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A Phase I Study of Entinostat in Children and Adolescents with Recurrent or Refractory Solid Tumors, Including CNS Tumors: Trial ADVL1513, Pediatric Early Phase-Clinical Trial Network (PEP-CTN)[1]

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Abstract

Background: Entinostat is an oral small molecule inhibitor of Class I Histone Deacetylases (HDAC) which has not previously been evaluated in pediatrics. We conducted a phase 1trial to determine the maximum tolerated dose/recommended phase 2 dose (MTD/RP2D), toxicity profile,

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Data Sharing
The data that supports the findings of this study are available in the supplementary material of this article

pharmacokinetics (PK), and pharmacodynamics (PD) of entinostat in children with relapsed or refractory solid tumors including central nervous systems malignancies.

Methods: A rolling six dose escalation design evaluated two dose levels. Entinostat oral tablet formulation was administered once per week, four doses per 28 day cycle. PK and PD studies were performed.

Results: Twenty-one eligible patients median (range) age 14 (6–20). Six subjects were treated at 3mg/m^2 dose level and fifteen were treated in 4mg/m^2 dose level. The study included patients with CNS tumors (n=12), sarcomas (n=6), or other solid tumors (n=3). Eight patients were not fully evaluable for toxicity due to progression of disease prior to receiving the required percentage of protocol therapy. No cycle one dose limiting toxicity (DLT) was observed at either dose level. A threefold higher Area Under the Curve (AUC) was achieved in our cohort compared to adults using a similar dosing schedule. The PD studies showed increase in acetylated lysine in peripheral blood leukocytes at both doses.

Conclusions: Entinostat was well tolerated with no DLT observed. All patients experienced progression within the first two cycles except one patient with ependymoma with stable disease. Based on pharmacokinetics and pharmacodynamics, the R2PD in pediatric patients with solid tumors is 4mg/m^2 orally administered once weekly.

Keywords

Ewing Sarcoma; Glioma low grade; oncology general; pediatric hematology oncology; phase I; phase 1 clinical trial agents; phase 1 study; rhabdomyosarcoma; osteosarcoma; neuro-oncology; pediatric oncology

Introduction

Histone Deacetylases (HDACs) play an important role in cell differentiation and modulate the balance between pro- and anti-apoptotic processes in cells. Dysregulation of histone acetylation may lead to aberrant repression of tumor suppressor genes, resulting in maintenance of the malignant phenotype. Many cancer cells show high levels of HDAC expression and hypoacetylation of histones. [1,2]

Entinostat (SNDX-275 Syndax Pharmaceuticals, Waltham, MA) is an oral small molecule inhibitor of class I HDACs (HDACs I, II, III, and XI). [3] Pediatric solid tumors commonly exhibit alterations in transcription factors whose target genes regulate cell proliferation, differentiation, and survival. HDAC inhibitors (HDACi) have the potential to regulate expression of genes repressed through epigenetic mechanisms. For this reason, entinostat has been tested *in vitro* against a variety of pediatric cancer cell lines including neuroblastoma, Ewing sarcoma, retinoblastoma, osteosarcoma, and medulloblastoma with inhibition of growth at low micromolar concentrations.[4] Entinostat demonstrated *in vitro* inhibition in pediatric solid tumor cell lines, including the Rh30 rhabdomyosarcoma and TC-71 Ewing sarcoma. The entinostat IC_{50}'s were ≤ 0.1 M compared to the class I/II HDACi vorinostat IC_{50}'s > 1 μM in these cell lines. [4,5] Treatment of Ewing sarcoma cells with entinostat leads to histone acetylation, growth inhibition, and apoptosis.[6] The effect of entinostat on cell growth may be due to the silencing of protein and mRNA expression of

Pax3:Foxo1 in both mouse and human alveolar rhabdomyosarcoma (ARMS) cells.[7] In cell lines derived from osteosarcoma lung metastases, entinostat induces Fas ligand mediated cell death through downregulation of the apoptosis inhibitor, c-FLIP. *In vivo* treatment with entinostat resulted in decreased c-FLIP expression and induced tumor regression in nu/nu-mice with osteosarcoma lung metastases. [8]

Entinostat has been administered as monotherapy in adults with cancer in six completed clinical studies to date and two ongoing trials (6 studies in patients with solid tumors and 2 studies in patients with hematologic malignancies; in one study the monotherapy was followed by combination therapy). [9] In addition, 19 combination studies have been completed to date. [9] In a phase 1 study in adults with refractory solid tumors and lymphoma, daily oral administration was not tolerable as the first two patients experienced DLT at the first dose level of 2 mg/m^2. [10] In another phase I trial in adults with refractory solid tumors and lymphoid malignancies, entinostat was administered orally once weekly for 4 weeks of a 6-week cycle. [11] Among 19 evaluable patients, no grade 4 toxicities were observed. DLT were grade 3 hypophosphatemia, hyponatremia, and hypoalbuminemia. The maximum tolerated dose (MTD) was 6 mg/m^2 on this schedule. An additional phase I trial or entinostat evaluated multiple schedules. [12] Toxicities were similar to other phase I trials, and DLTs were hypophosphatemia and asthenia. A twice-weekly schedule was found to not be tolerable, and the RP2D was 4 mg/m^2 when entinostat is given weekly for 3 weeks of a 28-day cycle. [12] There is no previous clinical trial experience in pediatrics.Given that entinostat was tolerable at $6mg/m^2$ for four weekly doses only when provided with a 2-week break between cycles, and that the R2PD in adults was ultimately determined to be $4mg/m^2$ for 3 weekly doses of a 28 day cycle, this trial was specifically designed to determine a pediatric dose that could be delivered on a continuous weekly schedule without breaks in an attempt to achieve persistent HDAC inhibition..

The primary objectives of this trial were to estimate the MTD or R2PD, characterize the toxicity profile, and describe the PK of entinostat in pediatric patients with refractory or recurrent solid tumors. Additional objectives were to describe the PD of entinostat within this patient population using flow cytometry measuring acetylated lysine within peripheral blood mononuclear cells.

Patients and Methods

This multi-institutional trial was conducted at Children's Oncology Group (COG) Pediatric Early Phase-Clinical Trial Network (PEP-CTN) Institutions (Formerly known as the COG Phase I and Pilot Consortium) and approved by National Cancer Institute's Pediatric Central IRB. Informed consent, and assent as appropriate, was obtained from patients and their guardians prior to enrollment. The trial is registered on clinicaltrials.gov as NCT02780804 [13]

Eligible patients were between the ages of 12 months and 21 years and had a body surface area of greater than or equal to $1.17m^2$ and subjects were required to swallow intact tablets; dosing was limited by the available formulation. Subjects had relapsed or refractory solid tumors including central nervous system tumors (CNS) or lymphoma. Patients were required

to have measurable or evaluable disease; no known curative therapy or therapy proven to prolong survival with an acceptable quality of life; a Lansky (≤16 y) or Karnofsky (>16 y) performance status ≥ 50%; and were recovered from the acute toxic effects of prior anticancer therapy. In addition, patients were required to have adequate organ function defined as: absolute neutrophil count (ANC) ≥ 1000/mm^3, platelet count ≥ 100,000/mm^3, Hemoglobin ≥ 8g/dl with or without transfusion, creatinine clearance or radioisotope GFR ≥ 70 ml/min/1.73 m^2 or normal serum creatinine (Cr; mg/dl) based on age and gender; total bilirubin ≤ 1.5 × upper limit of normal (ULN) for age, ALT ≤ 135 U/l, serum albumin ≥ 2 g/dL. Patients were not eligible if they had a history of congenital prolonged QTc syndrome, QTc >480 msec, or significant cardiac arrhythmias.

Entinostat (1mg and 5mg tablets were supplied by Syndax Pharmaceuticals and distributed by the National Cancer Institute's Division of Cancer Treatment and Diagnosis) was administered orally in a fasted state (2 hours after and 1 hour before food) on days 1, 8, 15, and 22 of a 28-day cycle. Due to limitations of available tablet strengths, dosing was based on a dosing nomogram. Two dose levels were evaluated 3mg/m^2 and 4mg/m^2 with an option to de-escalate dose in the setting of toxicity. The study used a rolling six study design for dose escalation, and PK expansion occurred at the R2PD. [14]

Toxicities were graded according to CTCAE version 5. Patients were monitored with weekly physical examinations, blood chemistries including liver function tests, and complete blood counts. Subsequent cycles were administered if the patient met eligibility laboratory parameters, did not experience study related adverse events (AE) warranting discontinuation, and had no evidence of disease progression.

Radiographic disease evaluation was conducted at baseline, at the end of cycle 1, cycle 3, and cycle 5, and then every 3 cycles. Radiographic response was assessed using RECIST v1.1 by the treating physician investigator. In addition, objective responses or prolonged stable, defined as ≥ 6 cycles, were centrally reviewed by an independent radiologist.

DLT was defined as any event that was possibly, probably, or attributable to protocol therapy. The DLT observation period for the purpose of dose escalation was the first cycle of therapy. Hematologic DLT was defined as Grade 4 decrease in ANC or platelet count. Additionally, grade 3 thrombocytopenia with clinically significant bleeding, petechiae or purpura persisting for ≥ 7 days or requiring platelet transfusion on ≥ 2 separate days within a 7-day period was consider dose limiting. Grade 3 or 4 fever and neutropenia was not considered a DLT.

Non-hematologic DLT was defined as any Grade 3 or 4 nonhematological toxicity with the exception of Grade 4 fever and the following Grade 3 toxicities: nausea and vomiting of <3 days duration, fever or infection < 5 days duration, and diarrhea ≤ 3 days duration. Additionally the following Grade 3 laboratory abnormalities were not considered a DLT provided they resolved to baseline levels within 7 days of drug interruption and did not occur with re-challenge: liver enzyme elevation, electrolyte abnormalities, and elevation in amylase and lipase. Non-hematological toxicity that persisted for ≥ 7 days and was considered sufficiently medically significant or intolerable by patients that it required

treatment interruption for ≥ 7 days beyond the scheduled dose or which recurred upon drug challenge was considered a DLT.

The MTD, determined from toxicity during cycle 1, was defined as the dose at which fewer than one-third of patients experienced a DLT. One dose reduction was permitted if the toxicity resolved within 14 days of drug discontinuation.

Blood samples for pharmacokinetics were obtained on all patients during cycle 1 prior to and at 0.5, 1, 3, 6, 24, 48, and 96 hours after the day 1 dose; pre-dose on day 8, pre-dose and 1-hour post dose on day 22, and on day 28 of cycle 1. Plasma was isolated by centrifugation at 1500 to 2000 x g for 15 minutes and stored frozen at −70˚C until analysis. Entinostat plasma concentrations were measured using a validated, previously described method. [15] A descriptive analysis of the pharmacokinenic parameters of Entinostat were preformed to define systemic exposure, and drug clearance. The PK parameters were summarized in simple summary statistics including means, medians, ranges, and standard deviations. Pharmacokinetic parameters were calculated by standard non-compartmental analysis using Phoenix Winnonlin 7.0.0 (Certara, L.P. St Louis, MO).

In consenting patients, exploratory pharmacodynamic assessment of global protein lysine acetylation in peripheral blood mononuclear cells (PBMC) was performed using a flow cytometric technique which has been previously described in detail.[15,16,17] Briefly, peripheral blood samples were collected in Cell Preparation Tubes (CPT) with sodium citrate (BD Biosciences) on day 1 pre-dose, 6 h, 24 h and day 8 post- treatment of cycle 1, and mononuclear cells were isolated, viably frozen and stored in liquid nitrogen. For the assessment of acetylated lysine, the cells were thawed, washed and stained for surface antibodies (anti-CD3 clone OKT3, anti-CD14 clone HCD14, anti-CD19 clone H1B19 and anti-CD56 clone 5.1H11) and viability dye (LIVE/DEAD® Fixable Aqua Dead, Invitrogen). After fixation and permeabilization, the cells were stained for acetylated lysine (anti-acetylated lysine antibody, clone 15G10). All antibodies are from Biolegend. Multiparametric flow cytometry was performed (MACSQuant; Miltenyi Biotec.), and data were analyzed using FlowJo software v.10.0.7 (FlowJo, LLC). Global protein lysine residue acetylation change (fold change of median value of MFI) of subjects was compared to pharmacokinetic data across the patient samples using Pearson correlation

Results

Twenty-one patients were enrolled on the study; all were eligible and 13 were evaluable for DLT assessment. Those who were inevaluable did not receive 85% of protocol-prescribed drug during cycle 1 as the result of early disease progression (n=7) or were removed from protocol therapy due to physician preference (n=1). Table 1 presents patient demographics and disease characteristics. The median (range) number of cycles administered was 1.5 (1–17).

Hematologic and non-hematologic adverse evets as well as laboratory abnormalities that were assessed by investigator to be at least possibly attributed to Entinostat are summarized in Supplemental Table 1. No cycle 1 DLTs were observed among three evaluable patients

enrolled at 3mg/m^2. Ten evaluable patients enrolled at 4mg/m^2 (including the PK expansion cohort), and no DLT were observed in cycle 1. One later cycle DLT of neutropenia was observed at the 4mg/m^2 dose level. No dose reductions were required throughout the study. During cycle 1 for both dose levels, Grade ≥ 3 toxicities were hematologic including decreased white blood cell count (n=1), neutropenia (n=4), and lymphopenia (n=1). Other frequent toxicities during cycle 1 were non-dose limiting changes in laboratory evaluations. Grade ≤ 2 toxicities included fatigue (n=4), vomiting (n=3), diarrhea (n=2), thrombocytopenia (n=1), lymphopenia (n=1) and nausea (n=2). At the R2PD, toxicities noted after cycle 1 were primarily hematologic and included decreased white blood cell count (n=4), neutropenia (n=3), anemia (n=3), lymphopenia (n=3), thrombocytopenia (n=3), and hemoglobin increase (n=1). Neutropenia was the only grade three adverse event (n=2). A complete listing of all toxicities that occurred in later cycles is detailed in Supplemental Table 2.

Response

One patient with an ependymoma continues on protocol therapy in cycle 28 based on institutional determination of radiographically stable disease. At the time of blinded central review of imaging, the patient was found to have progression of leptomeningeal disease at the cycle 10 disease evaluation; however, the patient remains on protocol therapy based on institutional assessment of response. Two patients experienced disease progression in cycle 3 of therapy (Ewing Sarcoma and hepatocellular carcinoma). The remainder of the patients experienced disease progression within the first two cycles of therapy.

Pharmacokinetics were evaluated in nineteen patients and are summarized in Table 2. The median time to peak concentration was 1 hour (range 0.5–25 hours), and similar across dose levels. The median peak concentration 53 ng/ml in our study was similar across dose levels. Area under the curve (AUC) appeared to increase in proportion to dose, and the median values of clearance (3.4 L/hr/m^2) and half-life (45.9 hours) were similar across the dose levels. The median clearance values for males and females were 3.5 L/hr/m^2 and 3.4 L/hr/m^2, respectively. The median clearance values for children <12 y.o. and ≥12 y.o. were 2.8 L/hr/m^2 and 3.5 L/hr/m^2, respectively.

Global protein lysine residue acetylation change (median value of MFI (Mean Florescence Intensity)) from baseline was evaluated in PBMC subsets including CD3$^+$ T-cell lymphocytes, CD14$^+$ monocytes, CD19$^+$ B-cell lymphocytes, and CD56$^+$ NK cells from consenting patients. A two-fold increase in MFI was noted 24 hours post dose which then decreased to a 1.7-fold increase by day 8 across all cell types tested (p=0.0167 student's t-test) (Figure 1 Panel A). There was no significant difference noted between dose level 1 and dose level 2 (Figure 1 panel B). Global protein lysine residue acetylation change (fold change of median value of MFI) of subjects was compared to pharmacokinetic data across the patient samples using Pearson correlation. Although there was no statistical correlation between AUC or Cmax and MFI acetylation, there appeared to be a direct trend in all but B-cells (Supplemental Figure 1). Interestingly, one of these patients with higher MFI and AUC remains on study receiving entinostat with stable disease. In prior studies, histone

acetylation as a surrogate marker of HDACi has been linked to clinical activity. [17] The small numbers in our cohort preclude meaningful statistical analysis.

Discussion

Entinostat was very well-tolerated in the pediatric population, and no DLTs were noted during cycle 1 in either of the dose levels tested (3mg/m^2 or 4mg/m^2). The primary adverse events associated with entinostat at the 4 mg/m^2 dose level were hematologic, however, this was not dose limiting. The MTD of entinostat in children and adolescents was not achieved given how well the drug was tolerated. Entinostat 4mg/m^2 per week of a twenty-eight-day cycle was tolerable in adults and was associated with increased histone acetylation in the peripheral blood leukocytes. [10, 11, 12] The median entinostat C_{max} value of 53 ng/ml (0.141 uM) was higher than the IC50 values for the Rh30 rhabdomyosarcoma cell line (IC50 = 0.062 μM) and the TC-71 Ewing sarcoma cell line (IC50 = 0.10 μM).[4,5] No differences in clearance were noted for gender in our cohort, however, there was a small age difference in clearance. Children <12 y.o. treated with 4 mg/m^2 entinostat had lower clearance (2.8 L/hr/m^2) and higher AUC (1422 hr*ng/ml) than children ≥12 y.o. (clearance 3.5 L/hr/m^2 and AUC 1076 hr*ng/ml) as shown in Table 2. Interestingly, differences were noted in the pharmacokinetics in pediatric patients in our study compared to the previously published adult series as shown in Table 2.[11,12] While the C_{max} values for the 4 mg/m^2 dose were similar between the pediatric and adult studies, we observed a greater than three-fold higher AUC in pediatric patients compared to adults. This effect did not appear to be related to the fasted vs fed state. The reasons for the differences in PK are unclear but may be related to the differences in assays, the duration of sampling and extrapolation of the exposure. In our study, plasma samples for entinostat measurements were collected for 168 hours after the first dose and the measured AUC was ≥ 70% of the total AUC ($AUC_{0-\infty}$). In the PK reported from the adult study by Kummar et al, PK samples were obtained for 72 hours after the first dose and up to 50% of the AUC_∞ was extrapolated after the first measured exposure (AUC_{72hr}) and therefore was not a robust measure of exposure. In addition, the adult phase I trials were preformed more than 10 years before our pediatric trial started, and entinostat measurements were made using a different methodology. Thus, the difference in PK in children and adults may be due to study methodology. However, we cannot rule out that a physiological difference may contribute to the difference in PK between children and adults since a small difference was noted between younger and older children. Given the small cohort of patients that participated in PK studies, additional studies are warranted in future investigations of entinostat.

There is no difference in the side effect profile in adults compared to children receiving entinostat 4mg/m^2/week with Grade ≥ 3 toxicities occurring in less than 20% of patients for all courses. Toxicity is primarily hematological. [10, 11, 12] Interestingly, the series published by Ryan et al demonstrated that dose reductions were required in 6 adult patients after the first cycle of treatment but the reasons for dose reductions are not listed. Each of the adult studies notes an increase in fatigue, nausea and vomiting compared to a relative paucity of these toxicities observed in our pediatric cohort. Given this favorable toxicity profile despite a greater measured AUC compared to adults receiving entinostat 4 mg/m^2, we have established the same RP2D as in prior adult studies. [10,11,12] The entinostat

recommended dose in children and adolescents with solid tumors is 4mg/m^2 dose per week continuously, without the need for a period of rest, and was associated with a global and persistent increase in protein lysine residue acetylation. In our study, entinostat did not show evidence of single agent activity in common pediatric solid tumors. Recently, entinostat was also evaluated in combination with chemotherapy in mouse models for rhabdomyosarcoma and was not found to enhance the standard therapy. [17] Preclinical studies with entinostat have found a synergistic effect with check point inhibitors and in combination with hormonal therapy for patients with homone receptor positive, HER2 negative breast cancers but clinical trials have yet to demonstrate a clinical benefit. [19–21] As more pre-clinical data in immunomodulation in pediatric solid tumors becomes available the role of entinostat in immunomodulation and if a synergistic effect will need to be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations Key:

AUC	Area Under the Curve
CNS	Central Nervous System
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-Limiting Toxicity
HDACs	Histone Deacetylases
HDACi	Histone Deacetylase Inhibitors
MTD	Maximum Tolerated Dose
mFI	Mean Fluorescence Activity
PBMC	Peripheral Blood Mononuclear Cells
PD	Pharmacodynamics
PK	Pharmacokinetics
y	Years

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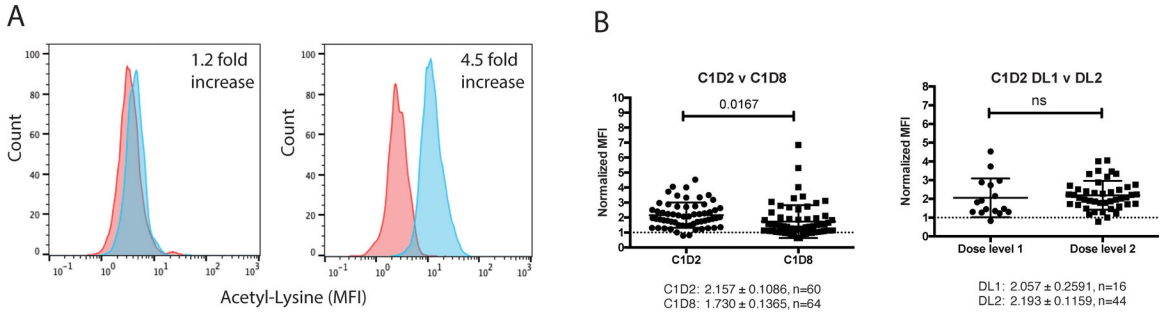

Figure 1:

Pharmacodynamic analysis of global protein lysine acetylation by multiparameter flow cytometry. A) Histogram representation of lysine acetylation in CD19+ B cells pre- and post-entinostat. Red histogram represents baseline acetylation from CD19+ cells drawn on day 1 pre-entinostat. Blue histogram represents acetylation from CD19+ cells at day 2 post exposure to entinostat. Left panel represents a sample with minimal increase in acetylation whereas the right panel represents a sample with a 4.5x increase. B) Comparison of the fold change of acetylated (MFI) from baseline from all the cells assessed on day 2 versus day 8. There was a mean increase of 2.1 at day 2 with a decrease to 1.7 by day 8 (Left Panel, *p=0.0167 by Student's t-test). There was no significant difference in comparing fold change for acetylated lysine (MFI) dose level 1 versus dose level 2.

TABLE 1:

Patient demographics and disease characteristics

Characteristic	Number (%)
Age (years)	
Median	14
Range	6–20
Sex	
Male	10 (48)
Female	11 (52)
Race	
White	15 (71)
Asian	0 (0)
American Indian or Alaska Native	0 (0)
Black or African American	2 (10)
Unknown	4 (19)
Ethnicity	
Non-Hispanic	16(76)
Hispanic	4 (19)
Unknown	1 (5)
Diagnosis	
Diffuse Intrinsic Pontine Glioma	1 (4.8)
Embryonal Rhabdomyosarcoma	1 (4.8)
Ependymoma	2 (9.5)
Ewing Sarcoma	1 (4.8)
Glioblastoma	5 (23.8)
Glioma	4 (19)
Hepatocellular Carcinoma, fibrolamellar	2 (9.5)
Osteosarcoma	1 (4.8)
Paraganglioma	1 (4.8)
Sarcoma	1 (4.8)
Synovial Sarcoma	2 (9.5)
Prior Therapy	
Number of patients receiving prior chemotherapy	N=18
Median number of cycles	2
Range	1–11
Radiation Therapy Courses	N=17
Median	1
Range	1–3

Table 2

Pharmacokinetics of Entinostat in Children (ADVL1513) Compared to Published Results in Adults.

	ADVL1513 Median (range)		Adult Studies	
	3 mg/m² weekly	**4 mg/m² weekly**	**4 mg/m² [1] weekly**	**4 mg/m² [2] weekly**
N	6	13	7	6
T_{max} (hr)	1 (0.5– 6)	1 (0.5– 25)	0.5 (0.5– 2.0)	0.5 (0.25– 1)
C_{max} (ng/mL)	54 (5– 136)	53 (14– 293)	31.7	60.5
Half-life (hr)	51 (27– 104)	45 (30– 104)	10.2	83.7
AUC_{∞} (ng/mL•hr)	834 (262–1408)<12 y.o.: NE ≥12 y.o.: 834 (262–1408)	1162 (839–2737) <12 y.o.: 1422 (1113–2737) ≥12 y.o.: 1076 (839–1378)	67.6 [3]	316
Cl/F (L/hr/m²)	3.5 (2.2– 10.7) <12 y.o.: NE ≥12 y.o.: 3.5 (2.2, 10.7)	3.3 (1.5– 5.2) <12 y.o.: 2.8 (1.5–3.7) ≥12 y.o.: 3.6 (2.7, 5.2)	112.8	12.7

1. Kummar et al 2007(values are shown as the mean) Reference 11

2. Gore et al 2008 (values are shown as the geometric mean) Reference 12

3. $AUC_{0–72h}$