).

Alisertib and Irinotecan Pharmacokinetics

Estimates of alisertib and irinotecan pharmacokinetic parameters by dose level are provided in Table 4. Alisertib pharmacokinetic parameters showed significant interpatient variability. Peak plasma concentrations and exposure increased as the alisertib dose increased. Day 4 trough concentration did not correlate with

Table 3. Percent of Hematologic and Nonhematologic Toxicities Observed in Evaluable Patients According to Dose Level and Grade

Toxicity	Any Grade Toxicity (244 Courses in 22 Patients), No. (%)	Dose Level and Toxicity Grade															
		1 (59 Courses in Six Patients)				1B (67 Courses in Six Patients)				2B (101 Courses in Six Patients)				3B (17 Courses in Four Patients)			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Hematologic toxicities																	
Anemia	92 (22)	54	19	10	0	31	54	6	0	26	37	33	2	6	53	41	0
Decreased WBC count	87 (21)	27	34	25	5	33	25	27	0	27	40	15	5	6	24	41	18
Decreased platelet count	84 (20)	27	15	20	10	64	10	4	3	41	12	17	20	12	12	35	35
Decreased neutrophil count	69 (22)	5	25	31	14	1	12	45	9	17	20	14	14	0	24	29	35
Decreased lymphocyte count	43 (15)	3	29	22	0	19	3	9	0	18	9	11	0	12	35	35	0
Non-hematologic toxicities																	
Diarrhea	55 (22)	64	5	7	0	31	7	0	0	36	9	4	0	53	24	0	0
Nausea	54 (20)	29	25	2	0	42	13	0	0	48	3	0	0	35	18	6	0
Increased ALT	48 (17)	47	14	0	0	40	3	0	0	34	7	6	2	6	0	6	0
Increased AST	46 (18)	69	2	0	0	30	0	0	0	40	4	5	0	6	0	6	0
Vomiting	44 (20)	41	8	0	0	45	10	1	0	24	9	0	0	24	12	6	0
Alopecia	42 (9)	22	42	0	0	0	3	0	0	1	50	0	0	0	65	0	0
Anorexia	24 (14)	8	3	2	0	5	2	0	0	6	28	4	0	6	35	6	0
Hypocalcemia	23 (10)	39	7	0	0	1	1	0	0	25	1	0	0	6	0	0	0
Cough	20 (16)	14	0	0	0	18	0	0	0	26	1	0	0	6	6	0	0
Fatigue	20 (15)	15	2	0	0	7	1	0	0	16	6	0	0	53	12	0	0
Abdominal pain	18 (11)	17	12	0	0	12	0	0	0	11	7	1	0	0	0	0	0
Increased GGT	18 (6)	12	2	0	0	0	48	0	0	3	0	0	0	0	0	0	0
Hypokalemia	18 (12)	36	0	12	0	3	0	1	0	9	0	1	0	12	0	0	0
Hypoalbuminemia	16 (12)	27	3	0	0	7	0	0	0	16	1	0	0	0	0	0	0
Weight loss	16 (8)	31	7	0	0	0	1	0	0	11	0	0	0	18	0	6	0
Increased creatinine	12 (5)	0	0	0	0	7	0	0	0	23	2	0	0	0	0	0	0
Increased blood bilirubin	11 (5)	24	5	0	0	9	0	0	0	5	0	0	0	0	0	0	0
Headache	11 (11)	3	1	0	0	12	1	0	0	5	5	0	0	29	0	0	0
Pain in extremity	11 (6)	2	2	0	0	3	1	0	0	11	10	0	0	0	0	0	0
Proteinuria	10 (2)	2	0	0	0	0	0	0	0	1	22	0	0	0	0	0	0
Weight gain	10 (2)	0	0	0	0	0	0	0	0	8	16	1	0	0	0	0	0
Epistaxis	2 (3)	0	0	0	0	0	0	0	0	1	0	0	0	24	0	6	0

NOTE. Percentage of affected courses shown for all courses of therapy. Only toxicities occurring in greater than 20% of courses in at least one dose level are shown. Toxicities attributed as unrelated to protocol therapy are not shown.
Abbreviation: GGT, γ-glutamyl transferase.

occurrence of first-course DLT. In contrast, three of five patients with first-course DLTs had exposures of greater than 60 μM·hr (one each at dose levels 1, 2B, and 3B), whereas none of the patients who did not have first-course DLTs had exposures of greater than 60 μM·hr .

Irinotecan exposure and clearance did not change as a function of alisertib dose level. Likewise, SN-38 and SN-38G peak plasma concentration and exposure seemed to be similar across alisertib dose levels. Peak APC concentration and exposure decreased with increasing doses of alisertib. SN38 exposure was not associated with occurrence of first-course DLT.

UGT1A1 Pharmacogenomic Studies

Twenty of 22 eligible patients agreed to participate in this optional study. Eighteen patients had sufficient DNA for analysis. Nine patients were wild type at both alleles, seven were heterozygous for the *UGT1A1*28* allele (seven promoter TA repeats), and two were homozygous for the *UGT1A1*28* allele. One (11%) of nine wild-type patients had first-course DLTs compared with three (33%) of nine patients with at least one copy of *UGT1A1*28*. One of two patients homozygous for *UGT1A1*28* had a first-course DLT. *UGT1A1*28* genotype was not associated with alisertib exposure, alisertib day 4 trough concentration, or SN38 exposure.

Antitumor Activity

The overall objective response rate was 31.8%, with a CR rate of 22.7% (Table 5). Only two patients had PD as their best response. At the MTD, three of six patients responded (two CRs). All responders were irinotecan-naïve, such that the response rate in this group was 41.2% (seven of 17) versus 0% (zero of five) for patients previously treated with irinotecan ($P = .14$). The response rate was 35.7% for patients with *MYCN*-nonamplified tumors compared with 16.7% for patients with *MYCN*-amplified tumors ($P = .61$).

The median number of courses received across all dose levels was eight (range, two to 32), with 15 patients (68.2%) receiving six or more courses of therapy. PFS at 2 years was 52.4% (95% CI, 29.2% to 71.4%; Fig 1).

DISCUSSION

Aurora A kinase inhibition with alisertib as a single agent has displayed antitumor activity in preclinical models of neuroblastoma,[17] although zero of 11 patients with neuroblastoma had objective responses in a phase I monotherapy trial.[11] To explore effective combination-based therapy, we combined alisertib with irinotecan and temozolomide in a phase I study for patients with relapsed or refractory neuroblastoma. We chose the irinotecan and temozolomide backbone for this evaluation because of its known activity in this setting, modest myelosuppression, and limited mucosal toxicity. This regimen was tolerable when 80% of the single-agent pediatric dose of alisertib was used along with supportive care with myeloid growth factor and cephalosporin for diarrhea prophylaxis. Despite a shared metabolic pathway through *UGT1A1*, our pharmacokinetic data do not support an irinotecan-alisertib interaction. *UGT1A1* genotyping results, which suggest that patients with at least one copy of the *UGT1A1*28* allele may be at higher risk of severe toxicity, are consistent with published data for irinotecan monotherapy.[20]

The proportion of patients with an objective response (31.8%) or on protocol therapy for six or more courses (68.2%) suggests significant antitumor activity of this combination. In a phase II

Table 4. Pharmacokinetic Parameters for Alisertib, Irinotecan, and Key Irinotecan Metabolites in 22 Patients According to Dose Level

Pharmacokinetic Parameter	Median Dose Level (Range)			
	1 (n = 6)	1B (n = 6)	2B (n = 6)	3B (n = 4)
Alisertib				
Day 4 trough, μM	0.48 (0.09-0.98)	0.35 (0.13-1.38)	0.3 (0.22-4.07)	0.73 (0.15-1.35)
Day 5 trough, μM	0.37 (0.12-1.36)	0.36 (0.18-0.65)	0.2 (0.05-3.31)	0.69 (0.25-1.27)
C_{max}, μM	2.56 (1.91-5.92)	2.39 (1.72-5.92)	3.77 (2.16-6.76)	4.94 (3.99-6.22)
t_{max}, h	2.04 (1.12-6.93)	1.74 (0.65-2.9)	2.5 (0.97-4)	2.52 (2-3.03)
Half-life, h	7.20 (5.22-9.83)	8.61 (4.2-18.2)	6.19 (3.65-20.28)	8.54 (5.35-10.05)
AUC_{meas}, μM·hr	28.15 (16.9-65.5)	21 (15.4-44.9)	30.71 (19.61-117.03)	47.73 (32.71-84.67)
Irinotecan				
C_{max}, ng/mL	722 (502-880)	703 (642-5,371)	1,238 (493-1,757)	784 (546-987)
$AUC_{0\text{-}24\ h}$, h·ng/mL	3,702 (2,059-5,772)	2,680 (1,626-8,492)	3,957 (3,231-4,301)	2,615 (2,414-3,288)
Clearance, L/h	14.0 (8.8-29.4)	15.7 (4.6-27.3)	10.4 (9.0-31.4)	16.0 (12.6-20.1)
APC				
C_{max}, ng/mL	61.2 (23.1-480.4)	59.8 (28.2-105)	55.8 (25.7-76.3)	43.4 (29.1-49.4)
$AUC_{0\text{-}24\ h}$, h·ng/mL	571 (248-3,313)	477 (197-1,083)	511 (293-821)	418 (299-457)
SN-38				
C_{max}, ng/mL	9.5 (6.6-18.7)	12.6 (5.8-16.5)	12.0 (8.8-14.7)	11.7 (7.0-13.8)
$AUC_{0\text{-}24\ h}$, h·ng/mL	63.2 (20.5-294.6)	52.9 (28.4-119.6)	80.8 (35.0-109.9)	72.0 (62.6-102.0)
SN-38G				
C_{max}, ng/mL	18.2 (16.0-46.9)	16.9 (4.8-23.7)	13.8 (7.0-26.8)	13.0 (10.4-24.0)
$AUC_{0\text{-}24\ h}$, h·ng/mL	206.5 (124.3-325.3)	97.6 (53.8-243.6)	141.8 (77.5-298.1)	134.4 (101.1-237.8)

Abbreviations: APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin; $AUC_{0\text{-}24\ h}$, area under the serum concentration-time curve from start time to 24 hours; AUC_{meas}, area under the serum concentration-time curve measured; C_{max}, maximal drug concentration; t_{max}, time to maximal concentration.

Table 5. Best Overall Objective Responses (either CR or PR) on Protocol Therapy According to Dose Level and Sites of Disease Evaluable for Response

		Overall Response		MIBG Response		CT Response		BM Response	
Dose Level	No. of Patients	No./Total (%; 95% CI)	Response (No.)	No./Total (%; 95% CI)	Response (No.)	No./Total (%; 95% CI)	Response (No.)	No./Total (%; 95% CI)	Response (No.)
1	6	2/6	CR (1) PR (1) SD (4)	1/5	PR (1) SD (4)	1/4	CR (1) SD (3)	0/2	SD (2)
1B	6	1/6	CR (1) MR (3) SD (1) PD (1)	2/5	CR (2) SD (3)	2/4	CR (1) PR (1) SD (1) PD (1)	2/3	CR (2) SD (1)
2B	6	3/6	CR (2) PR (1) MR (1) SD (1)	1/4	CR (1) SD (3)	3/4	CR (1) PR (2) SD (1)	0/1*	SD (1)
3B	4	1/4	CR (1) SD (2) PD (1)	0/3	SD (3)	0/2	SD (1) PD (1)	2/3	CR (1) CRu† (1) SD (1)
All dose levels	22	7/22 (31.8; 14 to 55)	CR (5) PR (2) MR (3) SD (8) PD (2)	4/17 (23.5; 7 to 50)	CR (3) PR (1) SD (13)	6/14 (42.9; 18 to 71)	CR (3) PR (3) SD (6) PD (2)	4/9 (44.4; 14 to 79)	CR (4) SD (5)

Abbreviations: BM, bone marrow; CR, complete response; CRu, unconfirmed complete response; CT, computed tomography; MIBG, metaiodobenzylguanidine; MR, mixed response; PD, progressive disease; PR, partial response; SD, stable disease.

*An additional patient at this level had bone marrow involvement at study entry, but BM was not re-evaluated during the study.

†Response was unconfirmed because there was only one follow-up BM evaluation.

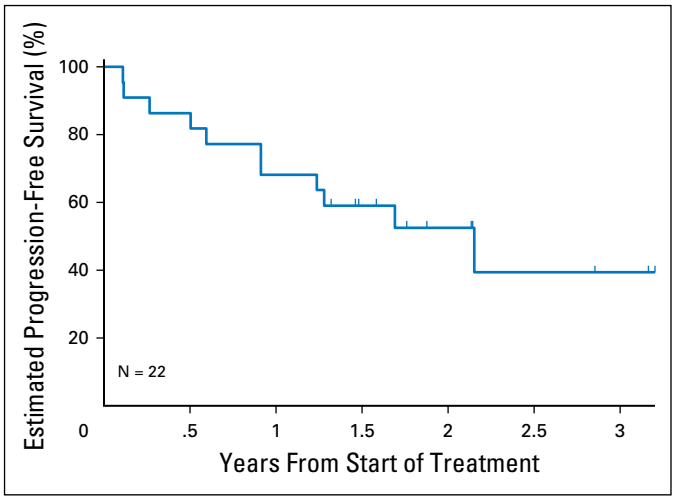

Fig 1. Estimated progression-free survival from start of protocol therapy for 22 eligible patients with relapsed or refractory neuroblastoma.

trial, patients with primary refractory or first relapsed neuroblastoma treated with irinotecan-temozolomide had a response rate of 15%.[2] In a subanalysis focused on patients without previous irinotecan therapy (as in the prior phase II trial of the backbone alone), we observed a more striking response rate of 41.2%. Our 2-year PFS estimate for the full cohort (52.4%) compares favorably with the 2-year event-free survival rate of 13% reported with the irinotecan-temozolomide backbone.[2]

A 5-day irinotecan-temozolomide schedule was chosen because studies in another pediatric solid cancer have shown that response rates do not differ with a more protracted regimen of 5 days per week for 2 weeks.[29] Because a 5-day regimen is more convenient and maximizes potential synergy by increasing the extent of coadministration with alisertib, this schedule was selected for evaluation. However, we note that a more protracted schedule is associated with less hematologic toxicity.[30]

Our embedded pharmacokinetic studies enhanced our understanding of this novel combination. SN-38 exposure and peak plasma concentrations were similar to those reported in children receiving irinotecan monotherapy at the same dose of 50 mg/m^2.[31] This finding, along with similar SN-38 exposures independent of alisertib dose level, argues against an increase in SN-38 exposure with concomitant alisertib. Likewise, alisertib exposure at the MTD of 60 mg/m^2 was similar to those reported in children receiving alisertib monotherapy at this same dose.[11] Preclinical work suggests that plasma concentrations of greater than 1 μM maintained for at least 8 hours lead to optimal pharmacodynamic effect of Aurora A kinase inhibition.[32] Our results indicate that dosing at the MTD achieved this goal in the majority of patients. Alisertib concentrations of 1 μM have also been shown to reduce Mycn protein stability in neuroblastoma,[12] although we did not observe a higher response rate in patients with *MYCN*-amplified tumors. Newer Aurora A kinase inhibitors have been designed to more potently destabilize Mycn protein,[13] but these are not yet in clinical testing.

In conclusion, we have determined that alisertib can be combined with irinotecan and temozolomide along with myeloid growth factor support and cephalosporin prophylaxis for diarrhea. The favorable pharmacokinetic results and promising response rate and PFS suggest that this regimen may provide an effective option for patients with advanced neuroblastoma. Completion of a phase II study and evaluation of a liquid formulation are ongoing.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase I Study of the Aurora A Kinase Inhibitor Alisertib in Combination With Irinotecan and Temozolomide for Patients With Relapsed or Refractory Neuroblastoma: A NANT (New Approaches to Neuroblastoma Therapy) Trial

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Appendix

Details of Response Assessment

Soft tissue lesions were evaluated by using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria. Patients with metaiodobenzylguanidine (MIBG)-avid disease were assessed by using a relative Curie score derived from Curie scores from subsequent scans divided by the score from the baseline scan. Patients were coded as having a complete response (CR) if all areas of uptake completely resolved and no new lesions were seen (relative score, 0). Patients were coded as having a partial response if the relative score was 0.1 to 0.5. Patients were coded as having progressive disease (PD) if they developed a new MIBG-avid lesion. All other patients with MIBG-avid disease who did not meet any of the above criteria were coded as having stable disease.

Patients with MIBG nonavid disease were assessed by using fluorodeoxyglucose (FDG) positron emission tomography scans. Patients were coded as having a CR if all areas of uptake completely resolved and no new lesions were seen. Patients were coded as having PD if they developed new FDG-avid lesions. All other patients being evaluated by FDG positron emission tomography scans who did not meet any of the above criteria were coded as having stable disease.

Bone marrow (BM) response was assessed by using the following NANT (New Approaches to Neuroblastoma Therapy) criteria. Patients were coded as having CR if they had morphologic evidence of BM disease at study entry by using hematoxylin and eosin staining and then no tumor cells seen on two subsequent aspirate/biopsy procedures separated by at least 3 weeks. Patients were coded as having PD if they enrolled with BM involvement and a subsequent sample showed greater than 25% tumor cells and there was at least a doubling in the amount of tumor cells compared with baseline. Patients who enrolled with no BM involvement were coded as PD if they had morphologic evidence of BM involvement on two consecutive aspirate/biopsy procedures separated by at least 3 weeks. Patients with BM involvement at baseline who did not meet any of the above criteria were coded as having stable BM disease. The response at each site of disease was used to derive a patient's overall response according to Appendix Figure A1 (online only).

RECIST Response	MIBG Response	Bone Marrow Response	Overall Response
Progressive disease by any parameter			Progressive disease
Complete response in one parameter with all other parameters coded as complete response or not involved			Complete response
Complete response, partial response, or not involved	Partial response	Complete response or not involved	Partial response
Partial response	Complete response, partial response, or not involved	Complete response or not involved	Partial response
Stable disease in one parameter with all other parameters coded as stable disease or not involved			Stable disease
Complete response or partial response for at least one parameter, stable disease for a second parameter, and response other than disease progression for the third parameter (if evaluable)			Mixed response

Fig A1. Derivation of overall response according to response at each potential disease parameter. MIBG, metaiodobenzylguanidine.