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Phase II study of alisertib as a single agent for treating recurrent or progressive atypical teratoid/rhabdoid tumor

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Abstract

Background. Recurrent atypical teratoid/rhabdoid tumor (AT/RT) is, most often, a fatal pediatric malignancy with limited curative options.

Methods. We conducted a phase II study of Aurora kinase A inhibitor alisertib in patients aged <22 years with recurrent AT/RT. Patients received alisertib once daily (80 mg/m^2 as enteric-coated tablets or 60 mg/m^2 as liquid formulation) on Days 1–7 of a 21-day cycle until progressive disease (PD) occurred. Alisertib plasma concentrations were measured in cycle 1 on Days 1 (single dose) and 7 (steady state) and analyzed with noncompartmental pharmacokinetics. Trial efficacy end point was ≥10 participants with stable disease (SD) or better at 12 weeks.

Results. SD (n = 8) and partial response (PR) (n = 1) were observed among 30 evaluable patients. Progression-free survival (PFS) was 30.0% ± 7.9% at 6 months and 13.3% ± 5.6% at 1 year. One-year overall survival (OS) was 36.7% ± 8.4%. Two patients continued treatment for >12 months. PFS did not differ by AT/RT molecular groups. Neutropenia was the most common adverse effect (n = 23/30, 77%). The 22 patients who received liquid formulation had a higher mean maximum concentration (C_{max}) of 10.1 ± 3.0 μM and faster time to C_{max} (T_{max} = 1.2 ± 0.7 h) than those who received tablets (C_{max} = 5.7 ± 2.4 μM, T_{max} = 3.4 ± 1.4 h).

Conclusions. Although the study did not meet predetermined efficacy end point, single-agent alisertib was well tolerated by children with recurrent AT/RT, and SD or PR was observed in approximately a third of the patients.

Key Points

- Aurora kinase A inhibitor alisertib is a potential therapeutic option in AT/RT.
- Alisertib yielded stable disease in a proportion of children with recurrent AT/RT.
- Treatment response did not vary by AT/RT molecular groups.

Importance of the Study

Atypical teratoid/rhabdoid tumor (AT/RT) is a devastating pediatric cancer with a high mortality. It is characterized by loss of *SMARCB1/INI1* tumor suppressor function and subsequent activation of Aurora kinase A, which regulates mitotic spindle and cell division. In this phase II study, children with recurrent AT/RT received the Aurora kinase A inhibitor alisertib once daily for 7 days of a 21-day cycle until disease progression occurred or intolerable toxicity. The trial efficacy end point of ≥10 participants with stable disease or better at 12 weeks was not reached. Nevertheless, 9 of 30 participants had stable disease, including one with partial response by central independent imaging review and 2 who were on treatment for >12 months. Neutropenia was the most common side effect. These findings indicate that alisertib may help some children with AT/RT by prolonging survival and/or serving as a bridge to other therapies.

Atypical teratoid/rhabdoid tumor (AT/RT) is a highly malignant cancer that occurs predominantly in children aged <3 years.[1] Despite exhibiting very aggressive behavior, AT/RT is thought not to be affected by recurrent genetic alterations, except in members of the chromatin remodeling SWI/SNF complex genes *SMARCB1* (*INI1*) or *SMARCA4* (*BRG1*).[2–4] Although our understanding of the biological underpinnings of AT/RT has expanded[5], novel therapies targeting the mechanisms of cancer development in AT/RT are lacking.

Aurora kinase A (encoded by *AURKA*) regulates the formation and stability of the mitotic spindle during the cell cycle.[6] Aurora kinase A inhibition, thus, causes mitotic delays, chromosome mis-alignment, and segregation, leading to cell death.[7] *AURKA* expression is upregulated in AT/RT and malignant rhabdoid tumors following inactivation of *SMARCB1* tumor suppressor gene. *SMARCB1* encodes the INI1 protein, which represses expression of *AURKA*, among other genes, in a cell type–specific manner, and knockdown of *AURKA* induces mitotic arrest and apoptosis in rhabdoid cell lines but not in healthy nonmalignant cells.[8] Although INI1 represses *AURKA* expression in tumor and healthy cells, its repression of *AURKA* in healthy diploid cells does not induce cleavage of caspase-3 and does not decrease their survival, thereby potentially sparing healthy tissues of any adverse effects. Alisertib (MLN8237) is a selective, potent, and reversible small-molecule inhibitor of Aurora kinase A that has been studied in adult cancers.[7,9,10] Alisertib has also been studied in children with solid tumors and leukemias, and a maximum tolerated and recommended phase II dosing has been established.[11,12] We previously reported durable response with use of alisertib in the treatment of 4 children with recurrent AT/RT.[13] However, no efficacy data with alisertib for treatment of pediatric brain and spinal cord cancers from clinical trials have been reported.

We conducted a phase II study (SJATRT, NCT02114229) of single-agent alisertib in patients aged <22 years with recurrent/progressive AT/RT or malignant rhabdoid tumors who experienced treatment failure to at least one frontline therapy. Here, we report the outcomes of the AT/RT cohort (A1 stratum) as a whole, as well as by AT/RT molecular groups: ATRT-MYC (MYC), ATRT-SHH (SHH), and ATRT-TYR (TYR). We also report treatment outcomes for patients with germline alterations leading to cancer predisposition syndromes.

Materials and Methods

Study Design

SJATRT is a multi-arm Phase II trial sponsored by St. Jude Children's Research Hospital (St. Jude) and Takeda Pharmaceuticals. Eligible patients include AT/RT and extra-CNS MRT and are enrolled in separate strata based on disease stage (newly diagnosed vs. recurrent), clinical risk classification, and disease location. In this manuscript we report the final results of Stratum A1 which enrolled patients younger than 22 years with recurrent or progressive AT/RT. The study design assessed the efficacy of alisertib

against fixed thresholds for the dual primary outcomes of 12-week disease stabilization or sustained objective response rates (ie, complete response [CR] and partial response [PR] rate). Patients must have had radiographically measurable disease, which was defined by at least one lesion measurable in two dimensions or a positive CSF cytology for tumor cells within 2 weeks before enrollment. Enrollment in the trial required immunohistochemical confirmation of the loss of INI1 or BRG1 protein expression or molecular confirmation of biallelic *SMARCB1* or *SMARCA4* loss of function mutation in tumor cells either at the time of initial diagnosis or at progression. The study participants must have had adequate organ function, a performance score of at least 60, and recovery from acute adverse effects of prior chemotherapy, immunotherapy, or radiation therapy. Written informed consent was obtained from parents or legal guardians and from participants aged 14–17 years. In addition, assent was obtained from participants 7–13 years old. The study was conducted in accordance with the World Medical Association Declaration of Helsinki principles and ethical guidelines. The study was approved by the Institutional Review Board (IRB) of St. Jude and five other participating hospitals.

CR was defined as the disappearance of all radiologically discernible lesions and two consecutively negative CSF cytology findings (for those with initially positive cytology findings). We defined PR as ≥50% reduction in size of the target lesions, as measured by the products of maximum perpendicular diameters. In the presence of more than one target lesion, we defined PR as a >50% reduced sum of the products of the maximum perpendicular diameters of all measurable target lesions or two consecutively negative CSF cytology findings (for those with initially positive cytology findings) plus a <50% reduced tumor size. Progressive disease (PD) was defined as >25% increase in the size of any measurable lesion, appearance of new radiographic lesion, or conversion of negative CSF cytology to positive, confirmed by two consecutive cytologic evaluations. Responses not meeting criteria for CR, PR, or PD were defined as stable disease. A CR or PR confirmed by repeat MRI and/or CSF sampling after at least two additional courses of therapy was termed sustained objective response.

Tumor DNA methylation, molecular subgroups, and germline analysis were performed as previously described.[14] We classified AT/RTs to the MYC, SHH, and TYR molecular groups by using Infinium Methylation EPIC BeadChips arrays (Illumina) and the German Cancer Research Center classifier (https://www.molecularneuropathology.org/mnp).[15] All patients with adequate tumor tissues for DNA methylation profiling and paired germline samples were included in our biological analyses, and the associated patient outcomes were correlated to molecular group and pathogenic or likely pathogenic (P/LP) germline alterations (GLA), as specified in the exploratory analyses of the study.

Aurora Kinase A Immunohistochemistry

Immunohistochemistry on human tumor samples was performed using a 1:100 dilution of anti-Aurora A Kinase (Abcam, ab52973) diluted in BOND antibody diluent (Roche Tissue Diagnostics, 251-018) and detected using BOND Refine Polymer DAB (Leica Biosystems, DS9800) detection kits. The qualitative staining levels were scored as 0 (no staining), 1 (weak/focal staining), and 2 (widespread staining) immunoreactivity as previously described.[13]

Treatment Plan

Alisertib was provided and distributed by Takeda (formerly Millennium) Pharmaceuticals. Alisertib was administered p.o. once daily on Days 1–7 of a 21-day cycle at a dose of 80 mg/m^2 in enteric-coated tablets (ECTs) or at a dose of 60 mg/m^2 as liquid solution (p.o. or nasogastric/G-tube), followed by 14 days of rest. Up to 35 total cycles were to be administered to achieve a total duration of therapy of 24 months or until PD occurred or unacceptable toxicities developed. The liquid was administered at least 1 h before or 2 h after a meal and was rounded off to the nearest milligram. ECTs were administered whole and without any restriction of oral intake. If vomiting occurred within 30 min of taking the ECT or within 15 min of the liquid, the dose was repeated. Coadministration of alisertib with proton pump inhibitors or H2 blockers was not permitted due to the possibility of increased absorption of alisertib.

The criteria to begin a new cycle of therapy included an absolute neutrophil count ≥500/mm^3, hemoglobin >8 g/dL (with or without transfusion support), and platelet count ≥50 000/mm^3 (without transfusion support). In addition, all other clinical toxicities considered by the investigator to be related to alisertib therapy must have resolved to ≤Grade 2 before a new cycle of therapy was initiated. If treatment was delayed >2 weeks (ie, a rest period >4 weeks) because of incomplete recovery from treatment-related toxicity, the dose of alisertib was reduced to 60 mg/m^2 for ECTs and to 45 mg/m^2 for the liquid solution when therapy resumed. If start of subsequent cycles of therapy was delayed again for 2 additional weeks beyond the stated recovery period (ie, 4-week rest period) after the first dose reduction, treatment was discontinued.

Safety

All adverse effects were graded according to the Common Terminology Criteria for Adverse Events v4.0 of the National Cancer Institute for toxicity and performance reporting.

Pharmacokinetics

Plasma pharmacokinetic studies of p.o. alisertib administered as ECT or liquid solution were performed for all consenting patients to evaluate the pharmacokinetics of alisertib. On Day 1 of the first cycle, serial blood samples were collected at the following times: predose, 0.5, 1, 1.5, 4, 6 (±0.5), 24 (±4), and 48 (±6) h (prior to the Day 3 dose). To obtain the 24- and 48-h time points, the alisertib dose on Day 2 was held. On Day 7 of the first cycle, alisertib steady-state serial blood samples were collected at the following time points: predose, 1.5, 4, and 24 (±4) h after the final dose. Whole-blood samples

(1 ml/sample) were collected in K_2-EDTA tubes, inverted several times to mix, immediately aliquoted, and centrifuged at 10 000 rpm for 2 min. The plasma supernatant was stored at –80°C within 1 h of sample collection until further analysis.

CSF pharmacokinetic studies were performed in consenting patients with external access to CSF (ie, ommaya reservoir). On Days 1 and/or 7 of the first cycle, either a single or serial CSF samples were collected predose and at 1.5, 4 (±0.5), and 24 (±4) h after the alisertib dose. A simultaneous plasma sample was obtained at the time of all CSF sample collections. The CSF samples (0.5 ml/sample) were stored at –80°C within 1 h of sample collection until further analysis. All plasma and CSF samples were assayed at St. Jude for alisertib with a previously published high-performance liquid chromatography with tandem-mass spectrometric detection method.[16] The lower limit of quantification was 5 ng/ml (ie, 0.0096 μM) for analysis of plasma and CSF concentrations.

Alisertib concentration–time data were analyzed with classic noncompartmental pharmacokinetic techniques with Phoenix WinNonlin v8.0 (Certara USA, Inc., Princeton, NJ). The peak concentration (C_{MAX}) and time to C_{MAX} (T_{MAX}) were determined directly from the concentration–time profile. The last three measurable concentration–time data points in the serial sampling window were used to define the log-linear terminal slope (β), and the terminal half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\beta$. The area under the concentration versus time curve from time zero to the last measurable sampling time point ($AUC_{0–Tlast}$) was calculated by using the linear-up/log-down trapezoidal rule. The $AUC_{Tlast–\infty}$ was calculated as the ratio of the last measurable time point to β. The total $AUC_{0–\infty}$ was obtained by using the sum of $AUC_{0–Tlast}$ and $AUC_{Tlast–\infty}$. The bovine serum albumin (BSA)-normalized apparent oral clearance (CL/F) was calculated as the BSA-normalized dose divided by $AUC_{0–\infty}$. Alisertib plasma C_{MAX}, T_{MAX}, and AUC obtained after a single alisertib dose and at steady state were compared between the two drug formulations (liquid solution vs ECT) with Mann–Whitney tests (significance determined as $P < .05$).

Study Endpoint and Statistical Analysis

The primary aims of the A1 stratum of the SJATRT study were to estimate the sustained objective response rate and the rate of disease stabilization, and to determine whether the efficacy signal was sufficient to merit continued investigation of alisertib in patients with recurrent AT/RT. Sustained objective responses were defined as CR or PR occurring within the first 10 cycles (approximately 30 weeks) of treatment and sustained for 2 additional cycles (approximately 6 weeks). To count towards success criteria, disease stabilization needed to last a minimum of 12 weeks as confirmed by MRI and CSF analysis, when indicated. The design was based on a bivariate binomial outcome [17,18] with unacceptable and versus desirable sustained objective response rates were of 5% versus 25%, respectively, and unacceptable vs desirable 12-week SD rates of 20% versus 45%, respectively. During incorporation of prolonged disease stabilization into design of the trial, given dismal outcomes for children with recurrent AT/RT, 45% disease stabilization rate for 12 weeks or more was determined to be a meaningful signal, as a complementary measure to objective response, to consider alisertib promising. With 10% type 1 error and 90% power, the targeted sample size was 30 with an interim analysis planned at 15 subjects. Therapy was considered promising if ≥10 patients were without PD by centrally reviewed MRI at 12 weeks or if ≥4 patients had sustained PR/CR. All eligible patients who received at least one dose of alisertib were considered evaluable.

Outcome distributions were estimated with Kaplan–Meier plots. Overall survival (OS) was defined as the time interval from treatment start date to date of death from any cause or to date of last contact for survivors. Progression-free survival (PFS) was defined as time interval from treatment start date to date of progression or date of death or to date of last contact for patients without PD or death. Median PFS was estimated based on reverse Kaplan–Meier approach. We used log-rank tests to compare outcome distributions among patient groups. We used exact Wilcoxon–Mann–Whitney tests to examine the association between age and germline status. Cox regression was used to examine association between PFS and time from initial diagnosis to study enrollment.

Results

Study Participants

Stratum A1 of SJATRT study enrolled 32 patients between May 2014 and July 2019. The following data are current as of November 4, 2021. Two patients who did not start alisertib during the study period (one because of PD after enrollment and the other because of alternate therapy opted by the family) were excluded from all analyses. We analyzed data from 30 patients representing the three AT/RT molecular groups: SHH, (n = 10), MYC (n = 10), TYR (n = 6), and unknown (n = 4). All patients were off therapy at the time of this report because of PD (n = 25), patient/family decision (n = 4), and physician request (n = 1). Twenty-seven of 30 patients had germline data available, 5 of whom (18.5%) had P/LP GLAs. Aurora A kinase staining results were available for 25 of the 30 patients (Table 1).

Pharmacokinetics

After single-dose alisertib, we observed higher mean C_{MAX} values (10.61 ± 3.90 μM) and faster time to C_{MAX} (T_{MAX} = 1.32 ± 0.97 h) in 22 patients who received liquid formulation than those who received ECTs (C_{MAX} = 5.67 ± 2.84 μM and T_{MAX} = 4.73.4 ± 1.64 h). Drug exposure did not differ between formulations ($AUC_{0–\infty}$ = 62.9 ± 22.1h·μM for liquid vs 70.5 ± 17.1 h·μM for ECT). The average apparent oral clearance was 1.91 ± 1.10 L/h per m^2. Serial CSF samples were collected in two patients, and the CSF-to-total plasma $AUC_{0–24h}$ ratios were 1.2% to 2.7%. The plasma pharmacokinetics parameters are detailed in Table 2.

Table 1 Patient/tumor characteristic and adverse effects

Variable	No. (%)
Age at enrollment, years	
Median (range)	3.1 (1.1–20.2)
Sex	
Female	19 (63)
Male	11 (37)
Race	
White	20 (67)
Black	6 (20)
Asian	1 (3)
Mixed/multiple/other	3 (10)
Number of alisertib courses	
Median (range)	2 (1–30)
1	3
2	14
4	2
6–11	7
12–30	4
Molecular groups ($n = 26$)	
ATRT-MYC	10 (38)
ATRT-SHH	10 (38)
ATRT-TYR	6 (24)
Germline ($n = 27$)	
Positive	5 (16.7)
Negative	22 (73.3)
NA	3 (10.0)
Aurora A staining result ($n = 25$)	
Negative	4 (13)
Positive (1+)	10 (33)
Positive (2+)	11 (37)
NA	5 (17)
Common grade 3+ toxicity	
Neutropenia	23 (77)
Anemia	10 (33)
Lymphocytopenia	8 (27)
Thrombocytopenia	8 (27)
Febrile Neutropenia	7 (23)
Hypokalemia	3 (10)
Oral mucositis	3 (10)

Abbreviations: NA, not available.

Toxicity

The most common Grade 3/4 toxicities were neutropenia (n = 23/30, 77%), anemia (33%), lymphopenia (27%), thrombocytopenia (27%), and febrile neutropenia (23%). Hypokalemia and mucositis occurred in 10% of patients. Alisertib is structurally related to benzodiazepines (BZDs) and induces BZD-like effects by binding to GABA receptors, such as somnolence, confusion, and memory loss.[19] Two patients developed Grade 3 somnolence- one after the first dose, prompting parents to remove their child from study and another during the first course of treatment. Rarely paradoxical reactions were noted, with one patient reporting delirium and agitation requiring therapeutic intervention for delirium and dose reduction of alisertib. Patients were, hence, observed for any neurological status changes after their first dose of alisertib for 6 h and re-evaluated at 24- and 48-h post administration.

Outcomes

Median treatment duration for 30 evaluable participants on the study was 45 days (range, 2–653 days). The median number of alisertib cycles administered for the entire cohort was 2 (range, 1–30). We observed one PR and eight patients with SD at 12 weeks of treatment, as determined by central independent imaging review (Figures 1 and 2). There was insufficient statistical evidence to suggest a difference in age at time of study enrollment between patients with SD/PR [n = 9, median age 4.1 years (range, 1.9–20.2)] and the rest [n = 21, median age 2.8 years (range, 1.1–11)] (P = .077). The 6-month and 1-year PFS estimates were 30.0% ± 7.9% and 13.3% ± 5.6%, respectively. The 1-year OS was 36.7% ± 8.4% (Figure 3A). The median PFS for all patients was 59 days (95% confidence interval, 41–172 days). The median time on therapy for nine patients with SD/PR was 7.6 months (range, 5.1–21.5 months) with 2/9 patients staying on therapy for >12 months, one of whom received treatment for 21.5 months. Median time to study enrollment from initial diagnosis was 2.7 years for patients with SD or PR (n = 9) and 1 year for rest (n = 21). However, we did not observe an association between time to study enrollment from diagnosis versus PFS (P = .28) [hazard ratio = 0.89 (95% confidence interval, 0.72–1.10)]. Two additional patients continued treatment for 6.2 and 9 months based on response assessment at 12 weeks by enrolling sites as SD. This assessment, though, was not confirmed by central independent imaging review (Table 3). Therefore, our study failed to meet the predefined success criteria of ≥10 patients without PD at 12 weeks or ≥4 patients with PR or CR.

P/LP GLAs occurred in *SMARCB1* in four patients and *TP53* in one patient (Supplementary Table S1 and Figure S1). Outcomes for the study cohort did not differ by germline status or by molecular group (Figure 3B–E). Additionally, there was no difference in outcomes by molecular groups between patients who met efficacy criteria (n = 9) compared to those who did not (n = 17) (P = .46).

Four patients were alive at the time of our analysis (TYR = 3, MYC = 1). Three of these patients had PD, and one without PD was removed from treatment at the treating physician and family's request because the family pursued a different therapy after being treated in the SJATRT study for approximately 11 months during which she had SD. Three patients with PD are alive at 3.8, 4.4, and 5.0 years from date of progression (Table 3 and Supplementary Figure S2).

Adequate tumor samples for Aurora A kinase staining were available for 25 patients. The staining was negative in

Table 2 Blood Pharmacokinetic Parameters of Alisertib

		Median (Range)		
PK Parameters	Days	Liquid ($n = 22$)	ECT ($n = 8$)	Liquid vs ECT *P*-Value[a]
C_{MAX} (μM)	1	10.8 (4.6–20.8)	5.9 (1.0–9.7)	.0014
	7	9.2 (1.3–23.1)	5.7 (1.0–18.6)	
TMAX (h)	1	1.0 (0.5–4.0)	5.0 (1.5–6.0)	<.0001
	7	1.5 (1.5–4.0)	4.0 (1.5–4.0)	
AUC0-T (h·μM)	1	57.8 (29.7–110)	66.0 (48.9–91.2)	.41
	7	83.8 (17.0–261)	89.8 (20.2–143)	
CL/F (L/h/m^2)	1	2.0 (1.1–3.9)	2.3 (1.7–3.4)	.27
	7	1.2 (0.4–6.0)	1.0 (0.5–2.8)	

Abbreviations: $AUC_{0\text{-}T}$, area under the concentration curve from 0 to infinity for Day 1 and from 0 to 24 h for Day 7; C_{MAX}, maximum concentration; CL/F, apparent oral clearance; ECT, enteric-coated tablets; PK, pharmacokinetic; T_{MAX}, time to reach C_{MAX}.
[a]Mann–Whitney tests based on Day 1 PK parameters.

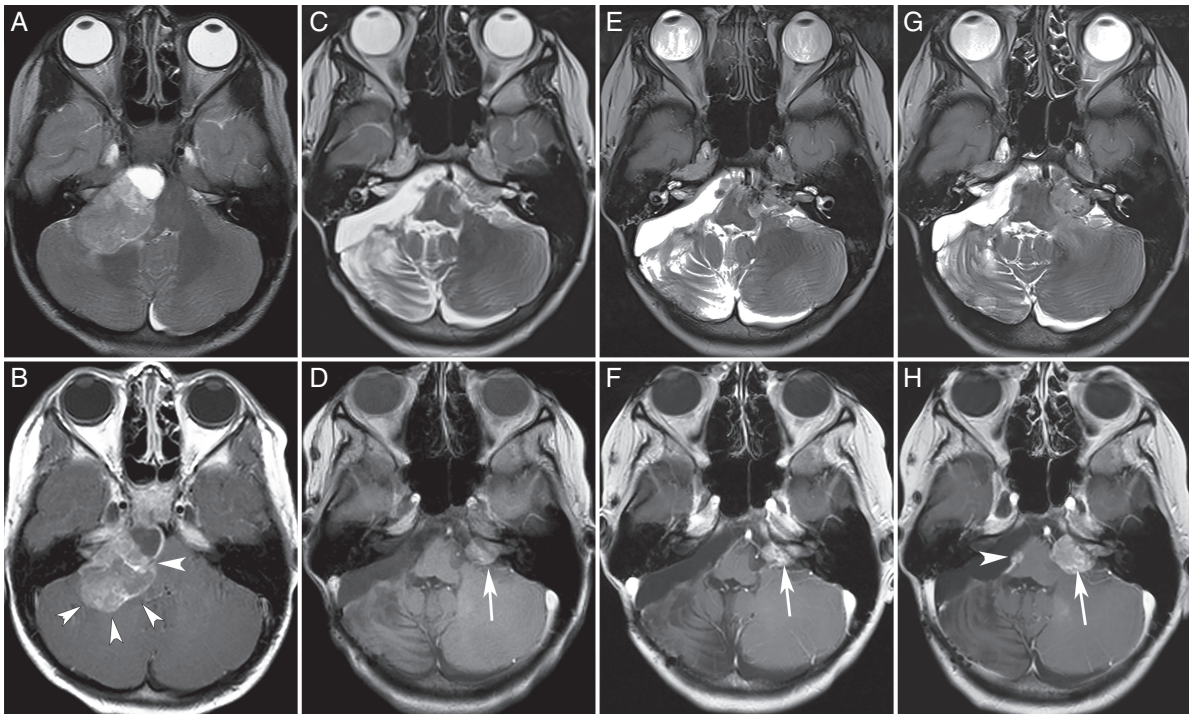

Fig. 1 Magnetic resonance images of a female patient with stable disease as the best response on the trial. Axial T2-weighted image (A) and postcontrast axial T1 weighted (B) images at the time of initial diagnosis at 5 years of age showing a well-defined dominantly solid tumor in the right cerebellopontine angle cistern with avid but inhomogeneous enhancement. Axial T2-weighted (C) and postcontrast axial T1 weighted (D, arrow) images 12 years later showing metastatic disease recurrence within the contralateral cerebellopontine angle cistern. Follow-up study after 4 months of alisertib therapy shows a stable disease on axial T2-weighted (E) and axial T1-weighted images (F, arrow). Progressive disease 3 months later (following 7 months of therapy on the trial) as evidenced by increase in size of the lesion in both T2-weighted (G) and postcontrast axial T1-weighted (H, arrow) images, along with a new lesion along the surface of the brainstem on the right side (H, arrowhead).

4 patients, focal (ie, weak) in 10 patients, and widespread (ie, high) in 11 patients (Supplementary Figure S3). The PFS and OS rates did not differ according to staining pattern (Supplementary Figure S4).

Salvage therapies were pursued for the four children alive at the time of analysis. This included craniospinal irradiation in one patient; focal radiation therapy and ifosfamide, carboplatin, and etoposide chemotherapy

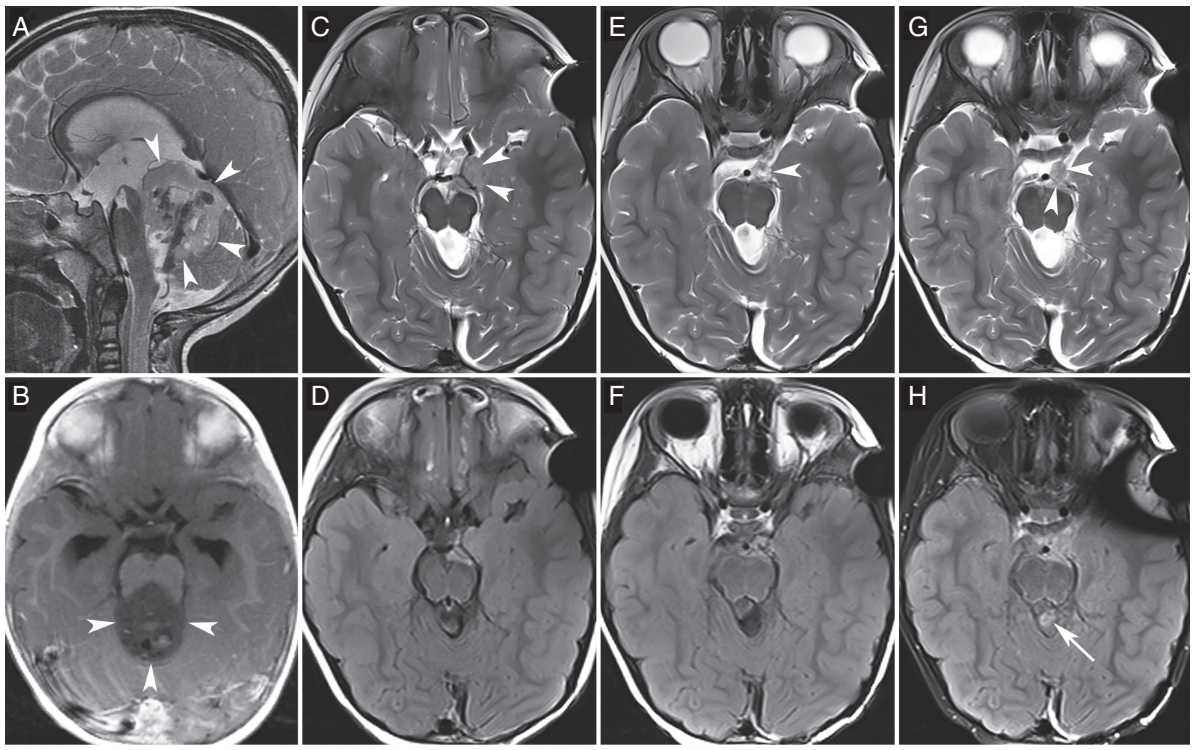

Fig. 2 Magnetic resonance images of a female patient with partial response as the best response in the trial. At the time of initial diagnosis, sagittal T2-weighted image (A, arrowheads) shows a voluminous, heterogeneous tumor in the pineal and supravermian location with no perceptible enhancement on postcontrast T1-weighted image (B, arrowheads). Axial T2-weighted (C, arrowheads) and T2 FLAIR (D) images imaging 2 years later show metastatic recurrence in the premesencephalic and suprasellar cistern surveillance imaging after 4 cycles of treatment with alisertib demonstrating almost complete disappearance of the lesion in axial T2-weighted image (E, arrowhead) although still recognizable as an ill-defined faintly hyperintense structure in the axial contrast-enhanced T2 FLAIR image (F). Follow-up study 3 months later shows recurrent disease on T2-weighted image anterior to the left cerebral peduncle (G, arrowheads) and a new metastasis lesion along the upper aspect of the cerebellar vermis in axial contrast-enhanced T2 FLAIR images (H, arrow).

for 6 cycles in one patient; surgery, focal radiotherapy, and off-protocol alisertib treatment in one patient; and other clinical trials for one patient.

Discussion

The SJATRT trial using single-agent alisertib is the largest study to date to report the tolerability and efficacy of a molecularly targeted therapy in children with recurrent or progressive AT/RT, including an analysis of outcomes according to molecular groups, GLA status, and tumor Aurora kinase A expression. Alisertib was well tolerated in most patients. Clinical benefits included SD and PR in approximately one-third of study participants. However, the study did not meet its predetermined efficacy objective in this patient population based on central imaging review.

In this patient population, oral alisertib displayed a similar pharmacokinetic profile as that previously reported in adults and children, with moderate interpatient variability.[11,12,20–23] The alisertib overall exposure (ie, AUC) did not differ between the liquid and ECT formulations. However, when administered as ECTs, the alisertib pharmacokinetic profile exhibited a slower rate of absorption with lower concentration peaks than that of the liquid formulation. The ECT absorption profile in our patients was similar to that previously reported in pediatric patients receiving 80 mg/m^2 alisertib as ECTs, with a T_{MAX} of 2–3 h and a C_{MAX} of 3.6–7.5 μM.[11,12] The alisertib average apparent oral clearance was 1.91 L/h/m^2 in our population, which is similar to the previously published median and range values in children of 2.04 L/h/m^2 and 0.96–3.54 L/h/m^2, respectively, and approximately half that reported in adults (4.11–4.25 L/h).[20,21] A population-based pharmacokinetic analysis will be performed to further characterize alisertib disposition in the pediatric population and to determine the potential influence of patient covariates on pharmacokinetic variability.

Of the four children alive at the time of our analysis, two received treatment for >12 months and another one for approximately 11 months. All four children have since received salvage therapies. Alisertib, consequently, either prolonged survival or acted as a bridge to other salvage therapies in a proportion of the study participants, as previously reported in a small pilot study of alisertib in recurrent ATRT patients.[13] However, most of the children with

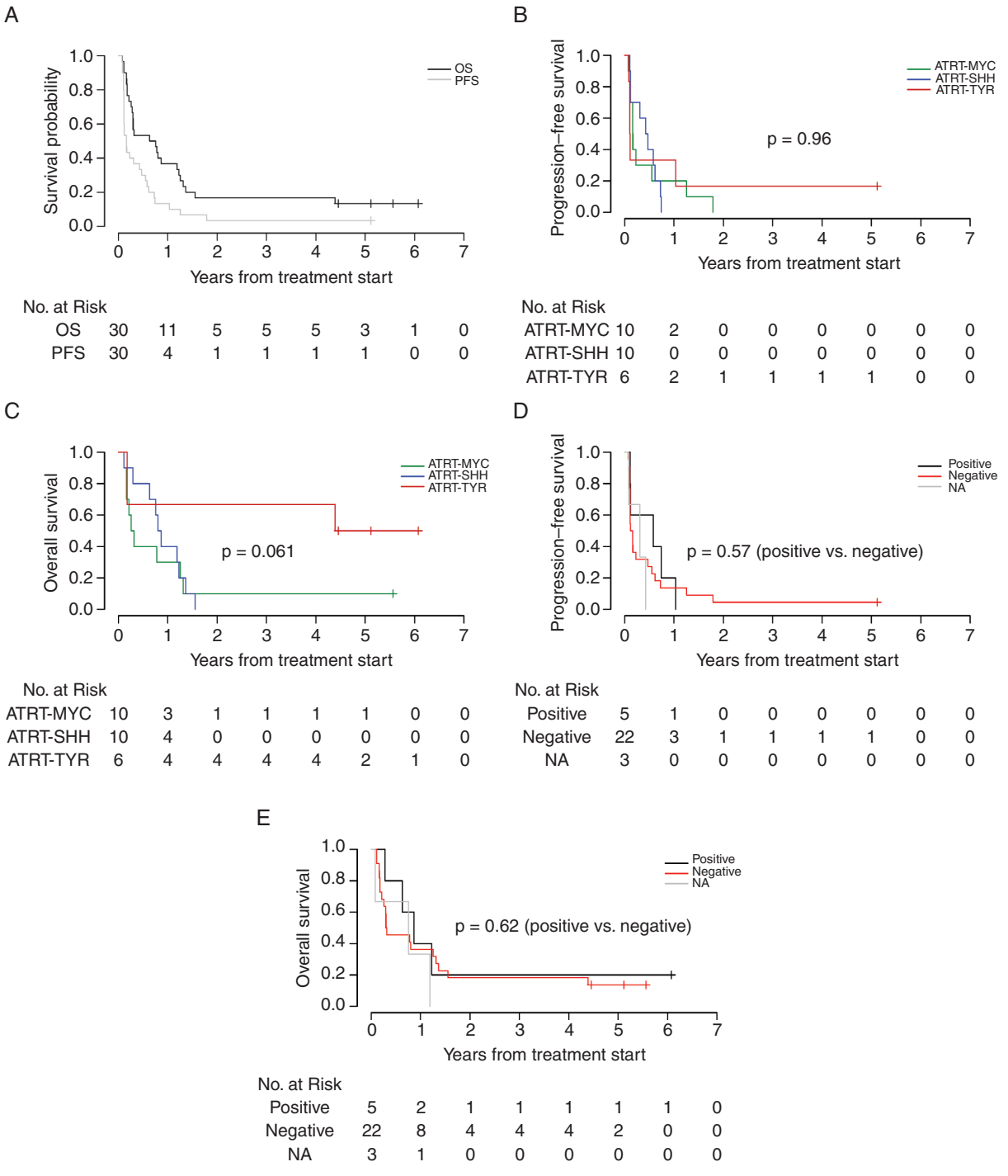

Fig. 3 Kaplan–Meier survival curves for all patients and according to molecular subgroup. (A) Progression-free survival (PFS) and overall survival (OS) for all participants ($n = 30$). (B) PFS and (C) OS categorized by molecular group ($n = 26$). (D) PFS and (E) OS categorized by germline status ($n = 27$).

recurrent or relapsed AT/RT in our study did not benefit from single-agent alisertib treatment and experienced rapid progression while receiving treatment. Moreover, the response to treatment and PFS did not differ by molecular group.

The very limited data on the availability and efficacy of salvage therapies for those with recurrent or relapsed AT/RT suggest dismal outcomes for most of these patients. In a single-institution experience of 68 eligible patients treated at St. Jude, the 5-year OS from recurrence or

Table 3. Patient Level Details

Patient number	Age at Enrollment (Years)	Gender	Molecular Group	Treatment Duration (Months)[a]	Off Treatment Reason	12-Week Response Assessment by Central Imaging Review	12-Week Response Assessment by Enrolling Site	Time to PD or Time to Last Follow-up (Months)[a]	Survival Status	Time to Death or Time to Last Follow-up (Months)[a]	Formulation Used
1	1.8	Female	ATRT-MYC	2.7	Disease progression	PD	PD	2.7	Dead	3.8	Liquid
2	2.5	Male	NA	0.2	Family decision	–	–	1.0	Dead	1.0	Liquid
3	9.7	Male	ATRT-MYC	1.5	Disease progression	–	–	1.4	Dead	3.1	ECT
4	1.9	Female	ATRT-SHH	1.3	Disease progression	–	–	1.3	Dead	1.4	Liquid
5	1.2	Male	NA	1.3	Disease progression	–	–	1.3	Dead	3.4	Liquid
6	1.9	Female	ATRT-TYR	1.3	Disease progression	–	–	1.3	Dead	52.8	Liquid
7	5.1	Female	ATRT-SHH	1.4	Disease progression	–	–	1.4	Dead	7.6	ECT
8	3.2	Female	ATRT-TYR	12.4	Disease progression	SD	SD	12.4	Alive	73.0	ECT (1 cycle) & liquid
9	2.9	Male	NA	1.3	Disease progression	–	–	1.3	Dead	3.6	Liquid
10	5.9	Female	ATRT-MYC	0.1	Family decision	–	–	2.0	Dead	2.0	Liquid
11	2.1	Male	ATRT-TYR	1.3	Disease progression	–	–	1.3	Alive	53.5	Liquid
12	16.5	Male	ATRT-MYC	21.5	Disease progression	SD	SD	21.5	Alive	66.8	ECT
13	6.2	Female	ATRT-SHH	5.1	Disease progression	SD	SD	5.1	Dead	14.3	Liquid
14	2.3	Female	ATRT-SHH	9.0	Disease progression	PD	SD	8.9	Dead	10.4	Liquid
15	1.9	Female	ATRT-TYR	10.9	Family decision	SD	SD	61.5	Alive	61.5	ECT (1 cycle) & liquid
16	4.4	Male	NA	1.4	Disease progression	–	–	1.3	Dead	3.6	Liquid
17	4.7	Female	ATRT-SHH	3.8	Disease progression	–	–	3.8	Dead	9.1	Liquid
18	4.1	Female	ATRT-SHH	5.7	Disease progression	PR	PR	5.7	Dead	16.4	ECT (1 cycle) & liquid
19	2.3	Male	ATRT-SHH	7.0	Disease progression	SD	SD	7.0	Dead	14.7	Liquid
20	17.7	Female	ATRT-MYC	6.7	Disease progression	SD	SD	6.6	Dead	15.8	Liquid (1cycle) & ECT
21	1.1	Male	ATRT-MYC	0.9	Disease progression	–	–	0.9	Dead	1.3	Liquid
22	11.0	Female	ATRT-MYC	6.2	Physician Request	PD	SD	15.1	Dead	15.1	ECT
23	2.9	Male	ATRT-MYC	1.3	Disease progression	–	–	1.3	Dead	9.4	Liquid
24	1.1	Male	ATRT-TYR	1.0	Disease progression	–	–	1.0	Dead	2.1	Liquid
25	20.2	Female	ATRT-SHH	8.8	Disease progression	SD	SD	8.8	Dead	18.7	ECT
26	5.6	Female	ATRT-MYC	1.9	Disease progression	–	–	1.9	Dead	2.6	ECT
27	2.2	Female	ATRT-SHH	1.4	Disease progression	–	–	1.4	Dead	3.6	Liquid
28	4.0	Female	ATRT-SHH	7.6	Disease progression	SD	SD	7.4	Dead	9.6	Liquid

Table 3. continued

Patient number	Age at Enrollment (Years)	Gender	Molecular Group	Treatment Duration (Months)[a]	OffTreatment Reason	12-Week Response Assessment by Central Imaging Review	12-Week Response Assessment by Enrolling Site	Time to PD or Time to Last Follow-up (Months)[a]	Survival Status	Time to Death or Time to Last Follow-up (Months)[a]	Formulation Used
29	7.9	Female	ATRT-TYR	1.3	Disease progression	–	–	1.3	Dead	2.1	ECT
30	2.8	Female	ATRT-MYC	1.4	Family decision	–	–	1.9	Dead	1.9	Liquid

Abbreviations: ECT, enteric-coated tablet; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease.
The two patients who had SD by site assessments but PD by central review are highlighted in green.
[a]Duration from start of treatment.

relapse was <10%, reflecting the lack of curative treatment options for most patients with relapsed disease.[24] Peyrl and colleagues reported the outcomes of children with recurrent embryonal tumors who were treated with multi-agent antiangiogenic therapy and intrathecal chemotherapy, in which three children with AT/RT, including one with multiple recurrent tumors, were alive after 10 to 42 months.[25]

Previous reports of molecularly targeted treatment of children with recurrent AT/RT include the St. Jude institutional experience with alisertib and a phase I study of the CDK4/6 inhibitor ribociclib (LEE011), but these studies were limited by small numbers of patients. Both studies reported prolonged SD with alisertib (n = 4/4) and ribociclib (n = 2/13).[13,26] The results of a phase I study of tazemetostat, a selective inhibitor of enhancer of zeste homolog-2 (encoded by *EZH2*), were presented at the 2020 American Society of Clinical Oncology conference. In this study, 7 of 30 patients with AT/RT experienced SD or better with tazemetostat treatment, including one patient with a CR (median duration of response, 28 weeks; range, 24–75 weeks). Tazemetostat was well tolerated by most patients, but one patient developed T-cell lymphoblastic lymphoma.[27] Together, these findings indicate that most patients with recurrent AT/RT do not experience durable responses to the currently available, very limited salvage treatment options.

Alisertib is a selective, potent, and reversible small-molecule inhibitor of Aurora kinase A, which belongs to a highly conserved family of serine/threonine protein kinases that includes Aurora B and C kinases.[28] Aurora kinase A and B are expressed in all actively dividing cells. Aurora kinase A localizes to the centrosomes and proximal mitotic spindle during mitosis, where it functions in a diverse set of mitotic processes. Consequently, alisertib functions primarily as a cytostatic drug. In accordance with this function, SD was the main response in eight patients, and only one patient experienced a PR in our study. Molecular group and GLAs did not affect outcomes in our patients. This finding is similar with our previous observations for molecular groups and GLA in newly diagnosed AT/RT treated with risk-adapted therapy.[14] Alisertib penetrated the blood–brain barrier, albeit in low concentration. This finding is consistent with a recent study in a rat model that alisertib had a CNS penetration of 3.12%.[29] Whether this factored into lack of efficacy in our study is impossible to determine because of the small number of patients with CSF PK data.

Alisertib toxicity was generally manageable, with only one patient requiring dose reduction and one patient withdrawing from the study because of adverse effects. Nine patients who met efficacy criteria stayed on treatment >5 months, with two receiving alisertib longer than a year. Two other patients continued treatment for 6.2 and 9 months based on the assessment by enrollment sites. This finding is of relevance because lack of second-line curative therapies for most patients with recurrent or relapsed AT/RT translates into rapid disease progression and death. Alisertib, hence, may be considered as a palliative treatment option under such circumstances. Additionally, alisertib therapy bridged three of four survivors to other treatment options that resulted in durable disease remission at the time of our analysis. While PFS did not differ by molecular groups, OS approached significance (P = .06) with children with TYR group having longer survival (Figure 3C). This is similar to our recent report on children with newly

diagnosed AT/RT treated in St. Jude multi-institutional studies[14]demonstrating better OS for children with TYR group, suggesting a more indolent course and possibly better outcomes for these children compared to MYC or SHH group. Whether alisertib improves survival in patients with newly diagnosed AT/RT who receive alisertib combined with chemo-radiotherapy will be determined in the ongoing portions of the SJATRT trial (NCT02114229).

AT/RT is a difficult disease to treat, especially in children who experience frontline therapy failure. Although molecular advances have increased our understanding of the biological underpinnings of AT/RT, these advances have not translated into improved outcomes for children affected by this devastating disease. Ongoing clinical trials of molecularly targeted therapies and immunotherapies hold a promise of better outcomes for these children: NCT04897880, NCT02962167, NCT02601937, NCT04416568, NCT04185038, NCT03500991, and NCT03434262.

Study Limitations

We could not determine any biological correlates of response in the nine patients who demonstrated a response to treatment by molecular groups, germline alterations or by *Aurora A* expression and thus are unable to recommend alisertib for any specific subset of children with recurrent AT/RT. Additionally, due to the availability of alisertib PK study results in only 2 patients, a correlation between CSF concentration of the drug and response to treatment is not possible from the current study.

Conclusions

The activity of single-agent alisertib was deemed not promising in children with recurrent/progressive AT/RT according to the predetermined efficacy criteria in our trial. Nevertheless, SD or PR by central imaging review occurred in approximately one-third of patients, with a manageable alisertib adverse effect profile. Alisertib ECTs exhibited slower absorption rates and lower peak concentrations than the liquid formulation, but the overall exposure did not differ between the two formulations. Alisertib benefited some children with recurrent or relapsed AT/RT as a form of palliative therapy or as a bridge to other treatment modalities that may produce durable remission.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

KeyWords

alisertib | alisertib pharmacokinetics | atypical teratoid/rhabdoid tumor | Aurora kinase A

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References

1. Ostrom QT, Patil N, Cioffi G, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2013-2017. *Neuro Oncol.* 2020; 22(12 Suppl 2):iv1–iv96.
2. Biegel JA, Zhou JY, Rorke LB, et al. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. *Cancer Res.* 1999; 59(1):74–79.
3. Hasselblatt M, Gesk S, Oyen F, et al. Nonsense mutation and inactivation of SMARCA4 (BRG1) in an atypical teratoid/rhabdoid tumor showing retained SMARCB1 (INI1) expression. *Am J Surg Pathol.* 2011; 35(6):933–935.
4. Versteege I, Sevenet N, Lange J, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature.* 1998; 394(6689):203–206.

5. Ho B, Johann PD, Grabovska Y, et al. Molecular subgrouping of atypical teratoid/rhabdoid tumors-a reinvestigation and current consensus. *Neuro Oncol.* 2020; 22(5):613–624.
6. Nikonova AS, Astsaturov I, Serebriiskii IG, Dunbrack RL, Jr., Golemis EA. Aurora A kinase (AURKA) in normal and pathological cell division. *Cell Mol Life Sci.* 2013; 70(4):661–687.
7. Hoar K, Chakravarty A, Rabino C, et al. MLN8054, a small-molecule inhibitor of Aurora A, causes spindle pole and chromosome congression defects leading to aneuploidy. *Mol Cell Biol.* 2007; 27(12):4513–4525.
8. Lee S, Cimica V, Ramachandra N, Zagzag D, Kalpana GV. Aurora A is a repressed effector target of the chromatin remodeling protein INI1/hSNF5 required for rhabdoid tumor cell survival. *Cancer Res.* 2011; 71(9):3225–3235.
9. Goff LW, Azad NS, Stein S, et al. Phase I study combining the aurora kinase a inhibitor alisertib with mFOLFOX in gastrointestinal cancer. *Invest New Drugs.* 2019; 37(2):315–322.
10. Lim KH, Opyrchal M, Acharya A, et al. Phase 1 study combining alisertib with nab-paclitaxel in patients with advanced solid malignancies. *Eur J Cancer.* 2021; 154:102–110.
11. Mosse YP, Fox E, Teachey DT, et al. A Phase II study of alisertib in children with recurrent/refractory solid tumors or leukemia: children's oncology group Phase I and pilot consortium (ADVL0921). *Clin Cancer Res.* 2019; 25(11):3229–3238.
12. Mosse YP, Lipsitz E, Fox E, et al. Pediatric phase I trial and pharmacokinetic study of MLN8237, an investigational oral selective small-molecule inhibitor of Aurora kinase A: a Children's Oncology Group Phase I Consortium study. *Clin Cancer Res.* 2012; 18(21):6058–6064.
13. Wetmore C, Boyett J, Li S, et al. Alisertib is active as single agent in recurrent atypical teratoid rhabdoid tumors in 4 children. *Neuro Oncol.* 2015; 17(6):882–888.
14. Upadhyaya SA, Robinson GW, Onar-Thomas A, et al. Relevance of molecular groups in children with newly diagnosed atypical teratoid rhabdoid tumor: results from prospective St. Jude multi-institutional trials. *Clin Cancer Res.* 2021; 27(10):2879–2889.
15. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature.* 2018; 555(7697):469–474.
16. Lipsitz E, Moorthy G, Mosse Y, Fox E, Adamson PC. A sensitive and selective liquid chromatography/tandem mass spectrometry method for determination of MLN8237 in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010; 878(25):2369–2373.
17. Kocherginsky M, Cohen EE, Karrison T. Design of Phase II cancer trials for evaluation of cytostatic/cytotoxic agents. *J Biopharm Stat.* 2009; 19(3):524–529.
18. Sill MW, Rubinstein L, Litwin S, Yothers G. A method for utilizing co-primary efficacy outcome measures to screen regimens for activity in two-stage Phase II clinical trials. *Clin Trials.* 2012; 9(4):385–395.
19. Sells TB, Chau R, Ecsedy JA, et al. MLN8054 and alisertib (MLN8237): discovery of selective oral Aurora A inhibitors. *ACS Med Chem Lett.* 2015; 6(6):630–634.
20. Zhou X, Mould DR, Takubo T, et al. Global population pharmacokinetics of the investigational Aurora A kinase inhibitor alisertib in cancer patients: rationale for lower dosage in Asia. *Br J Clin Pharmacol.* 2018; 84(1):35–51.
21. Venkatakrishnan K, Zhou X, Ecsedy J, et al. Dose selection for the investigational anticancer agent alisertib (MLN8237): pharmacokinetics, pharmacodynamics, and exposure-safety relationships. *J Clin Pharmacol.* 2015; 55(3):336–347.
22. Dees EC, Cohen RB, von Mehren M, et al. Phase I study of aurora A kinase inhibitor MLN8237 in advanced solid tumors: safety, pharmacokinetics, pharmacodynamics, and bioavailability of two oral formulations. *Clin Cancer Res.* 2012; 18(17):4775–4784.
23. Cervantes A, Elez E, Roda D, et al. Phase I pharmacokinetic/pharmacodynamic study of MLN8237, an investigational, oral, selective aurora a kinase inhibitor, in patients with advanced solid tumors. *Clin Cancer Res.* 2012; 18(17):4764–4774.
24. Carey SHJ, Acharya S, et al. Outcomes for children with recurrentatypical teratoid rhabdoid tumor: a single institution study. *Pediatric Blood Cancer.* 2021; 68(S3):e29060.
25. Peyrl A, Chocholous M, Kieran MW, et al. Antiangiogenic metronomic therapy for children with recurrent embryonal brain tumors. *Pediatr Blood Cancer.* 2012; 59(3):511–517.
26. Geoerger B, Bourdeaut F, DuBois SG, et al. A phase I study of the CDK4/6 inhibitor ribociclib (LEE011) in pediatric patients with malignant rhabdoid tumors, neuroblastoma, and other solid tumors. *Clin Cancer Res.* 2017; 23(10):2433–2441.
27. Chi SB, Bourdeaut F, Willis T, et al. Phase I study of tazemetostat, an enhancer of zeste homolog-2 inhibitor, in pediatric pts with relapsed/refractory integrase interactor 1-negative tumors. *J Clin Oncol.* 2020; 38(15):10525.
28. Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. *Nat Rev Mol Cell Biol.* 2001; 2(1):21–32.
29. Power E, Oh J, Zhang L, Elmquist W, Daniels D. HGG-13. Brain distribution and clearance of alisertib is limited by Pgp and BCRP efflux pumps and dependent upon delivery method. *Neuro-Oncology.* 2021; 23(Supplement_1):i20–i20.