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Population Pharmacokinetics and Exposure-Safety Relationships of Alisertib in Children and Adolescents With Advanced Malignancies

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Abstract

Population pharmacokinetic (PK) and exposure-safety analyses of alisertib were performed in children enrolled in 2 clinical trials: NCT02444884 and NCT01154816. NCT02444884 was a dose-finding study in children with relapsed/refractory solid malignancies (phase 1) or neuroblastomas (phase 2). Patients received oral alisertib 45 to 100 mg/m^2 as powder-in-capsule once daily or twice daily for 7 days in 21-day cycles. Serial blood samples were collected up to 24 hours after dosing on cycle 1, day 1. NCT01154816 was a phase 2 single-arm study evaluating efficacy in children with relapsed/refractory solid malignancies or acute leukemias. Patients received alisertib 80 mg/m^2 as enteric-coated tablets once daily for 7 days in 21-day cycles. Sparse PK samples were collected up to 8 hours after dosing on cycle 1, day 1. Sources of alisertib PK variability were characterized and quantified using nonlinear mixed-effects modeling to support dosing recommendations in children and adolescents. A 2-compartment model with oral absorption described by 3 transit compartments was developed using data from 146 patients. Apparent oral clearance and central distribution volume were correlated with body surface area across the age range of 2 to 21 years, supporting the use of body surface area–based alisertib dosing in the pediatric population. The recommended dose of 80 mg/m^2 once daily enteric-coated tablets provided similar alisertib exposures across pediatric age groups and comparable exposure to that in adults receiving 50 mg twice daily (recommended adult dose). Statistically significant relationships ($P < .01$) were observed between alisertib exposures and incidence of grade $\geq$2 stomatitis and febrile neutropenia, consistent with antiproliferative mechanism-related toxicities.

Keywords

alisertib, Aurora A kinase, exposure-safety, pediatric, population pharmacokinetics

Alisertib is a selective small-molecule inhibitor of Aurora A kinase that is being developed for the treatment of hematologic and nonhematologic malignancies.[1,2] An Aurora A kinase inhibitor would be expected to have potential applications across a broad range of human tumors, given the essential role of mitosis in tumor proliferation.[3–7] Indeed, alisertib has demonstrated activity against a broad range of tumors in vitro and in vivo.[3,4,8–12] Alisertib is also expected to be toxic to bone marrow and gastrointestinal epithelium, where Aurora A kinase is expressed and active.[13]

The antitumor activity of alisertib has also been studied in multiple nonclinical models of pediatric cancers under the National Cancer Institute's Pediatric Preclinical Testing Program.[14,15] Significant antitumor activity was observed in multiple solid-tumor (neuroblastoma, Wilms tumor, rhabdoid tumor, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, and medulloblastoma) and acute lymphoblastic leukemia xenograft models. Combination effects have also been observed with alisertib and standard-of-care agents in nonclinical models of pediatric leukemia, medulloblastoma, and neuroblastoma.[16,17]

Alisertib is a weak acid and demonstrates pH-dependent solubility. Coadministration of gastric acid–reducing agents, such as esomeprazole, increased alisertib systemic exposure by ≈30% in adult cancer

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patients following administration of alisertib as enteric-coated tablets.[18] Results from a mass balance study in adult patients with advanced solid tumors indicated that alisertib was predominantly cleared via hepatic metabolism. Renal excretion accounted for <3% of apparent total clearance.[19] Subsequent metabolite profiling of excreta indicated that phase 1 oxidative metabolism pathways were involved in >68% of alisertib metabolism.[20] In vitro cytochrome P450 (CYP) reaction phenotyping studies indicated that CYP3A4, with 86% contribution, was the major enzyme involved in oxidative metabolism of alisertib.[20] Alisertib exposure increased by ≈40% in the presence of itraconazole, a strong CYP3A/P-glycoprotein inhibitor, and decreased by 50% in the presence of rifampin, a strong CYP3A inducer, based on drug-drug interaction studies in adult patients with advanced solid tumors.[18] Adult patients with moderate or severe hepatic impairment have ≈150% higher unbound alisertib exposures compared with patients with normal hepatic function.[21]

A population pharmacokinetic (PK) analysis was performed on data from 671 adult patients with cancer in Western countries and in Japan/East Asia over a wide dose range and multiple dosing schedules. The PK of alisertib was described by a 2-compartment model with 4-transit compartment absorption and linear elimination. The final model included a covariate effect of region on relative oral bioavailability of alisertib, with adult patients in the East Asian region estimated to have ≈50% higher bioavailability compared with Western patients.[13] Mild hepatic impairment (total bilirubin ≤1.5× the upper limit of normal) and mild or moderate renal impairment (creatinine clearance ≤30 mL/min) did not result in clinically relevant effects on alisertib PK.[13]

This article describes a population PK analysis of data from 2 studies in pediatric patients with cancer. The aims of this analysis are to describe the PK of alisertib in the pediatric population, to assess the influence of relevant covariates on its PK, and to evaluate the relationship between alisertib exposure and key safety outcomes.

Methods

Study Design

Two clinical studies were conducted by the National Cancer Institute Cooperative Group, the Children's Oncology Group: studies ADVL0812 (NCT02444884)[22] and ADVL0921 (NCT01154816).[23] The final protocol, any amendments, and informed consent documents were reviewed and approved by the institutional review board or research ethics board at each of the participating Children's Oncology Group institutions (see Supplemental appendix for list of study centers). Both studies were conducted in accordance with the protocol, Declaration of Helsinki, International Conference on Harmonisation Good Clinical Practice guideline, and applicable local regulations.

Study ADVL0812[22] was a single-agent, dose-finding study in children and adolescents with relapsed or refractory solid tumors (phase 1) or neuroblastomas (phase 2). The phase 1 used a rolling-6 design, which has been described previously.[22,24] In summary, patients in both phases received alisertib orally (as powder-in-capsules) either once daily or twice daily on days 1 to 7 of a 21-day cycle. Alisertib dose regimens in phase 1 included 45, 60, 80, and 100 mg/m^2 once daily, and 30 and 40 mg/m^2 twice daily. In phase 2, all patients received alisertib 80 mg/m^2 once daily.

Study ADVL0921[23] was a phase 2, single-arm, single-agent study in pediatric patients with relapsed or refractory solid malignancies or acute leukemias (acute lymphoblastic leukemia and acute myeloid leukemia). All patients received alisertib orally (as enteric-coated tablets), 80 mg/m^2 once daily on days 1 to 7 of a 21-day cycle.

Patients

In both studies, patients were aged >12 months and ≤21 years. Written informed consent was obtained from the patients (or their parents/guardians if they were children) before participation in the studies. Patients were required to have histologic verification of malignancy at original diagnosis or relapse (except for certain solid tumors in ADVL0812[22]), and a Karnofsky (for patients >16 years) or Lansky (for patients ≤16 years) performance status score of ≥50, and must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy. Adequate bone marrow, renal, and hepatic function were also required. Exclusion criteria included uncontrolled infection, pregnancy/lactation, concurrent use of other anticancer agents, growth factors, investigational drugs, certain P-glycoprotein substrates, and daily benzodiazepines.

Assessments

In both studies, blood samples for plasma PK analysis were collected before dosing on days 1, 4(±1), and 7(±1) of cycle 1. In ADVL0812, additional samples were collected on cycle 1, day 1 at 0.5, 1, 2, 3, 4, 6 to 8, and 24 hours after dosing in patients ≥10 kg, and at 1, 2, 4, 6 to 8, and 24 hours after dosing in patients <10 kg.[22] In study ADVL0921, 3 additional samples were collected on cycle 1, day 1, at 1 to 2, 3 to 4, and 6 to 8 hours.[23] Plasma concentrations of alisertib were measured using a previously published validated liquid chromatography–tandem mass spectrometry assay,

with a lower limit of quantification of 10 nmol/L.[25] The intraday precision based on the standard deviation of replicates of quality control samples ranged from 0.2% to 4% and with accuracy ranging from 96% to 102%. The interday precision ranged from 0.5% to 7% and the accuracy ranged from 93% to 105%.

Adverse events were recorded throughout each study and grades were assigned using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Population PK Modeling

Population modeling used NONMEM version 7.3 (Icon Development Solutions, Dublin, Ireland) with Intel Visual Fortran Intel 64 Compiler XE, version 12.0.0.104, build 20101006 (Santa Clara, California). SAS version 9.4 (SAS Institute Inc, Cary, North Carolina) was used for most graphical outputs and data manipulation. The population PK profile of alisertib was modeled extensively in a previous analysis of data from adult patients with cancer and was described by a linear, 2-compartment model with transit absorption over the 5 to 200 mg/d range.[13] This information was used as a starting point for structural model development in the analysis in pediatric patients. One-, 2-, and 3-compartment linear models with first-order absorption defined by a rate constant and an absorption lag, or by a series of transit compartments, were examined to ensure that the current data set both supported and was best reflected by a 2-compartment model with transit absorption. The 2 pediatric studies used different alisertib formulations (powder-in-capsules and enteric-coated tablets). Although the data in adults and the population PK model did not indicate a discernible difference in bioavailability between these 2 formulations,[2,26] the relative bioavailability of the capsule and tablet has not been characterized in the pediatric population. Therefore, a potential difference in bioavailability of these 2 formulations in the pediatric population was evaluated in the base model and carried forward as an intrinsic aspect of alisertib pediatric PK.

Models were selected on the basis of goodness of fit as judged by changes in the minimum objective function. The likelihood ratio test was used to compare nested covariate models to base models with significance levels of $P = .01$ for forward addition of covariates, and $P = .001$ for backward deletion of covariates. The addition of parameters and covariates was also assessed by their ability to reduce interindividual variability terms.

Age, weight, body surface area (BSA), race, ethnicity, sex, and drug formulation were assessed as predictors of PK variability using a standard forward addition ($P < .01$), backward elimination ($P < .001$) strategy. The choice of the final covariate model was based on models that had a statistically significant improvement in the objective function value, passed the covariance step and had a condition number <20 (computed as the square root of the ratio of the largest to smallest eigenvalue), had precise estimates of the covariate parameter (asymptotic standard error <51.2%), and reduced the between-subject variability (BSV) of the associated population parameters to a clinically important extent (>5% reduction in BSV).

Base, full, and final models were evaluated using standard goodness-of-fit diagnostic plots and visual predictive checks against the evaluation data set. The final population model was used to simulate 200 versions of the analysis data set based on final parameter values including residual variability. The alisertib concentration-time course for the observed data, together with the median and 90% prediction intervals of the simulated concentrations, were plotted by dose regimen. When sparse sampling resulted in too few observations for a visual predictive check to be informative, data were pooled by dividing concentration by dose, which allowed data from all subjects to be combined. The predictive performance of the model was considered acceptable if the time course of the median simulated and observed data were similar, with no important systematic deviations, and the majority of the original data points lay inside the prediction intervals.

The primary method for determining the confidence intervals (CIs) of the model parameters was by use of the asymptotic standard errors returned by the covariance step of NONMEM. Nonparametric bootstrapping (N = 1000) was also used as a secondary check of precision of parameter estimates.

Simulations were conducted and typical value and empirical Bayes prediction of the interindividual random effect (eta)-corrected post hoc apparent clearance (CL/F) values were used to estimate the area under the concentration–time curve (AUC) at steady state (AUC_{ss}) for patients in the pediatric and adult population data sets based on the respective population PK models. Doses and dose regimens used in clinical trials in children and adults[26–29] were evaluated for exposure similarity at the respective maximum tolerated doses across the patient populations.

An external validation of the model was conducted by using the final model to predict adult alisertib PK data from a number of studies.

The final model was used to evaluate 80 mg/m^2 administered once daily in children and adolescents, which is the maximum tolerated dose and recommended phase 2 dose for alisertib in this patient population. Alisertib exposure in children and adolescents at 80 mg/m^2 once daily was further evaluated in relation

to exposure in adult patients at 50 mg twice daily (the recommended clinical dose of alisertib in adult patients).[2]

Exposure-Safety Analysis

The relationship between alisertib exposure and safety outcomes was evaluated across trials ADVL0812[22] and ADVL0921[23] in children and adolescents.

The following adverse events (toxicity end points) were selected on the basis that they were representative of the key adverse events involving the mechanism of action of alisertib (ie, the effect on proliferative tissues): grade ≥2 stomatitis, grade ≥2 neutrophil count decreased, grade ≥2 febrile neutropenia, grade ≥2 anemia, and grade ≥2 adverse events in the infections and infestations system organ class.

The average concentration of alisertib at steady state ($C_{ss,avg}$) was used as the exposure metric and was calculated using the following equation:

$$C_{ss,avg}\,(\mu M) = \frac{\text{Dose (mg)} * 1000}{\text{CL/F (L/h)} * 518.92 * \text{tau}}$$

Tau represents the alisertib dosing interval (12 hours for twice-daily dosing and 24 hours for once-daily dosing). Dose was the administered starting dose (mg) of alisertib. The CL/F for each individual patient was estimated from the population PK analysis considering the formulation administered and the relative bioavailability if estimated as significantly different from 1.0 in the model. A relative bioavailability of 0.671 for alisertib enteric-coated tablet in reference to the capsule in the pediatric population was estimated by population PK analysis on the basis of data from these 2 studies. The molecular weight of alisertib is 518.92 g/mol.

Exposure–adverse event relationships for the toxicity endpoints were estimated by logistic regression. Age, sex, performance score, number of prior therapy regimens, cancer type, and number of treatment cycles while on study were explored as covariates.

Results

Data from 146 patients with a total of 606 alisertib concentration records contributed to the population PK and exposure-response analyses. Of the 146 evaluable patients, 77 were aged 2 to 11 years, 40 were aged 12 to 16 years, and 29 were aged 17 to 21 years. A summary of continuous and categorical covariates (baseline characteristics) is presented in Table 1.

Population PK Analysis

Alisertib PK in children and adolescents was best described by a linear, 2-compartment model, parameterized for CL/F, apparent central (V1/F), and peripheral volumes of distribution, and apparent intercompartment clearance. Oral absorption was best described with 3 transit compartments expressed via an absorption transit rate constant. The final model is illustrated in Figure S1, and the final model parameters are presented in Table 2. The final model evaluation showed that the precision of the parameter estimates and the residual variability were acceptable. Inclusion of the covariate effects decreased the BSV for CL/F and V1/F in the final model. Bootstrap runs were performed to estimate the 95%CI of the parameters. Overall, mean values were generally in the center of the 95%CI of the parameters (data not shown).

Covariate effects noted in the base model were resolved in the final model. Body size was identified as the significant predictor of CL/F and V1/F BSV. While body weight was identified as the most significant predictor in terms of objective function changes, BSA provided comparable results based on all goodness-of-fit criteria (Figure 1). Because current alisertib dosing regimens adjust for BSA (mg/m^2) in children and adolescents with cancer, BSA was carried forward in the final model in this analysis. When BSA-normalized CL/F is plotted against age group, the dependence of age on alisertib CL/F is no longer readily apparent (Figure 2). The distributions of BSA-normalized CL/F values substantially overlap across the age groups, supporting the adequacy of BSA-based dosing in normalizing exposures over the ≥2-year pediatric age range (Table S1).

The bioavailability of the enteric-coated tablet formulation relative to powder-in-capsule was significantly different: 68% (bootstrap 90%CI, 55%-86%) relative bioavailability of enteric-coated tablet vs powder-in-capsule in pediatric patients (Figure 3). Results of simulations from the model are presented for the enteric-coated tablet formulation, as this is the formulation of alisertib used in ongoing pediatric clinical trials.

An external validation was conducted by using the final model to predict alisertib PK in adult patients. Visual predictive checks (Figure 4) demonstrated good reproducibility of the analysis data set and of the external validation data set in adults (Figure 4), suggesting that the model can be used to derive exposure metrics in support of alisertib pediatric dose selection.

An 80 mg/m^2 once-daily (the recommended phase 2 dose for alisertib in children and adolescents) dosing regimen of the enteric-coated tablet formulation provided approximately comparable exposure to that in adults receiving 50 mg twice daily (ie, 100 mg/d, recommended clinical dose of alisertib for adult patients; Figure 5).

Exposure-Safety Analysis

The incidences of each of the selected adverse events are presented in Table S2. The incidence of grade ≥2

Table 1. Summary of the Population PK and Exposure-Safety Analysis Data Set and Distributions of Continuous and Categorical Covariates

	Statistic	ADVL0812 N = 46	ADVL0921 N = 100	Total N = 146
Population PK				
Dose, mg/m^2		45, 60, 80, 100	80	
Dose, mg		25-150	40-160	
Continuous covariates				
Age, y	Mean (range)	10.7 (4-21)	11.3 (2-21)	11.1 (2-21)
Weight, kg	Mean (SD)	39.5 (21.19)	42.9 (25.98)	41.9 (24.55)
BSA, m^2	Mean (SD)	1.22 (0.41)	1.26 (0.48)	1.25 (0.46)
Dose, mg	Mean (SD)	76.5 (32.74)	99.4 (36.3)	92.2 (36.7)
Categorical covariates				
Male		25	54	79
Race				
White	n	31	58	89
Black	n	8	16	24
Asian	n	2	4	6
Other/unknown	n	5	22	27
Ethnicity				
Non-Spanish/non-Hispanic	n	41	69	110
Mexican	n	1	4	5
Puerto Rican	n	1	2	3
Spanish/Hispanic	n	1	18	6
Unknown	n	2	7	9
Formulation				
Capsule	n	46	0	46
Enteric-coated tablet	n	0	100	100
Exposure-safety				
Age				
2 to ≤12 years		30	55	85
12 to ≤16 years		10	22	32
>16 years		6	23	29
ECOG performance score				
0		27	64	91
>0		19	36	55
Number of prior therapy regimens				
≤1		2	60	62
≥2		44	40	84
Cancer type				
Hematologic		0	19	19
Nonhematologic		46	81	127
Number of treatment cycles while on study				
1		26	59	85
≥2		20	41	61

BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation.

Table 2. Final Model Parameters for the Pediatric Population PK Model of Alisertib in Patients With Advanced Malignancies

Code	Parameter	Unit	Population value	SE, %	BSV (ratio)	SE, %	Eta shrinkage, %
CL/F	Apparent clearance	L/h	1.84	12.0	0.581[a]	11.5	16.6
V1/F	Apparent central volume	L	24.1	14.4	0.699[a]	20.7	39.1
Q/F	Apparent intercompartment clearance	L/h	2.66	19.2	…	…	…
V2/F	Apparent peripheral volume	L	32.3	20.2	0.932	21.0	62.6
K_{tr}	Transit compartment rate constant	1/h	2.35	9.7	0.540	8.3	42.1
F_{ECT}	Enteric coated tablet relative bioavailability	%	67.1	12.5	…	…	…
BSA_{V1}	Effect of body surface area on V1/F	…	1.47	22.2	…	…	…
BSA_{CL}	Effect of body surface area on CL/F	…	0.742	19.5	…	…	…
CCV	Proportional residual error	Ratio	0.59	6.8	…	…	…

BSV, between-subject variability; eta, empirical Bayes estimate of the interindividual random effect; SE, standard error.
[a] CL/F–V1/F BSV correlation: 0.583.

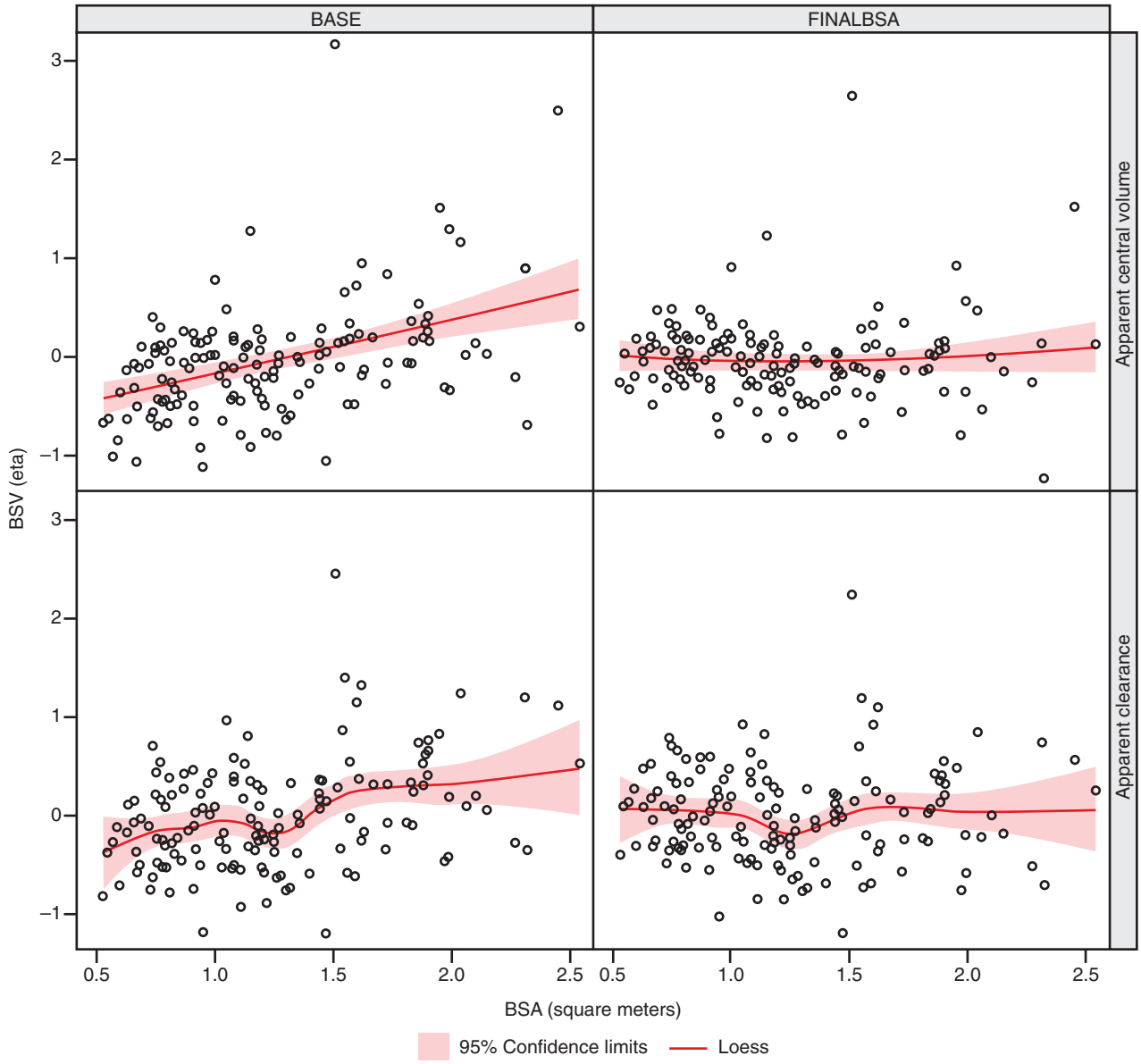

Figure 1. BSA covariate effect in the final model. BSA, body surface area; BSV, between subject variability; eta, empirical Bayes prediction of the interindividual random effect.

neutrophil count decreased, grade ≥2 anemia, grade ≥2 stomatitis, and febrile neutropenia in the safety population are 65%, 54%, 31%, and 20%, respectively. A statistically significant ($P = .0002$) relationship between alisertib $C_{ss,avg}$ and the incidence of grade ≥2 stomatitis was observed. The logistic regression fitted model and 95%CI are shown in Figure 6A. Age, sex, performance score, number of prior therapies, cancer types, and number of treatment cycles were evaluated as covariates, and none were identified as a statistically significant predictor. Based on this estimated relationship, at the geometric mean alisertib $C_{ss,avg}$ of 2.218 μM at 80 mg/m^2 (enteric-coated tablet formulation), the predicted probability of grade ≥2 stomatitis was 0.159 (95%CI, 0.092-0.260). The corresponding estimate for the level −1 reduced dose of 60 mg/m^2 (enteric-coated tablet) with alisertib $C_{ss,avg}$ of 1.66 μM was 0.143 (95%CI, 0.079-0.247).

A statistically significant ($P = .01$) relationship between alisertib $C_{ss,avg}$ and the incidence of febrile neutropenia was observed. The logistic regression-fitted model and 95%CI are shown in Figure 6B. Based on this estimated relationship, at the geometric mean $C_{ss,avg}$ of 2.218 μM at 80 mg/m^2 (enteric-coated tablet formulation), the predicted probability of febrile neutropenia was 0.131 (95%CI, 0.073-0.224). The corresponding estimate for the level −1 reduced dose of 60 mg/m^2 (enteric-coated tablet) was 0.122 (95%CI, 0.065-0.219).

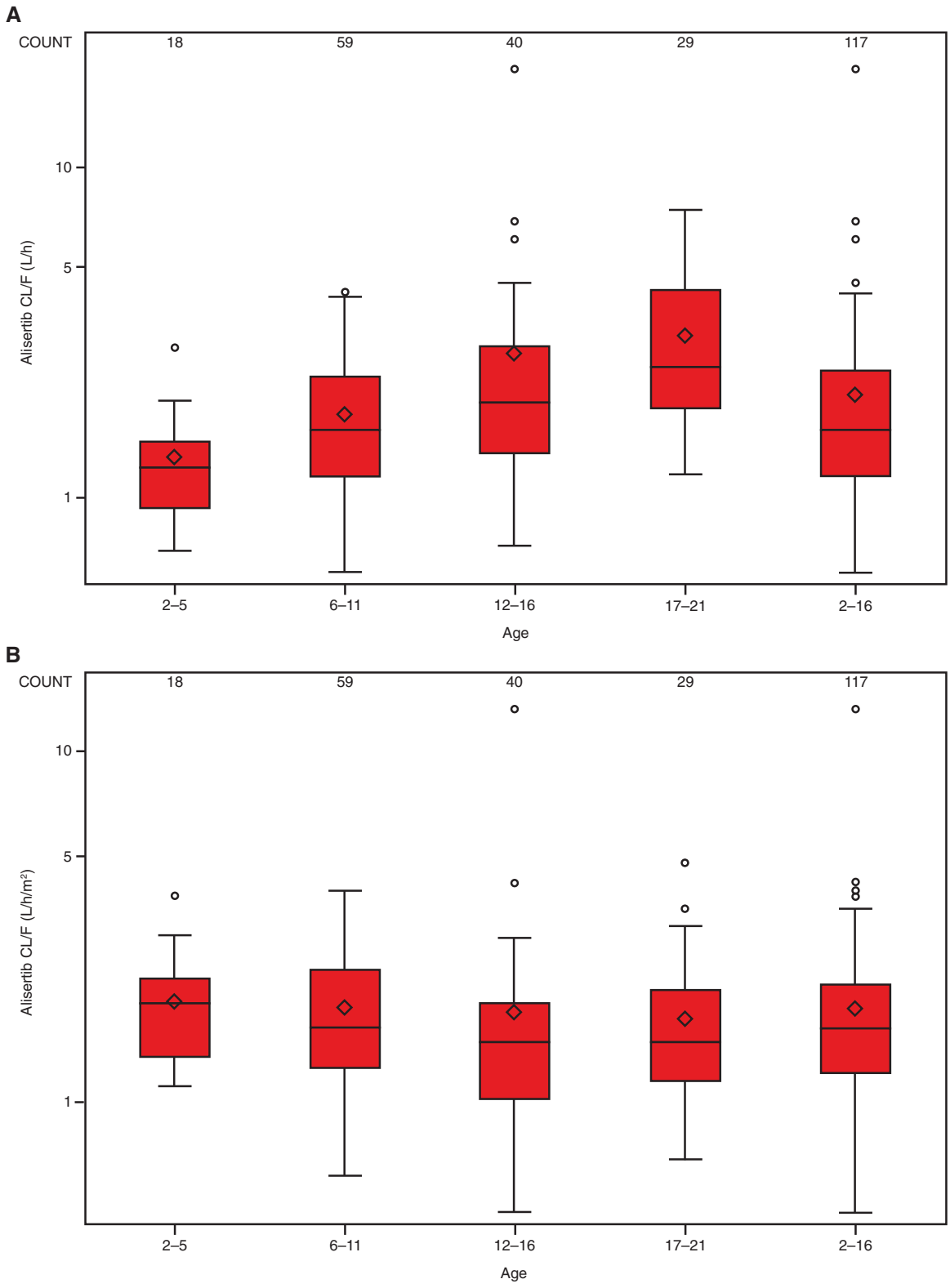

Figure 2. Alisertib CL/F (A) and BSA-normalized CL/F (B) by age group for pediatric patients. Estimates were obtained under the final model. Data for age groups 2 to 5, 6 to 11, and 12 to 16 are pooled for the group "2 through 16" box. Note that CL/F is presented as reference CL/F (ie, CL/F associated with capsule formulation). BSA, body surface area; CL/F, apparent clearance.

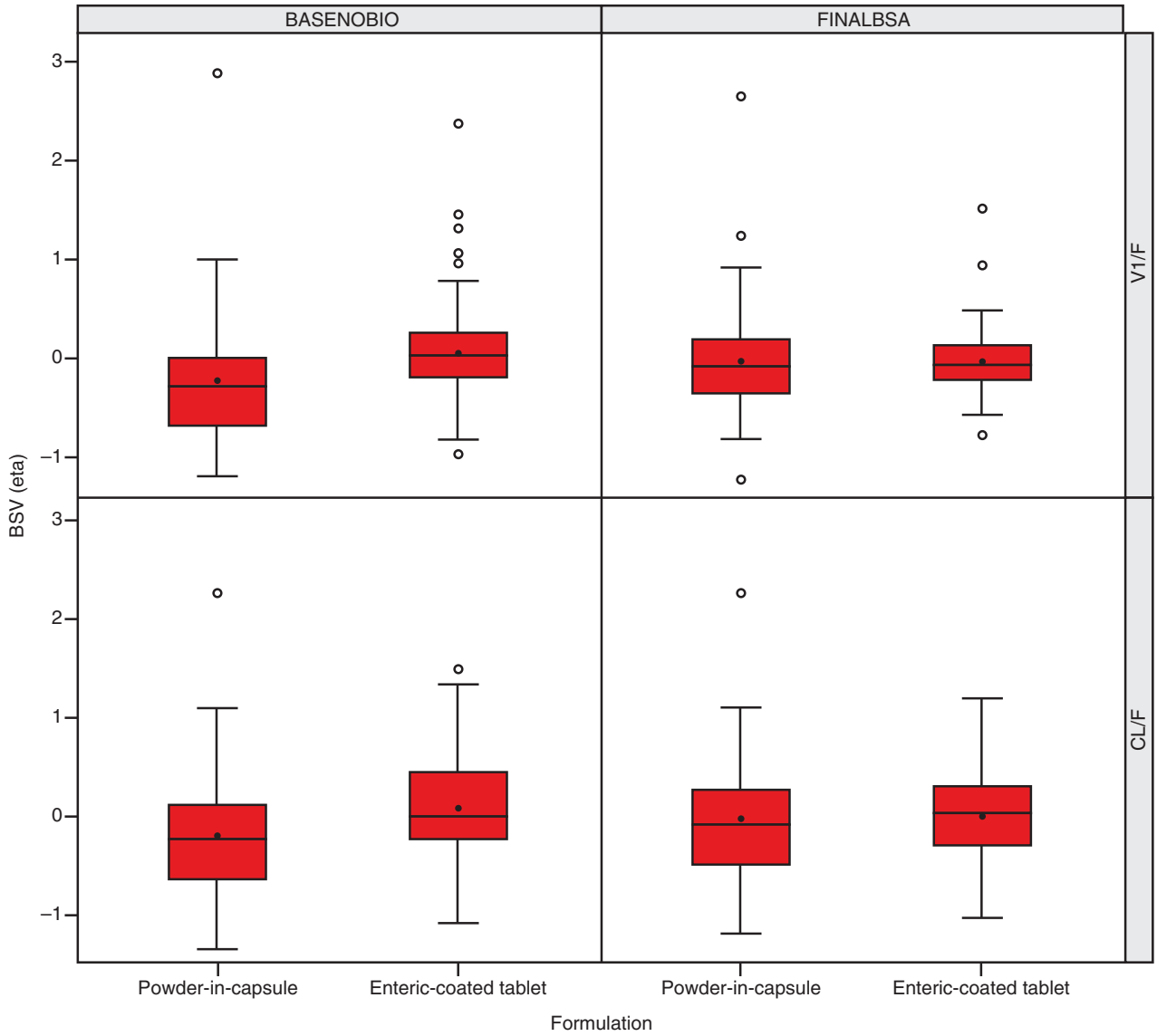

Figure 3. Formulation covariate effect in the final model. BASENOBIO refers to the base model without a bioavailability term for the enteric-coated tablet rather than the base model, which includes this term. The FINAL model includes the effects of formulation on F and of BSA on CL/F and V1/F. BSA, body surface area; BSV, between subject variability; CL/F, apparent clearance; eta, empirical Bayes estimate of the interindividual random effect; F, bioavailability; V1/F, apparent central volume.

Age, sex, performance score, number of prior therapies, cancer types, and number of treatment cycles were evaluated as covariates. The cancer type (solid tumor vs leukemia) was identified as a statistically significant predictor ($P < .0001$). The relationship between the incidence of febrile neutropenia and $C_{ss,avg}$ was significant in patients with solid tumors ($P = .01$) but did not reach statistical significance ($P = .36$) in patients with leukemia. However, the sample size of patients with leukemia was small (n = 19, which represents only 13% of the evaluable data set). Therefore, the results of this covariate analysis should be interpreted with caution. Accordingly, the results shown in Figure 6B are for the base model (ie, the overall population).

No statistically significant relationships were observed between alisertib $C_{ss,avg}$ and the incidence of grade ≥2 neutrophil count decreased ($P = .79$), grade ≥2 anemia ($P = .32$), or grade ≥2 infections and infestations system organ class events ($P = .27$).

Discussion

This study aimed to develop a population PK model to characterize the sources of variability and assess whether the BSA-based dosing of alisertib is an appropriate posology in children and adolescents with cancer aged 2 to 21 years based on data from studies ADVL0812 and ADVL0921. The PK of alisertib

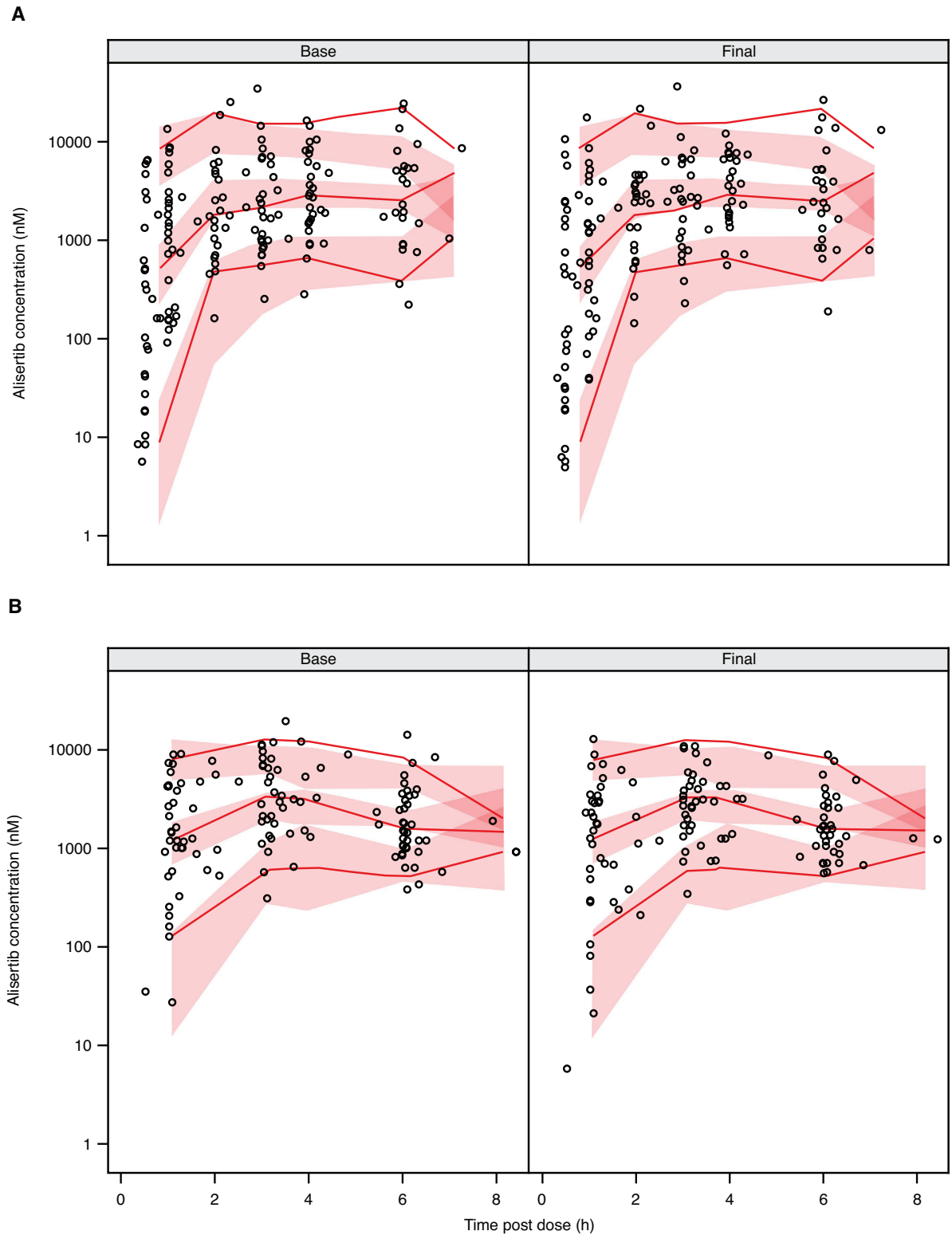

Figure 4. Visual predictive check for base and final models for (A) powder-in-capsule and (B) enteric-coated tablet formulations for day 1 of once daily regimen. The observed analysis data (symbols) were compared to 200 data sets simulated using the base and final models. Black circles are observed data, red lines are observed data 5th, 50th, and 95th percentiles. Pink shaded regions are 95% prediction intervals for the 5th, 50th, and 95th percentiles of simulated data.

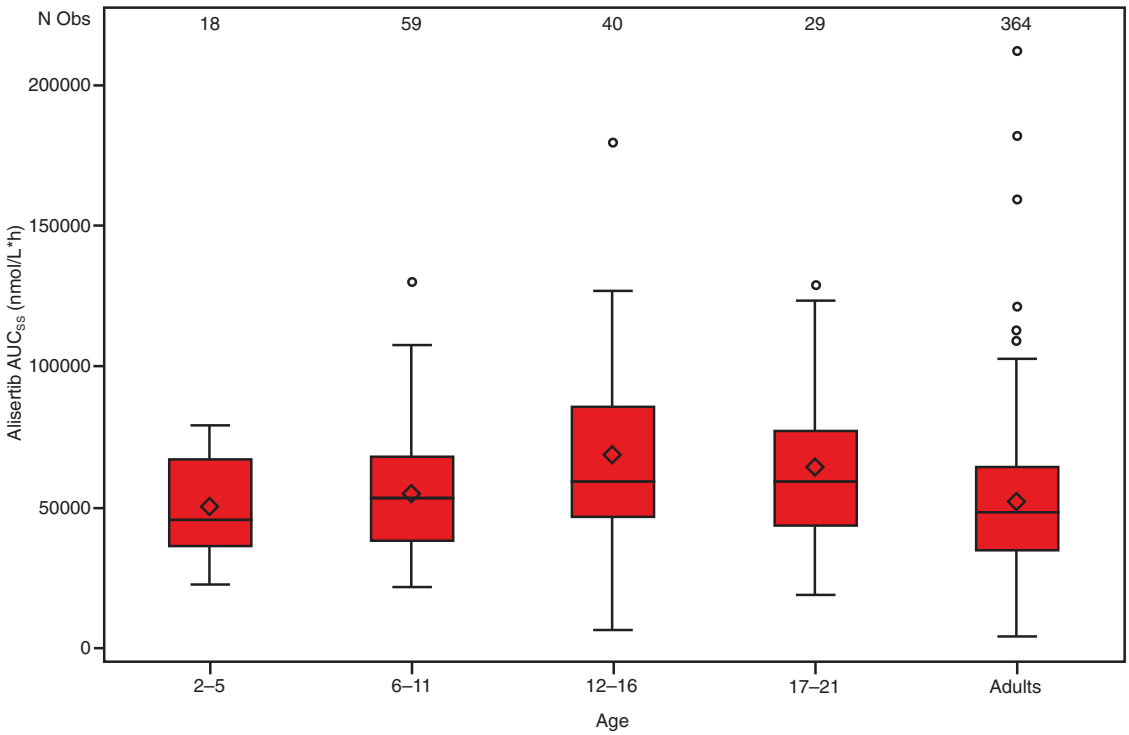

Figure 5. Alisertib AUC_{ss} by age group—pediatric simulated 80 mg/m^2. Post hoc clearance used to calculate AUC_{ss}, and enteric-coated tablet doses were adjusted for relative bioavailability. Results pooled across analyses. Results are presented for final pediatric model using 80 mg/m^2 (recommended pediatric dose) using the enteric-coated tablet formulation (F = 0.671). Results for adults are derived from the final adult model using 50 mg twice daily (100 mg total daily dose, recommended clinical dose for adults). Only adult data from Western region are presented. AUC_{ss}, area under the concentration time curve at a steady state.

was found to be best described by a 2-compartment model with 3 transit absorption compartments and a proportional residual error. A series of transit compartments with a single rate constant was considered more parsimonious than a lag time followed by the first-order rate constant to explain an absorption delay suggested by the data. A series of transit compartments with a single rate constant also resulted in more consistent convergence and alignment with the approach used in the alisertib population PK analysis in adult patients.[13]

BSA-normalized CL/F was similar across all ages, supporting BSA-based dosing of alisertib in children and adolescents with cancer. In adults, CL/F of alisertib was not affected by BSA over the range of BSA examined (1.34-3.28 m^2) (Table S3).[13] There is no discrepancy between the 2 models, however, because the influence of BSA on clearance appears to plateau in the pediatric population at the upper range of age and size. This is further supported by the good reproducibility between analysis data sets using pediatric data and external validation datasets with alisertib PK data from adults. The identification of BSA effect on CL/F is likely explainable by an underlying allometric relationship in the pediatric setting. BSA was evaluated for adequacy as a covariate on CL/F to enable relating the administered BSA-based doses to systemic exposure. As the patient population in this data set comprised pediatric patients of age >2 years, maturation of metabolic clearance mechanisms is to be expected in this analysis population, such that the BSA effect on CL/F is explainable by allometric rationale (ie, larger liver size and metabolic clearance capacity in patients with larger BSA).

Individual PK parameter values support BSA-based dosing as a fit-for-purpose posology in approximately normalizing total systemic exposures of alisertib in children and adolescents. Children and adolescent patients receiving the recommended dose (80 mg/m^2 once daily of the enteric-coated tablet)[22,23] produced a similar alisertib exposure to adults administered 50 mg twice daily (the maximum tolerated dose/recommended dose for clinical evaluation in adults),[26–29] indicating that 80 mg/m^2 once daily achieves pharmacologically active systemic exposures of alisertib in children and adolescent patients. These alisertib systemic exposures have been previously characterized to be associated with Aurora A kinase inhibition evidenced by decreases in chromosome alignment and spindle bipolarity in mitotic tumor cells based on exposure-pharmacodynamics relationships in adults, based on tumor biopsies assessed in the adult phase 1 studies.[2,27] Consistent with what

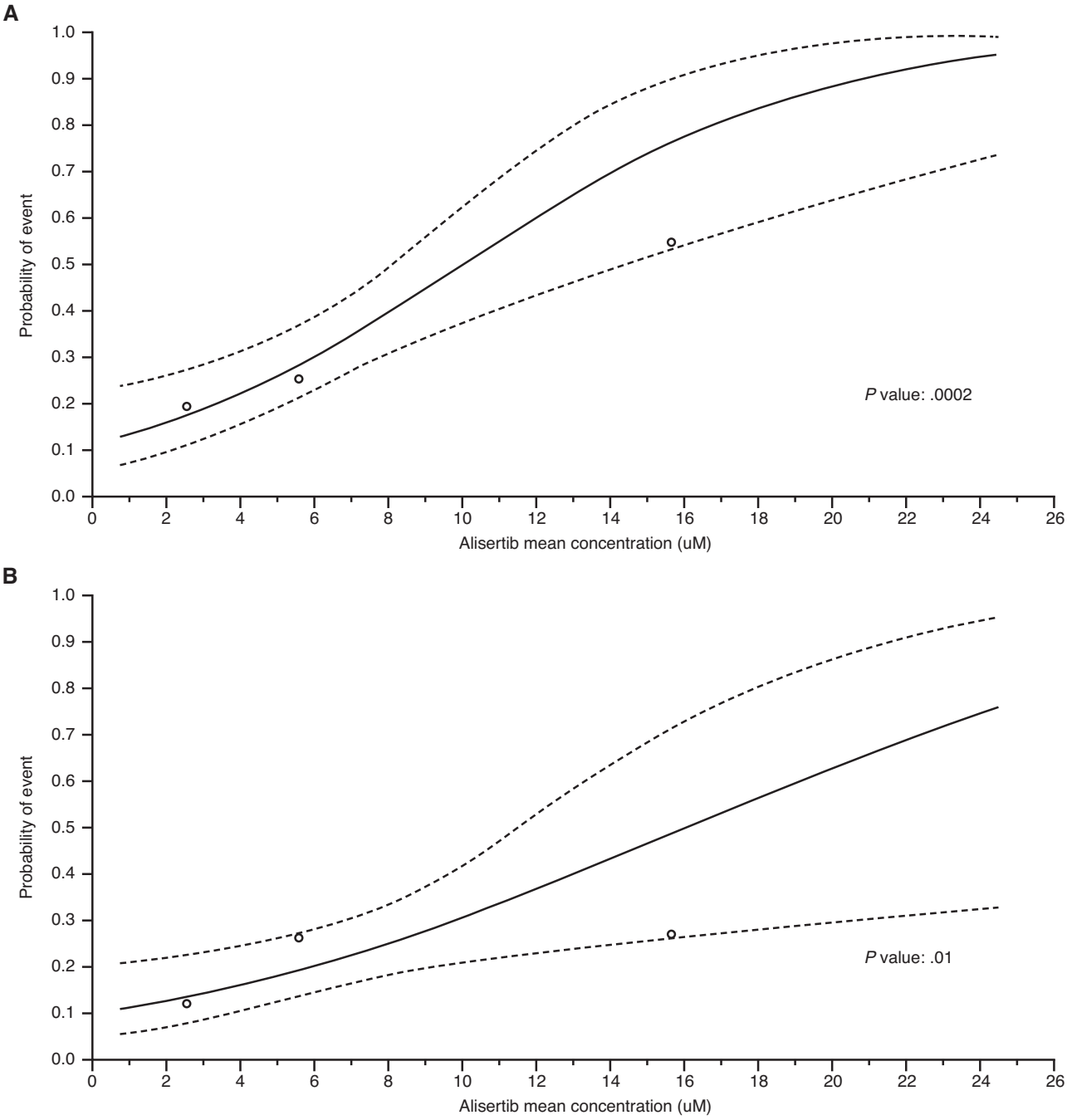

Figure 6. Logistic regression fitted model and 95% confidence intervals for (A) grade ≥2 stomatitis and (B) febrile neutropenia. Circles represent observed incidence in each tertile of alisertib average concentration. Logistic regression model was used to estimate adverse event incidence of clinical interest with alisertib average concentration as a predictor (base model). The covariate effects that may potentially modulate the exposure–safety relationship were examined in a full model via a stepwise procedure, where a covariate was entered into the model if it was significant at the 10% level. A covariate remained in the model at the backward elimination step when the covariate was significant at the 5% level. The covariates considered in the full model include age (2 to ≤12 years, >12 to ≤16 years, >16 years), sex (male, female), Eastern Cooperative Oncology Group performance score (0, >0), number of prior therapy regimens (≤1, ≥2), cancer type (hematologic, nonhematologic) and number of treatment cycles while on study (1, ≥2). For the adverse event end point "grade ≥2 stomatitis" (A) the full model is reduced to the base model after the stepwise selection procedure. For the adverse event end point "febrile neutropenia" (B), the full model with stepwise selection identified that the covariate of "cancer type" is significant. As the sample size of patients with hematologic malignancies was small (n = 19; 13% of the evaluable data set), the relationship between exposure and probability of febrile neutropenia for the base model is shown here.

was observed in adult patients, tumor types (solid tumors vs hematologic malignancies) did not impact alisertib apparent oral clearance (Figure S2).

Visual predictive checks of the time course of dose-normalized alisertib concentrations showed an acceptable agreement between observed and model simulated concentrations, suggesting the model can be used to derive exposure metrics in support of alisertib dose selection in children and adolescents. Simulations supported conclusion of similar alisertib exposure for children and adolescents administered 80 mg/m^2 once daily as enteric-coated tablet and adults administered a fixed 50 mg twice-daily dose.

Formulation had a significant effect on the bioavailability of alisertib in this study: the enteric-coated tablet was estimated to have 68% relative bioavailability compared with powder-in-capsule in pediatric patients. This result, however, should be interpreted cautiously as each formulation was used exclusively in 1 study (powder-in-capsule in ADVL0812[22] and enteric-coated tablet in ADVL0921[23]). As alisertib demonstrates pH-dependent solubility, factors such as gastric pH and emptying time, intestinal transit time, immaturity of secretion, and activity of bile and pancreatic fluid, among other factors, may have contributed to influencing oral bioavailability in pediatric patients following administration as an enteric-coated formulation.[30] In addition, difference in sampling schemes and, to some extent, patient populations may also contribute to the observed difference in bioavailability.

The subsequent exposure-safety analysis explored the relationship between alisertib exposures ($C_{ss,avg}$) after administration of alisertib at doses of 45 to 100 mg/m^2/d (7 days of dosing in 21-day treatment cycles) and clinical safety end points in children and adolescents using logistic regression. Statistically significant relationships between alisertib systemic exposures (ie, $C_{ss,avg}$) and the incidence of grade $\geq$2 stomatitis and febrile neutropenia were identified. The predicted probability of grade $\geq$2 stomatitis in pediatric patients receiving 80 mg/m^2 once-daily alisertib as enteric-coated tablet was 0.159 (95%CI, 0.092-0.260), which was comparable to an observed incidence of grade $\geq$2 stomatitis of 21% (Takeda data on file) in a phase 3 randomized trial in which adult patients received alisertib 50 mg twice daily (n = 138).[31] These findings were consistent with alisertib's antiproliferative effects, resulting from its mechanism of action as an antimitotic agent.[14,26] Based on a previously published alisertib exposure-safety analysis in adult patients,[2] adverse events such as neutropenia, febrile neutropenia, and stomatitis were associated with alisertib steady-state systemic exposures (AUC_{ss}), which were translated to $C_{ss,avg}$ ($AUC_{ss/\tau}$). Off-target central nervous system adverse events (eg, somnolence) experienced in adults, potentially from reversible binding of the benzodiazepine-like portion of alisertib molecule to central γ-aminobutyric acid receptors were better predicted by the peak plasma concentration of alisertib. While somnolence was not an adverse event of concern in the pediatric population, twice-daily dosing resulted in greater myelosuppression and hand-foot syndrome based on clinical evaluation during the phase 1 trial.[22] Accordingly, this regimen was not evaluated further and the vast majority of available pediatric data are from the QD regimen which was selected as the recommended phase 2 dosing regimen and schedule. Only 12 pediatric patients in this dataset received the twice-daily regimen. Accordingly, given the expected high correlation between peak plasma concentration, AUC, and minimum plasma concentration, alternate exposure metrics beyond $C_{ss,avg}$ were not evaluated in the exposure-safety analysis. It should thus be noted that the exposure-safety relationships evaluated in this analysis using AUC as the predictor are largely empirical and cannot be used to extrapolate across regimens and are only to be used to contextualize the expected safety profile with once-daily dosing on the studied dosing schedule.

Analyses of alisertib exposure-efficacy relationships were not performed in pediatric patients, mainly because of a lack of sufficient efficacy data. The phase 1 dose-finding study enrolled patients with advanced solid tumors across many different tumor types. Only 1 of 33 response-evaluable patients achieved an objective response (best response of partial response).[22] The phase 2 study enrolled patients across 12 types of hematologic and nonhematologic malignancies. Only 5 of the 137 response-evaluable patients across all 12 tumor types achieved a response (best response of complete or partial response).[23] The sample sizes for each type of cancer were too small to perform meaningful exposure-efficacy analyses.

Conclusions

In patients aged 2 to 21 years, the PK of alisertib was best described as a 2-compartment model with oral absorption described by 3 transit compartments. CL/F and V1/F were correlated with BSA, supporting the use of BSA-adjusted dosing in the children and adolescent patient population. An 80 mg/m^2 once-daily dosing regimen in pediatric patients provided exposure approximately similar across this pediatric population, and similar to that in adults receiving 50 mg twice daily, indicating that 80 mg/m^2 once daily achieves pharmacologically active systemic exposures of alisertib in children and adolescents. A statistically significant relationship was observed between alisertib $C_{ss,avg}$ and the incidence of grade $\geq$2 stomatitis. In patients with solid tumors, the relationship between the incidence

of febrile neutropenia and alisertib exposures was also significant. These findings were consistent with alisertib's mechanism-related antiproliferative toxicities. Taken together, the results of these population analyses on alisertib provide a valuable quantitative framework to support pharmacologic contextualization of the dosing regimens of this investigational Aurora A kinase inhibitor for pediatric clinical development.

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Conflicts of Interest

X.Z., Y.Y., and D.V.F. disclose employment by Millennium Pharmaceuticals, Inc, Cambridge, MA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. D.R.M. is a paid consultant for Millennium Pharmaceuticals, Inc, Cambridge, MA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. K. V. discloses previous employment by Millennium Pharmaceuticals, Inc. E.F. and E.G. have no disclosures to report.

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Data Sharing

Consistent with the policies of the National Institutes of Health, Children's Oncology Group (COG) considers requests from investigators who are not members of COG and to make its research data available to them. Investigators who wish to use individual patient data from one or more COG studies must make a formal request to the Group. The formal request must be in the form of a brief proposal per the guidance at https://childrensoncologygroup.org/index.php/data-sharing. The Group will then review the scientific merits and feasibility of the request.

References

1. Bolanos-Garcia VM. Aurora kinases. *Int J Biochem Cell Biol*. 2005;37(8):1572-1577.
2. 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.
3. Zullo KM, Guo Y, Cooke L, et al. Aurora A kinase inhibition selectively synergizes with histone deacetylase inhibitor through cytokinesis failure in T-cell lymphoma. *Clin Cancer Res*. 2015;21(18):4097-4109.
4. 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.
5. Ikezoe T, Yang J, Nishioka C, et al. A novel treatment strategy targeting Aurora kinases in acute myelogenous leukemia. *Mol Cancer Ther*. 2007;6(6):1851-1857.
6. Dar AA, Zaika A, Piazuelo MB, et al. Frequent overexpression of Aurora kinase A in upper gastrointestinal adenocarcinomas correlates with potent antiapoptotic functions. *Cancer*. 2008;112(8):1688-1698.
7. Mazumdar A, Henderson YC, El-Naggar AK, Sen S, Clayman GL. Aurora kinase A inhibition and paclitaxel as targeted combination therapy for head and neck squamous cell carcinoma. *Head Neck*. 2009;31(5):625-634.
8. Manfredi MG, Ecsedy JA, Chakravarty A, et al. Characterization of alisertib (MLN8237), an investigational small-molecule inhibitor of Aurora A kinase using novel in vivo pharmacodynamic assays. *Clin Cancer Res*. 2011;17(24):7614-7624.
9. Ding YH, Zhou ZW, Ha CF, et al. Alisertib, an Aurora kinase A inhibitor, induces apoptosis and autophagy but inhibits epithelial to mesenchymal transition in human epithelial ovarian cancer cells. *Drug Des Devel Ther*. 2015;9:425-464.
10. Wang F, Li H, Yan XG, et al. Alisertib induces cell cycle arrest and autophagy and suppresses epithelial-to-mesenchymal transition involving PI3K/Akt/mTOR and sirtuin 1-mediated signaling pathways in human pancreatic cancer cells. *Drug Des Devel Ther*. 2015;9:575-601.
11. Goldberg SL, Fenaux P, Craig MD, et al. An exploratory phase 2 study of investigational Aurora A kinase inhibitor alisertib (MLN8237) in acute myelogenous leukemia and myelodysplastic syndromes. *Leuk Res Rep*. 2014;3(2):58-61.
12. Melichar B, Adenis A, Lockhart AC, et al. Safety and activity of alisertib, an investigational Aurora kinase A inhibitor, in patients with breast cancer, small-cell lung cancer, non-small-cell lung cancer, head and neck squamous-cell carcinoma, and gastro-oesophageal adenocarcinoma: a five-arm phase 2 study. *Lancet Oncol*. 2015;16(4):395-405.
13. 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.
14. Carol H, Boehm I, Reynolds CP, et al. Efficacy and pharmacokinetic/pharmacodynamic evaluation of the Aurora kinase A inhibitor MLN8237 against preclinical models of pediatric cancer. *Cancer Chemother Pharmacol*. 2011;68(5):1291-1304.
15. Maris JM, Morton CL, Gorlick R, et al. Initial testing of the Aurora kinase A inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP). *Pediatr Blood Cancer*. 2010;55(1):26-34.
16. Muscal JA, Scorsone KA, Zhang L, Ecsedy JA, Berg SL. Additive effects of vorinostat and MLN8237 in pediatric leukemia, medulloblastoma, and neuroblastoma cell lines. *Invest New Drugs*. 2013;31(1):39-45.
17. Lipsitz EG, Nguyen V, Zhao H, et al. Modeling MLN8237, an Aurora kinase A inhibitor, with irinotecan (IRN) and temozolomide (TMZ) in neuroblastoma (NB). *J Clin Oncol*. 2010;28(15_suppl):10593-10593.
18. Zhou X, Pant S, Nemunaitis J, et al. Effects of rifampin, itraconazole and esomeprazole on the pharmacokinetics of

alisertib, an investigational Aurora A kinase inhibitor in patients with advanced malignancies. *Invest New Drugs*. 2018;36(2):248-258.
19. Zhou X, Pusalkar S, Chowdhury SK, et al. Mass balance, routes of excretion, and pharmacokinetics of investigational oral [14C]-alisertib (MLN8237), an Aurora A kinase inhibitor in patients with advanced solid tumors. *Invest New Drugs*. 2019;37(4):666-673.
20. Pusalkar S, Zhou X, Li Y, et al. Biotransformation pathways and metabolite profiles of oral [^{14}C]alisertib (MLN8237), an investigational Aurora A kinase inhibitor, in patients with advanced solid tumors. *Drug Metab Dispos*. 2020;48(3):217-229.
21. Zhou X, Lockhart AC, Fu S, et al. Pharmacokinetics of the investigational Aurora A kinase inhibitor alisertib in adult patients with advanced solid tumors or relapsed/refractory lymphoma with varying degrees of hepatic dysfunction. *J Clin Pharmacol*. 2019;59(9):1204-1215.
22. 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.
23. 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.
24. Skolnik JM, Barrett JS, Jayaraman B, Patel D, Adamson PC. Shortening the timeline of pediatric phase I trials: the rolling six design. *J Clin Oncol*. 2008;26(2):190-195.
25. 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.
26. 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.
27. 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.
28. Kelly KR, Shea TC, Goy A, et al. Phase I study of MLN8237–investigational Aurora A kinase inhibitor–in relapsed/refractory multiple myeloma, non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Invest New Drugs*. 2014;32(3):489-499.
29. Falchook G, Kurzrock R, Gouw L, et al. Investigational Aurora A kinase inhibitor alisertib (MLN8237) as an enteric-coated tablet formulation in non-hematologic malignancies: phase 1 dose-escalation study. *Invest New Drugs*. 2014;32(6):1181-1187.
30. Fernandez E, Perez R, Hernandez A, Tejada P, Arteta M, Ramos JT. Factors and mechanisms for pharmacokinetic differences between pediatric population and adults. *Pharmaceutics*. 2011;3(1):53-72.
31. O'Connor OA, Özcan M, Jacobsen ED, et al. Randomized phase III study of alisertib or investigator's choice (selected single agent) in patients with relapsed or refractory peripheral T-cell lymphoma. *J Clin Oncol*. 2019;37(8):613-623.
32. Battisti WP, Wager E, Baltzer L, et al. Good publication practice for communicating company-sponsored medical research: GPP3. *Ann Intern Med*. 2015;163:461-464.

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