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Phase 1 Trial of Ontuxizumab (MORAb-004) in Children with Relapsed or Refractory Solid tumors: A Report from the Children's Oncology Group Phase 1 Pilot Consortium (ADVL1213)

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Abstract

Background—Ontuxizumab is a humanized IgG mAb that targets the cell-surface glycoprotein endosialin (TEM-1/CD248) found on activated mesenchymal cells and certain tumors. Ontuxizumab binding to endosialin may interfere with platelet-derived growth factor signaling, prevent tumor-stroma organization, and prevent new vessel formation.

Methods—Ontuxizumab was administered intravenously on days 1, 8, 15, and 22 of a 28-day cycle at 3 dose levels (4, 8, and 12 mg/kg). Further dose escalation to 16 mg/kg was planned if the maximum tolerated dose (MTD) was not reached and the ontuxizumab systemic clearance (CL) was 30% higher in children compared to adults. Following determination of the MTD/recommended phase 2 dose, an additional cohort of 6 patients (<12y) was enrolled for further pharmacokinetic (PK) evaluation.

Results—Twenty-seven eligible patients (17 male, median age 15y, range 3 – 21y) were enrolled. Twenty-two patients [neuroblastoma (5), Ewing sarcoma (4), rhabdomyosarcoma (4), other tumors (9)] were fully evaluable for toxicity. Five patients did not complete cycle 1 due to tumor progression. Two of 10 patients experienced dose-limiting toxicity of bacteremia (n=1) and hyponatremia (n=1) at 12 mg/kg. Grade 2 fever or infusion related reactions occurred in 10 patients. Clearance was dose-dependent and within 30% of adult value at 12 mg/kg.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any relevant or important affiliations to disclose.

Conclusion—Ontuxizumab administered weekly at 12 mg/kg appears to be well tolerated in children with relapsed or refractory solid tumors. The PK of ontuxizumab does not appear to be significantly different in children compared to adults.

Keywords

Phase 1; solid tumors; Ontuxizumab

INTRODUCTION

Ontuxizumab (MORAb-004) is a humanized immunoglobulin G-1-kappa (IgG₁/κ) monoclonal antibody that targets endosialin [tumor endothelial marker-1 (TEM-1)]. Endosialin is a cell surface glycoprotein that is expressed on tumor-associated myofibroblasts, mural cells and on select human tumors.^{1–5} Preclinical investigations have demonstrated strong endosialin expression in pediatric malignancies including Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, synovial sarcoma, and neuroblastoma.^{6–8}

Endosialin has been shown to have a key role in tumor growth and neovessel formation.^{2,5–7,9} Endosialin may promote pericyte proliferation through the platelet derived growth factor (PDGF) signaling pathway and be involved in the development of the tumor neovascular network. Normal pericytes expressing high levels of endosialin have been shown to proliferate in response to PDGF-BB stimulation through phosphorylation of both the PDGF receptor and the mitogen-activated protein (MAP) extracellular signal-regulated kinase (ERK)-1/2 resulting in the expression of the transcription factor c-Fos.¹ Conversely, when endosialin expression is knocked down, PDGF-BB-induced pericyte proliferation is blocked. Disruption of human pericyte proliferation via inhibition of endosialin-dependent signal pathway may be a useful strategy to inhibit tumor angiogenesis. Blocking of endosialin may also have anti-angiogenic effects via prevention of vascular remodeling leading to decreased tumor blood flow.³

Endosialin has also been found to be integral to tumor-stroma organization. When ontuxizumab binds to human endosialin, it disrupts the interaction between endosialin and its ligands (fibronectin, collagen type I and IV), preventing protein-protein interactions that are critical for tumor-stroma organization.¹⁰ In addition, ontuxizumab may have direct inhibitory effects on mesenchymal cells expressing endosialin via antibody dependent cellular cytotoxicity.

Clinical trials in adults have demonstrated that ontuxizumab (0.0625 to 16 mg/kg) is safe and tolerable when administered on a weekly schedule.¹¹ The most common adverse events (AE) have included grade 1–2 fatigue, headache, and infusion toxicities. One dose limiting toxicity (DLT) of grade 3 vomiting was observed in patients who received 16 mg/kg of ontuxizumab. There were 9 additional reported serious AE of nausea (n=2), vomiting (n=1), diplopia (n=1), depressed level of consciousness (n=1), somnolence (n=1), speech disorder (n=1), pyrexia (n=1), and infusion reaction (n=1) observed in 3 of the 5 adults treated at this dose level. The maximum tolerated dose (MTD) was defined as 12 mg/kg. Based on pharmacokinetics, subsequent clinical trials have evaluated doses of 1 mg/kg to 8 mg/kg weekly to determine the optimal biologic dose of ontuxizumab¹².

We conducted this phase 1 dose escalation study to identify the MTD and/or recommended phase 2 dose (RP2D), to assess the safety profile of ontuxizumab, and to preliminarily define the antitumor activity of ontuxizumab in pediatric patients with relapsed or refractory solid tumors.

METHODS

Patient Eligibility

Patients >12 months and <22 years of age with recurrent or refractory solid tumors were eligible. Other eligibility criteria included a Karnofsky/Lansky performance score ≥ 50 ; recovery from prior therapy, and adequate organ function. Patients with primary CNS tumors or prior history of metastatic CNS disease were excluded. Additional exclusion criteria included history of clinically significant bleeding risk, major surgical procedures within 28 days of enrollment, or minor surgical procedures within 7 days of enrollment. Patients with grade ≥ 2 allergic reactions to prior monoclonal antibody were not eligible when the trial opened. This exclusion criterion was eliminated with Amendment 1. This trial was approved by the Institutional Review Boards of all participating sites. All patients or their legal guardians signed a document of informed consent and assent was obtained according to institutional guidelines.

Drug administration

Ontuxizumab was supplied by Morphotek, Inc. (Exton, PA) and administered intravenously once every 7 days (± 2 days). Infusion rates were: 0.03 mg/kg/min (max 2 mg/min) for the first 15 minutes, 0.07 mg/kg/min (max 5 mg/min) for the next 15 minutes, and 0.15 mg/kg/min (max 10 mg/min) for the remainder of the infusion. Patients were pretreated with acetaminophen, diphenhydramine, and ondansetron. All patients received dexamethasone [0.1 mg/kg (max dose 8 mg)] IV or PO 30 to 60 minutes prior to ontuxizumab on days 1 and 8 of cycle 1. Dexamethasone could be discontinued in absence of infusion reaction after the first 2 doses of ontuxizumab. The starting dose of ontuxizumab was 4 mg/kg on days 1, 8, 15, and 22 of a 28-day cycle with planned escalations in subsequent cohorts of patients to 8 and 12 mg/kg. An additional escalation to 16 mg/kg was to be evaluated if the MTD had not yet been reached and the mean antibody CL was at least 30% faster in children than in adults.

Study Design

The primary objectives of the study were to 1) estimate the MTD and/or RP2D of ontuxizumab administered as an intravenous infusion every week to children with refractory solid tumors, 2) describe the toxicities of ontuxizumab administered on this schedule, 3) characterize the pharmacokinetics (PK) of ontuxizumab in children with relapsed/refractory solid tumors, and 4) monitor for the development of ontuxizumab anti-drug antibody (ADA) in children receiving ontuxizumab. Secondary objectives were to 1) define the antitumor activity of ontuxizumab within the confines of a phase 1 study, 2) examine the change in plasma endosialin and PDGF receptor- β (PDGFR β) levels as potential biomarkers of ontuxizumab activity, and 3) correlate the baseline expression of endosialin and PDGFR β in

tissue and plasma with clinical parameters including disease response in an exploratory manner. To achieve these objectives, a rolling six dose-escalation scheme was used.¹³

Adverse Events and Definitions of Dose Limiting Toxicity

Required evaluations to monitor for regimen-related toxicity included physical examinations (weekly during cycle 1, then prior to subsequent cycles) as well as laboratory evaluations (complete blood count, electrolytes, and hepatic function panel) that were performed weekly during cycle 1 and on days 1 and 15 of subsequent cycles. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0 (<http://ctep.cancer.gov>). Hematologic DLT was defined as any grade 4 neutropenia or thrombocytopenia or any grade 3 neutropenia or thrombocytopenia that did not resolve to an ANC of $1000/\text{m}^3$ and platelets $75,000/\text{mm}^3$ within 7 days of the next scheduled dose. Non-hematologic DLT was defined as any grade 3 or 4 non-hematologic toxicity attributed to ontuxizumab with the exclusion of grade 3 nausea and vomiting of < 3 days duration or controllable with anti-emetics; grade 3 ALT/AST or GGT that returned to levels that meet initial eligibility or baseline criteria within 7 days of last dose of study drug; temperature greater than 40°C < 48 hours duration, grade 3 infection < 5 days duration; grade 3 electrolyte disturbances that were corrected with electrolyte supplementation to grade 1 within 72 hours; and grade 3 dehydration that was corrected to grade 1 within 48 hours with or without hospitalization. The MTD was defined as the highest dose at which less than one-third of patients experienced a DLT during cycle 1 of therapy. The study was designed to permit an expansion of each cohort to 12 patients if the two DLTs observed out of 6 evaluable patients were of different classes of AE and all of the following conditions were met: one of the DLTs did not appear to be dose related and the AE were readily reversible.

Response

Tumor response was reported using the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1).¹⁴ Disease assessment was required after cycle 1, 3, 5 and then after every 3rd cycle. Objective (partial or complete) responses had to be sustained for a minimum of two consecutive imaging evaluations at least 4 weeks apart. Responses and stable disease (≥ 6 cycles) were confirmed by central radiologist's review.

Pharmacokinetic Studies

During cycle 1, blood samples for PK analysis were collected prior to and at the end of each ontuxizumab infusion (days 1, 8, 15, and 22), 2 hours following infusion on days 1 and 22, and then on day 3 or 4. In addition, PK samples were obtained prior to and at the end of infusion on day 1 of cycles 2 and 3. Serum was isolated and stored at -80°C until analysis. Serum concentrations of ontuxizumab were measured using a validated endosialin antigen-based electrochemiluminescent (ECL) immunoassay to capture and quantify the serum concentration of free/partial or partially complexed antigen binding domains of ontuxizumab. The lower limit of quantitation for the method was 7 ng/mL in undiluted serum. Serum concentration-time data were analyzed by standard non-compartmental analysis (NCA) methods using Phoenix® WinNonlin® 6.3 (Certara USA, Inc., Princeton, NJ). Peak serum concentration (C_{max}), half-life ($t_{1/2}$), and area under the concentration-time curve from zero to day 8 ($\text{AUC}_{0-8\text{d}}$) were determined by NCA fitting on data from cycle 1.

Immunogenicity

Blood samples for the assessment of ADA were collected on all patients at baseline, prior to the day 15 infusion in cycle 1 and prior to the day 1 infusion in all subsequent cycles. Serum was isolated and stored at -80°C until analysis. Detection of ontuxizumab-specific antibodies in serum samples was accomplished using an immunoassay bridging format based on the formation of ontuxizumab ADA complexes detected in a quasi-quantitative manner via ECL assay. The sensitivity of this method using a surrogate anti-ontuxizumab IgG antibody as a positive control standard in human serum was 19 ng/mL in undiluted serum. Assay tolerance to residual ontuxizumab determined that low-level ADA was detectable in the presence of up to approximately 25 $\mu\text{g/mL}$ of ontuxizumab in human serum.

Biomarker Studies

Tissue from original diagnosis, relapse, or subsequent resection or biopsy prior to treatment with ontuxizumab was submitted for evaluation of baseline expression of endosialin and PDGFR β . Slides were prepared from formalin-fixed paraffin-embedded (FFPE) tissue and underwent endosialin and PDGFR β immunostaining using TEM-1, clone 9G5, lot 33432C and PDGFR β , clone 28E1 antibodies, respectively.¹⁵ Endosialin and PDGFR β immunostaining were evaluated as percentages of tumor cells with membranous and cytoplasmic staining as well as percentages of capillary, arterial, venous and lymphatic endothelial cells, perivascular cells, and non-vascular stromal cells with staining at intensities 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong).

In consenting patients, plasma samples for BA were collected at the following time points: day 1, day 3–4, and day 8 of cycle 1 prior to therapy, and prior to day 1 infusion in subsequent cycles. A sandwich immunoassay using Meso Scale Discovery® (MSD) ECL platform was used to quantitate the total level of endosialin and PDGFR β in plasma. Range of positive controls used for the endosialin and PDGFR β assays were 25–14,000 pg/mL and 5–2,500 pg/mL, respectively.

RESULTS

Twenty-seven patients were enrolled and received at least one dose of ontuxizumab between August 2013 and November 2014. Five of these 27 patients were not fully evaluable for toxicity because they did not complete cycle 1 due to progressive disease (PD). Baseline characteristics of eligible and evaluable patients are shown (TABLE 1). The median age of eligible patients was 15 years (range, 3–21y) and 17 patients (63%) were male. The most common cancers were Ewing sarcoma (19%), neuroblastoma (19%), and rhabdomyosarcoma (19%). Patients had received a median of 3 prior regimens of chemotherapy.

Maximum Tolerated Dose/Recommended Phase 2 Dose Determination

Cycle 1 DLTs are summarized in TABLE 2. No DLTs were observed among the 6 evaluable patients treated at 4 mg/kg dose level or the 6 evaluable patients at the 8 mg/kg dose level. Initially 6 patients were enrolled at the 12 mg/kg dose level. Two of these 6 patients were

removed from protocol therapy due to PD prior to receiving all 4 doses of cycle 1 and an additional 2 patients were enrolled. After interim PK analysis, the 12 mg/kg dose level was expanded to evaluate the PK of ontuxizumab in children < 12 years of age. Two of the 6 patients in the expansion cohort did not complete cycle 1 due to PD. Two of the 10 evaluable patients who received 12 mg/kg ontuxizumab experienced DLT. The first patient developed fever following the day 8 infusion of ontuxizumab. The patient was found to have *Staphylococcus epidermidis* bacteremia without any associated cardiovascular compromise. The patient completed a 14-day course of antibiotics and recovered without sequelae. Because the patient required intravenous antibiotic therapy for greater than 5 days and the proximity of the event to the infusion, this grade 3 AE was determined to meet criteria for DLT. The second patient developed grade 3 hyponatremia (sodium 129 mEq/L) prior to the day 22 dose in cycle 1. A normal saline bolus was given and the sodium increased to 131 mEq/L within 5 hours. The patient was subsequently removed from protocol therapy due to PD. Labs performed at the end of the cycle again demonstrated a grade 3 hyponatremia (sodium 128 mEq/L). No intervention was given due to prior decision to remove patient from therapy. Five days later, the hyponatremia resolved to grade 1 (130 mEq/L). In addition, a patient who received 12 mg/kg of ontuxizumab had grade 3 pleural effusion that contained malignant cells. This toxicity was determined not to be attributable to investigational drug. Based on the described toxicities, the 12 mg/kg dose level was determined to be the RP2D.

Additional Toxicities

Adverse events related to ontuxizumab are summarized in TABLE 3. Non-DLT, grade 3 regimen-related toxicities in cycle 1 were rare with only 1 grade 3 hypophosphatemia and 1 grade 3 anemia reported. The most common hematologic toxicity was anemia (11 events in 38 delivered cycles). The most common non-hematologic toxicities were AST increased (n=6), headache (n=6), hyperglycemia (n=6), nausea (n=6), vomiting (n=6), ALT increased (n=5), fatigue (n=5), hyponatremia (n=5), and grade 2 fever or infusion related reactions occurred in 10 patients.

Pharmacokinetics

All 27 patients participated in PK studies. Per protocol, since the MTD was not reached following escalation to 12 mg/kg, ontuxizumab exposure and CL in patients treated in the dose escalation cohort (n=6) at 12 mg/kg were compared to adult patients treated at this dose level in prior studies. The ontuxizumab CL was 20.0 ± 8.0 ml/h compared with 22.6 ± 10.1 ml/h in adults.¹⁶ The 7 day ontuxizumab exposure was 22150 ± 2940 $\mu\text{g}\cdot\text{h}/\text{ml}$ compared with 20200 ± 2720 $\mu\text{g}\cdot\text{h}/\text{ml}$ in adults.¹⁶ Thus, the mean ontuxizumab CL at 12 mg/kg in this trial was not significantly different than the reported CL in adults, supporting no further dose escalation. After expansion at the 12 mg/kg dose level, ontuxizumab PK were determined for all 25 patients who completed PK sampling after the first dose, while steady-state PK and accumulation were evaluable for 21 patients with trough samples on days 8, 15 and 22 (TABLE 4). The ontuxizumab half-life was similar across dose levels with a mean value for all patients of 84.4 ± 16.7 hours. Ontuxizumab CL appeared to decrease as the dose was increased from 4 mg/kg to 12 mg/kg; however, CL adjusted for body weight was similar

across dose levels with a mean value of 0.49 ± 0.18 mL/h/kg. Ontuxizumab trough concentrations increased 2.2-fold throughout the first cycle of treatment.

Immunogenicity

Baseline and treatment samples for ADA evaluation were available from 24 of the 27 enrolled patients. Two of 24 patients had a positive ADA detected at baseline. Neither of these subjects had detectable ADA following treatment. None of the subjects developed treatment-induced ADA response after ontuxizumab administration. However, 22 of the 24 patients had at least one sample that contained ontuxizumab serum concentrations that exceeded the drug tolerance limit of the assay. Therefore, it is possible that the presence of some low-level ADA could be masked, preventing definitive assignment of ADA status in these samples.

Biomarkers

Tissue-based biomarkers—Biopsy samples were available for evaluation of endosialin and PDGFR β staining from 21 of the 27 enrolled patients. Due to quality of submitted tissue, 17 of the 21 tissue samples were available for endosialin and PDGFR β staining analysis (TABLE 5). None of the samples demonstrated endosialin staining in the tumor cell membrane, arterial or venous endothelial cells. None of the samples demonstrated PDGFR β staining in venous endothelial cells. Only 2 tumor samples demonstrated 1+ membranous staining and 1 tumor had 1+ arterial cell staining for PDGFR β . In contrast, nearly all samples demonstrated 1+ to 3+ endosialin and PDGFR β staining in the non-vascular stromal cells, capillary endothelial cells, lymphatic endothelial cells and perivascular cells. There was no difference in the staining pattern of endosialin or PDGFR β in the 2 patients who had stable disease compared to the patients who had PD after cycle 1.

Plasma-based biomarkers—Plasma-based samples for measurement of endosialin and PDGFR β at baseline and following treatment with ontuxizumab were available from 25 of the 27 enrolled patients (Fig. 1). There was no apparent association between plasma endosialin or PDGFR β concentration and ontuxizumab concentrations. The range of baseline plasma endosialin levels across studied tumors was similar (44–199) ng/mL. However, the median baseline level was slightly higher in neuroblastoma patients compared to other patients [neuroblastoma 96 (range: 84–104) ng/mL, Ewing sarcoma 62 (range: 46–151) ng/mL, rhabdomyosarcoma 66 (range: 45–138) ng/mL, other 64 (range: 44–199) ng/mL]. Following treatment with ontuxizumab, the day 8 and day 29 (when available) plasma level of endosialin increased by a median (range) of 483 (307–787) and 799 (656–926) percent from baseline, respectively (Fig. 1A). In subjects who had serial plasma endosialin measured following cycle 2 (n=4), the endosialin level remained elevated from baseline but appeared to plateau. Baseline levels of plasma PDGFR β were highly variable (Fig. 1B). The 2 patients who demonstrated stable disease had the highest circulating level of PDGFR β at baseline and these levels remained relatively constant throughout treatment. Evaluation of plasma levels of PDGFR β following treatment demonstrated neither a significant change nor a consistent direction of change of PDGFR β plasma concentrations after ontuxizumab treatment.

Responses

Twenty-one of the 27 enrolled patients completed cycle 1. Five patients (desmoplastic small round cell tumor, Ewing sarcoma, hepatoblastoma, pancreatoblastoma, and rhabdomyosarcoma) did not complete cycle 1 due to PD. One patient did not complete cycle 1 due to DLT. There were no objective responses. Two patients with neuroblastoma had stable disease and each completed 5 cycles of ontuxizumab therapy prior to demonstrating PD. Three additional patients completed 2 cycles (undifferentiated sarcoma, clear cell sarcoma, neuroblastoma) and 1 patient (neuroblastoma) completed 3 cycles of therapy.

DISCUSSION

This is the first phase 1 study and pharmacologic evaluation of ontuxizumab in pediatric patients with relapsed solid tumors. Using a rolling 6 design, the dose escalation portion of the trial achieved complete accrual within 11 months. The study was then paused for assessment of toxicity and evaluation of pharmacokinetics. Six additional patients were enrolled and completed assessment in the PK cohort in 4 months. The trial completed accrual 16 months after initial activation.

Overall, ontuxizumab was well tolerated and demonstrated no significant myelosuppression. Using a controlled infusion rate and requiring pre-treatment with dexamethasone for the initial 2 doses and acetaminophen, diphenhydramine, and ondansetron prior to each dose, infusion related reactions were mild (grade 1 or 2), occurred in 27% of patients during cycle 1, and were seen in 16% of cycles delivered (6/38). Dose limiting toxicity (bacteremia and hyponatremia) occurred in two patients treated with ontuxizumab at 12 mg/kg intravenously weekly. During dose escalation, the patient with recurrent grade 3 hyponatremia during cycle 1 was not initially identified as meeting criteria for DLT because the subject discontinued protocol therapy on day 28 due to PD. We proceeded to PK expansion at 12 mg/kg dose level based on 1/6 patients with DLT (bacteremia) and similar CL of ontuxizumab in children and adults. Six patients were enrolled to PK expansion. Two patients experienced disease progression prior to the end of cycle 1. No patients in this cohort experienced DLT. Overall in evaluable patients who received 12 mg/kg, DLT occurred in 20% (2/10). Treatment with ontuxizumab was not associated with the development of measurable treatment-induced or boosted ADA response. Based on the toxicity and PK profile of ontuxizumab in pediatric patients, 12 mg/kg was identified as the recommended dose for further clinical development.

An important component of this trial was the incorporation of PK to compare CL in children and adults to inform dose escalation. We demonstrated that the CL of ontuxizumab is similar in children and adults. In this trial, the determination of RP2D was based on both the DLT and similar CL. However, for drugs in which no DLT is observed, we propose using CL as a parameter to determine if a dose in children is equivalent to the dose recommended in adults. We selected CL as the parameter because, for many drugs, CL is independent of dose. However, for ontuxizumab, saturation of CL may occur in adults and children.

Prior studies have demonstrated a potential role for circulating soluble cell surface proteins such as vascular endothelial growth factor (VEGF) receptor in the systemic circulation as

biomarkers for pharmacologic and clinical activity of anti-angiogenic therapy.^{17,18} Elevated levels of soluble PDGFR β in CSF have been found to correlate with brain pericyte injury, suggesting that soluble pericyte cell surface proteins, such as endosialin and PDGFR β , may be potential biomarkers for ontuxizumab activity.^{15,19} Biomarker evaluation demonstrated that circulating endosialin and PDGFR β in plasma were highly variable. The finding that the two subjects with stable disease had the highest serum PDGFR β levels, consistent with observations in other adult clinical trials (S. Weil, Morphotek Inc., personal communication), would support that PDGFR β levels may be a biomarker for pericyte injury. The data also demonstrate that plasma endosialin levels may increase with ontuxizumab treatment. Further investigation is required to determine circulating endosialin and PDGFR β may be a biomarker for pericyte injury or a compensatory response to overcome antibody blockade.

Using immunohistochemistry, archival tumor tissue samples from patients on this trial demonstrated endosialin and PDGFR β staining of nonvascular stromal cells as well as perivascular and capillary cells but not tumor cells. While other studies have demonstrated endosialin staining on pediatric tumor cells, there is often a high degree of variability of expression within and across tumor types.^{6,8} However, consistent with our findings, these studies demonstrate that even if endosialin is not expressed on the malignant cells, there is consistent and strong expression on the tumor stroma and vasculature, supporting a possible role therapeutic role of ontuxizumab in the disruption of tumor organization. Therefore, while no objective responses were observed in this study, based on the mechanism of action, lack of myelosuppression and tolerability, the evaluation of ontuxizumab in combination with chemotherapy or radiation with stratification based on serum PDGFR β levels may be considered.

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Abbreviations Key

ADA	Anti-drug antibody
AE	Adverse events
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AUC_{0-8d}	Area under the concentration-time curve

BA	Biomarker analysis
CL	Systemic clearance
C_{max}	Peak serum concentration
CNS	Central nervous system
COG	Children's Oncology Group
DLT	Dose limiting toxicity
ECL	Endosialin antigen–based electrochemiluminescent
ERK	Extracellular signal-regulated kinase
FFPE	Formalin-fixed paraffin-embedded
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
IgG_{1/κ}	G-1-kappa
MAP	Mitogen-activated protein
MSD	Meso Scale Discovery®
MTD	Maximum tolerated dose
NCA	Non-compartmental analysis
NCI	National Cancer Institute
NIH	National Institutes of Health
PD	Progressive disease
PDGF	Platelet derived growth factor
PDGFRβ	Platelet derived growth factor receptor-β
PDGF-BB	Platelet derived growth factor-BB
PK	Pharmacokinetic
RP2D	Recommended phase 2 dose
RECIST	Response Evaluation Criteria in Solid Tumors
t_{1/2}	Half-life
TEM-1	Tumor endothelial marker-1
ULN	Upper limit of normal

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Figure 1.

A. Individual endosialin plasma levels. **B.** Individual PDGFR β plasma levels. Subjects treated at Dose Level 1 (4 mg/kg) represented with ■ and; Subjects treated at Dose Level 2 (8 mg/kg) represented with ◆ and ---; Subjects treated at Dose Level 3 (12 mg/kg) represented with ● and —. Subjects with stable disease indicated in red. Subjects who received 3 cycles of therapy indicated in black. All other subjects with PD after cycle 1 depicted in grey.

TABLE 1

Patient Characteristics for Eligible and Evaluable Patients

Characteristic	Eligible Patients (n=27) Number (%)	Evaluable Patients (n=22) Number (%)
Age (years)		
Median	15	16.5
Range	3 – 21	3–21
Sex		
Male	17 (63)	14 (64)
Female	10 (37)	8 (36)
Race		
White	23 (85)	19 (86)
Asian	0 (0)	0 (0)
American Indian or Alaska Native	0 (0)	0 (0)
Black or African American		
Unknown	2 (7.5)	2 (9)
Ethnicity	2 (7.5)	1 (5)
Non-Hispanic	23 (85)	18 (82)
Hispanic	3 (11)	3 (14)
Unknown	1 (4)	1 (5)
Prior Therapy		
Chemotherapy Regimens		
Median	3	3
Range	1 – 6	1 – 6
Radiation Regimens		
Median	1	1
Range	1 – 4	1 – 2
Diagnosis		
Ewing Sarcoma	5 (19)	4 (18)
Neuroblastoma	5 (19)	5 (23)
Rhabdomyosarcoma	5 (19)	4 (18)
Clear Cell Sarcoma	2 (7)	2 (9)
DSRCT*	2 (7)	1 (5)
Osteosarcoma	2 (7)	2 (9)
Germ Cell Tumor	1 (4)	1 (5)
Hepatoblastoma	1 (4)	0
Pancreatoblastoma	1 (4)	0
Synovial Sarcoma	1 (4)	1 (5)

Characteristic	Eligible Patients (n=27) Number (%)	Evaluable Patients (n=22) Number (%)
Undifferentiated Sarcoma	1 (4)	1 (5)
Wilms Tumor	1 (4)	1 (5)

* DSRCT= Desmoplastic small round cell tumor

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TABLE 2

DLT Summary by Dose Level

Ontuxizumab Dose Level	No. Patients Entered	No. Patients Evaluable	No. Patients with DLT	Dose Limiting Toxicities (n)
4 mg/kg	6	6	0	
8 mg/kg	7	6	0	
12 mg/kg	8	6	2	Staph. epidermidis bacteremia (1) Hyponatremia (1 [*])
12 mg/kg (PK)	6	4	0	

* Toxicity reported after enrollment of PK cohort.

TABLE 3

Adverse Events Possibly, Probably, or Definitely Attributed to Protocol Therapy

Toxicity Type	Cycle 1 (Total, 22 Cycles)				Cycle 2 to 5 (Total, 16 Cycles)			
	Maximum Grade of Toxicity				Maximum Grade of Toxicity			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic Toxicities								
Anemia	6	2	1		1		1	
Lymphocyte count decreased	1	1			1			
Neutrophil count decreased		2				1		
Platelet count decreased	2							
White blood cell decreased	3				3			
Non-Hematologic Toxicities*								
Alanine aminotransferase increased	2	1			1			
Aspartate aminotransferase increased	5					1		
Fatigue	2	2				1		
Fever	3	1						
Headache	5	1						
Hyperglycemia	5				1			
Hypertension		3			1			
Hypokalemia	3				1			
Hyponatremia	2		1		2			
Hypophosphatemia	1	1	1					
Infusion related reaction	2	4						
Nausea	4	1			1			
Vomiting	2	2			1	1		

* Non-hematologic toxicities are those that occurred in > 10% of patients

TABLE 4

Summary Non-Compartmental Pharmacokinetics of Ontuxizumab

		Dose Level (mg/kg)		
		4 (n=6)	8 (n=7)	12 (n=14) *
C_{max} (µg/mL)		98.7 ± 14.7	141 ± 42	233 ± 37
$T_{1/2}$ (hr)		74.6 ± 17.4	78.5 ± 9.9	92.7 ± 16.3
AUC_{0-7d} (hr·µg/mL)		7800 ± 1760	11600 ± 4200	19200 ± 4300
$AUC_{0-\infty}$ (hr·µg/mL)		10100 ± 2700	14900 ± 5400	27800 ± 7800
CL (mL/hr)		25.9 ± 9.5	23.0 ± 8.6	17.0 ± 8.1
CL (mL/hr/kg)		0.422 ± 0.125	0.606 ± 0.246	0.455 ± 0.133
C_{trough} (µg/mL)	Day 8	20.4 ± 7.0	29.0 ± 10.4	64 ± 18
	Day 15	33.7 ± 12.9	51.5 ± 26.1	111 ± 38 ***
	Day 22	39.5 ± 15.2	65.3 ± 28.2 **	149 ± 55 ****
Accumulation (Day 22/Day 8)		1.9 ± 0.3	2.3 ± 0.4 **	2.2 ± 0.4 ****

* 12 patients were evaluable for PK after the first dose;

** n=6;

*** n=11;

**** n=9

TABLE 5

Tissue-based biomarkers

	Tumor Type [*]			
Endosialin 1⁺ to 3⁺ Staining 50% Cells	Neuroblastoma (n=5)	Rhabdomyosarcoma (n=4)	Ewing Sarcoma (n=3)	Other (n=5)
Tumor Cell Membrane [*]	0/5	0/4	0/3	0/5
Nonvascular Stromal Cells	4/4	1/2	2/3	5/5
Capillary Endothelial Cells	5/5	4/4	3/3	4/5
Lymphatic Endothelial Cell	5/5	2/3	2/2	4/5
Perivascular Cell	5/5	4/4	3/3	5/5
PDGFR-β 1⁺ to 3⁺ Staining 50% Cells	Neuroblastoma (n=5)	Rhabdomyosarcoma (n=4)	Ewing Sarcoma (n=2)	Other (n=6)
Tumor Cell Membrane	0/5	0/4	0/2	0/6
Nonvascular Stromal Cells	5/5	2/2	2/2	6/6
Capillary Endothelial Cells	5/5	4/4	2/2	6/6
Lymphatic Endothelial Cell	5/5	3/3	2/2	6/6
Perivascular Cell	5/5	4/4	2/2	6/6

^{*} Due to quality, not all submitted samples could be evaluated resulting in variability of the denominator within each subgroup