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RESEARCH ARTICLE

A phase 1/2 study of pepinemab in children, adolescents, or young adults with recurrent or refractory solid tumors: A children's oncology group consortium report (ADVL1614)

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Abstract

Purpose: Pepinemab, a humanized IgG4 monoclonal antibody, targets the SEMA4D (CD100) antigen to inhibit binding to its high-affinity receptors (plexin B1/PLXNB1, plexin B2/PLXNB2) and low-affinity receptor (CD72). SEMA4D blockade leads to increased cytotoxic T-cell infiltration, delayed tumor growth, and durable tumor rejection in murine tumor models. Pepinemab was well tolerated and improved T cell infiltration in clinical studies in adults with refractory tumors. SEMA4D was identified as a strong candidate proto-oncogene in a model of osteosarcoma. Based on these preclinical and clinical data, we conducted a phase 1/2 study to determine the recommended phase 2 dose (RP2D), pharmacokinetics, pharmacodynamics, and immunogenicity, of pepinemab in pediatric patients with recurrent/refractory solid tumors, and activity in osteosarcoma.

Experimental design: Pepinemab was administered intravenously on Days 1 and 15 of a 28-day cycle at 20 mg/kg, the adult RP2D. Part A (phase 1) used a Rolling 6

Abbreviations: ADAs, antidrug antibodies; ANC, absolute neutrophil count; AUC, area under the curve; C_{max}, maximum concentration; CTEP, Cancer Therapeutics Evaluation Program; DLT, dose-limiting toxicity; ELISA, enzyme-linked immunosorbent assay; MDSCs, myeloid-derived suppressor cells; NCI, National Cancer Institute; PK, pharmacokinetic; PLXNB1, plexin B1; PLXNB2, plexin B2; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; RP2D, Recommend Phase 2 Dose; SEMAs, semaphorins; TAMs, tumor associated macrophages; Teff:Treg, T-effector:T-regulatory; TME, immune tumor microenvironment.

design; Part B (phase 2) used a Simon 2-stage design in patients with osteosarcoma. Pharmacokinetics and target saturation were evaluated in peripheral blood.

Results: Pepinemab (20 mg/kg) was well tolerated and no dose-limiting toxicities were observed during Part A. There were no objective responses. Two patients with osteosarcoma achieved disease control and prolonged stable disease. Pepinemab pharmacokinetics were similar to adults.

Conclusions: Pepinemab (20 mg/kg) is safe, well tolerated and resulted in adequate and sustained target saturation in pediatric patients. Encouraging disease control in two patients with osteosarcoma warrants further investigation with novel combination strategies to modulate the tumor microenvironment and antitumor immune response.

Clinical trial registry: This trial is registered as NCT03320330 at Clinicaltrials.gov.

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KEYWORDS
pepinemab, phase 1, solid tumors

1 | INTRODUCTION

Semaphorins (SEMAs) are a diverse family of proteins including soluble and cell membrane-bound forms with known roles in development.[1,2] Although originally characterized as axon guidance molecules, they are now considered key regulators in many different cell types.[3] They signal and exert their effect through binding to plexin receptors.[4] SEMA4D plays a role in bone homeostasis by promoting differentiation of osteoblasts leading to bone formation. SEMA4D engagement with its cognate plexin receptor, plexin B (PLXNB), expressed on osteoblasts, inhibits bone formation.[5] SEMA4D inhibition has been shown in to suppress growth and oncogenic signaling of preclinical osteosarcoma models in which SEMA4D is highly overexpressed.[6,7]

Using a *Sleeping Beauty* forward genetic screen in which mice developed osteosarcoma via common insertion site-associated genes, SEMA4D was found to be a strong candidate proto-oncogene.[6] These tumors demonstrated upregulation of SEMA4D compared with normal osteoblasts. Overexpression of SEMA4D in osteosarcoma cell lines lead to activation of MET or ERBB2 and phosphorylation of AKT and/or ERK resulting in increased colony formation in soft agar. The oncogenic role of SEMA4D in osteosarcoma has been further confirmed by shRNA knockdown resulting in significantly decreased colony formation.[6]

SEMA4D is also expressed on cells within the tumor stroma and modulates the activity of the immune system. High levels of SEMA4D correlate with the presence of immunosuppressive tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) with concomitant exclusion of activated antigen-presenting cells and CD8+ cytotoxic T lymphocytes from the tumor, downregulating an immune-mediated antitumor reponse.[8]

In multiple tumor models, antibody neutralization of SEMA4D resulted in a redistribution of immune cells at the tumor invasive margins, including an increased frequency of activated tumor-infiltrating macrophages, intratumoral CD8+ T cells, and dispersion of M2 TAMs and MDSCs.[9–11] Increased interferon gamma and tumor necrosis factor alpha indicate a shift to a proinflammatory environment more supportive of an antitumor effect. Treatment with anti-SEMA4D antibody increased T-effector:T-regulatory (Teff:Treg) cell ratios within the tumor and tumor-specific cytotoxic T-cell activity.[9] Importantly, it has been demonstrated that efficient entry of functional tumor-specific T-cells into the tumor in response to SEMA4D blockade correlates with improved survival and response to immunotherapy in preclinical models and in patients with refractory cancers.[8,12]

Pepinemab (VX15/2503) is a humanized IgG4 monoclonal antibody that binds with strong affinity to the SEMA4D (CD100) antigen, blocking SEMA4D binding to its high affinity receptor, plexin B1 (PLXNB1), plexin B2 (PLXNB2), and low affinity receptor CD72, inhibiting the homeostatic function of SEMA4D.[13] Treatment of osteosarcoma cell lines with pepinemab resulted in decreased cell migration and colony formation. Additionally, treatment of a syngeneic mouse model of osteosarcoma with MAb67-2, a mouse analog of pepinemab, significantly reduced tumor growth (unpublished data from Dr. Branden Moriarity, University of Minnesota).

Pepinemab has been studied extensively in adults. In a first-in-human trial in adults with advanced solid tumors, pepinemab was well tolerated at doses up to 20 mg/kg.[12] Treatment-related adverse events were limited to Grades 1 and 2 and included nausea, fatigue, arthralgia, decreased appetite, infusion-related reaction, and pyrexia. One patient receiving 20 mg/kg achieved a partial response and 45.2% of patients exhibited stable disease for >8 weeks. In a more recent trial of patients with NSCLC, the combination of pepinemab with avelumab, a PD-L1-targeted immune checkpoint inhibitor, was well tolerated.[14] Antitumor activity was demonstrated in immunotherapy-resistant and PD-L1 negative/low tumors.[15] The loss of pepinemab saturation on circulating T-lymphocytes (target saturation) correlated with the minimal drug concentration needed for efficacy, with ≥80% saturation

associated with prolonged responses.[9,16] Pepinemab half-life was 20 days, with persistent cellular SEMA4D saturation for >155 days.[16]

The role of SEMA4D in tumorigenesis, control of the tumor microenvironment (TME) and its presence as a cell surface, soluble ligand, makes it an attractive therapeutic target. The established safety in adults and encouraging preclinical data in osteosarcoma provide rationale for the evaluation of pepinemab in children, adolescents, and young adults with recurrent/refractory solid tumors, with an emphasis on osteosarcoma. The primary objectives of this study were to determine if the adult Recommend Phase 2 Dose (RP2D) of 20 mg/kg is tolerated and deemed to biologically relevant and to preliminarily define the antitumor activity and disease control rate of pepinemab in patients with recurrent/refractory osteosarcoma.

2 | PATIENTS AND METHODS

2.1 | Patient eligibility

Patients aged ≥1 to ≤21 years (years) with recurrent or refractory solid tumors (Part A: phase 1), excluding central nervous system tumors, and patients aged ≥1 to ≤30 years with recurrent or refractory osteosarcoma (Part B: phase 2), were eligible for this study. Verification of malignancy at diagnosis or recurrence was required. Other criteria for eligibility included a Lansky or Karnofsky performance score of ≥50%; recovery from toxic effects of all prior anticancer therapy; adequate bone marrow function (absolute neutrophil count [ANC] ≥ 1000/mm^3, platelet count ≥ 100,000/mm^3, and hemoglobin ≥ 8.0 g/dL [with or without transfusion]), renal function [normal serum creatinine for age and gender, or creatinine clearance ≥ 70 mL/min/1.73 m^2], liver function (bilirubin ≤ 1.5 times upper limit of normal for age, ALT ≤ 135 U/L, and serum albumin ≥ 2 g/dL), and pulmonary function (no dyspnea at rest or exercise intolerance and pulse oximetry > 94% on room air). Patients were deemed ineligible if pregnant or breastfeeding, receiving corticosteroids and not on a stable or decreasing dose for at least 7 days, receiving other investigational drugs, anticancer agents, antigraft-versus-host-disease agents posttransplant, had uncontrolled infection or previously received a solid organ transplant.

The protocol was reviewed and approved by the Cancer Therapeutics Evaluation Program (CTEP) of the National Cancer Institute (NCI), the NCI CIRB and/or institutional review boards of participating institutions. Informed consent and child assent, when appropriate, were obtained from all participants and/or parents or legal guardians.

2.2 | Protocol therapy administration and study design

The goal of this study (Part A) was to determine if the adult RP2D is both tolerated and deemed a biologically appropriate dose. Our biologic endpoint was defined as adequate and sustained target saturation by pepinemab (≥80% saturation). A de-escalation in Part A to 10 mg/kg was planned if toxicity occurred at dose level 1 (20 mg/kg). No dose escalations were planned.

Pepinemab was supplied by Vaccinex, Inc. and distributed to participating institutions by CTEP. Pepinemab was administered intravenously over 60 min on Days 1 and 15 of each 28-day cycle. Subjects were allowed to continue protocol therapy until they experienced disease progression, met discontinuation criteria, or completed a maximum of 13 cycles.

This study had simultaneous enrollment in two parts. Using a Rolling 6 Design, Part A enrolled patients with relapsed or refractory extracranial solid tumors with measurable or evaluable disease.[17] Patients received pepinemab (20 mg/kg) IV every 14 days until disease progression or toxicity required treatment interruption. The RP2D was defined as a dose that was both tolerated and met criteria for adequate and sustained target saturation in at least five out of six patients. If the starting dose was found to be tolerated but target saturation was not adequate or sustained, the study would be suspended while further dose escalation was considered. If a dose level was determined to be intolerable, target saturation would be assessed and if saturation found to be adequate, the dose would be de-escalated (10 mg/kg).

Part B opened concurrently with Part A for patients ≥22 years and ≤30 years of age with relapsed or refractory osteosarcoma. Part B was expanded to include patients <22 years of age once Part A was complete and the initial six adult patients on Part B completed toxicity assessments. The primary aim of Part B was to define the antitumor activity of pepinemab for the treatment of relapsed or refractory osteosarcoma and to determine if pepinemab improved the disease control rate at 4 months or achieved an objective response rate.[18] In addition, a pharmacokinetics (PK) expansion cohort in children ≤12 years of age was conducted following completion of Part A.

Given the primary endpoints of disease control and response, patients enrolled on Part B were required to have measurable disease. Enrollment on Part B used a Simon 2-stage design.[19] Stage 1 planned to enroll 19 evaluable patients. If five or more patients achieved disease control or two or more patients had objective responses using the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), Stage 2 would open to enroll 10 additional patients.[20] Stage 2 would be declared a success if there were nine or more disease control successes or five or more RECIST responders. Similarly, if the probability of response was <5% and disease controlled <20%, this agent would not be considered for further development. If response was ≥22% or disease control ≥42% the agent would be considered for further development.

Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.[21] Hematologic dose-limiting toxicity (DLT) was defined as Day 15 Grade 4 neutropenia or Grade 3 thrombocytopenia that did not resolve to ANC ≥ 500/mm^3 and platelets ≥ 50,000 by Day 18, platelet count < 25,000/mm^3 on two separate days or requiring a platelet transfusion on two separate days within a 7 day period or myelosuppression that causes a delay of >14 days between treatment cycles. Nonhematologic DLT was defined as any Grade 3 or greater nonhematological toxicity attributable to protocol therapy with the specific exclusion of: Grade 3 nausea and vomiting <3 days duration, Grade 3 liver enzyme elevation, including ALT, that returns to Grade ≤ 1 or baseline prior to the time for the

TABLE 1 Patient characteristics for eligible patients ($N = 26$).

	Number (%)
Age (years)	
Median	15.5
Range	1–30
Sex	
Male	14 (54)
Female	12 (46)
Race	
White	12 (46)
Asian	1 (4)
American Indian or Alaska Native	0 (0)
Black or African American	3 (12)
Unknown	10 (38)
Ethnicity	
Non-Hispanic	17 (65)
Hispanic	6 (23)
Unknown	3 (12)
Diagnosis	
Chondroblastic osteosarcoma	2 (7.7)
Malignant rhabdoid tumor	1 (3.8)
Neuroblastoma, NOS	2 (7.7)
Osteosarcoma, NOS	19 (73.1)
Telangiectatic osteosarcoma	1 (3.8)
Undifferentiated sarcoma	1 (3.8)
Prior therapy	
Chemotherapy regimens ($N = 26$)	
Median	3
Range	1–7
Radiation therapy ($N = 9$)	
Median	1
Range	1–3

next treatment cycle, Grade 3 fever, Grade 3 infection and Grade 3 hypophosphatemia, hypokalemia, hypocalcemia, or hypomagnesemia responsive to supplementation. The DLT observation period for dose determination of MTD/RP2D was the first cycle.

Disease evaluations for Part A were performed after the first cycle then every other cycle × 2 and then every three cycles and for Part B, after cycle 2, 4, 6 and then every three cycles. Tumor response was reported using RECIST v1.1.

2.3 | Pharmacokinetics

Pharmacokinetics (PK) were performed in all patients. For patients >10 kg, blood samples for PK were obtained on cycle 1, Day 1 prior to start of infusion, at the end of infusion, and 2 h following the end of infusion. Additional timepoints included cycle 1, Days 4 (±1), 8 (±1) and cycle 1, Day 15 both prior to the start and at the end of infusion. Cycle 2 time points included Day 1 prior to start and at the end of infusion, 2 h following the end of infusion and Day 15 prior to start of infusion. Subsequent cycle samples were drawn on Day 1 prior to the infusion. In patients ≤10 kg, limited sampling strategy included: cycle 1, Day 1 prior to start of infusion (3.5 mL), end of infusion (2.5 mL), and 2 h following the end of infusion (2.5 mL), Day 15 prior to start of infusion (2.5 mL) and cycle 2, Day 1 prior to start of infusion (3.5 mL) and Day 15 prior to start of infusion (2.5 mL). Following cycle 2, a sample was collected on Day 1 of subsequent cycle. Pepinemab serum concentrations were analyzed by Covance Laboratories (Indianapolis, IN) via a validated enzyme-linked immunosorbent assay (ELISA) assay as previously described.[12,16]

The serum concentration–time data for pepinemab were analyzed by noncompartmental analysis using Phoenix® WinNonlin® Version 6.4 (Certara Corporation, Princeton, NJ). A descriptive analysis of PK parameters was performed, defining systemic exposure, half-life, concentration, and drug clearance.

2.4 | Pharmacodynamics and correlative biology

2.4.1 | Target saturation by pepinemab

Blood samples were analyzed for target saturation by pepinemab using a validated flow cytometry-based assay.[22] Patients had samples collected on cycle 1, Days 1 and 15, prior to the start of and at the end of infusion and cycle 1, Day 28 (or cycle 2 Day 1) regardless of whether or not patient proceeded to cycle 2. Patients who continued beyond cycle 2 had blood samples drawn prior to Day 1 infusion of each cycle.

2.4.2 | Total soluble SEMA4D

Blood and serum samples were evaluated for total soluble SEMA4D via a qualified ELISA assay. This assay detects both free sSEMA4D and the complex of pepinemab bound to sSEMA4D.[12,16] Patients >10 kg had samples drawn at cycle 1, Day 1 prior to start and at the end of infusion and on Day 1 of each subsequent cycle. Patients ≤10 kg had samples drawn at cycle 3, Day 1 and prior to start of infusion for each additional cycle.

2.4.3 | Antidrug antibodies

Serum samples were analyzed for antidrug antibodies (ADAs) through a qualified ELISA assay.[12,16] Patients ≤10 kg had limited sampling done on cycle 3 Day 1 prior to start of infusion and for each subsequent cycle. For patients >10 kg, samples were collected on cycle 1 Day 1 prior to the start and at the end of the infusion and prior to the start of the infusion in each subsequent cycle.

TABLE 2 Cycle 1 DLT summary.

Dose level	Part	Number of patients entered	Number of patients evaluable	Number of patients inevaluable	Number of patients with DLT	DLT types
20 mg/kg	A	6	6	0	0	
20 mg/kg	A PK	6	6	0	0	
20 mg/kg	B	14	14	0	1	Acute kidney injury, arthralgia, creatinine increased, myalgia

2.4.4 | Tumor expression of SEMA4D

Archival tumor tissue submission was required. Tumor expression of SEMA4D, PlexinB1, and other markers of immune cell infiltration were to be evaluated by immunohistochemistry.

3 | RESULTS

3.1 | Demographics

A total of 26 patients enrolled on the study and all were found to be eligible and evaluable for DLT assessment. The median age was 15.5 years (range 1—30 years) and 54% of patients were male. Twenty-two (84.6%) patients had a diagnosis of osteosarcoma. Additional characteristics of the patients are displayed in Table 1.

3.2 | Recommended phase 2 dose determination

The pepinemab RP2D in pediatric patients was confirmed to be 20 mg/kg IV q 14 days. One patient on Part B experienced cycle 1 DLT's including acute kidney injury/creatinine increased, myalgias, and arthralgias (Table 2). Grade 3 and higher cycle 1 toxicities that were not dose limiting included anemia, decreased lymphocyte count, and decreased neutrophil count (Table 3). Infusion-related reactions were common (40%), but all were ≤Grade 2 and did not interfere with administration of pepinemab. Table 3 displays toxicities that occurred beyond cycle 1. One part B patient experienced dose-limiting Grade 3 pericardial effusion during the second cycle of treatment. This remained unresolved at the time the patient came off protocol therapy for disease progression.

Twenty-three patients were evaluable for the biologic endpoint of target saturation with pepinemab and all had complete (>90%) and sustained saturation following the first dose of pepinemab. Two patients were unevaluable, one due to missing timepoints and one due to sample contamination secondary to being drawn from a central line.

Given that 20 mg/kg IV every 14 days was found to be safe, tolerable, and achieved sustained target saturation, this was determined to be the RP2D in pediatric patients to be further evaluated in the PK expansion cohort and Part B of the study.

3.3 | Response

Eight patients from Part A with osteosarcoma contributed to the response evaluation and 14 patients from Part B for a total of 22 response evaluable patients. The response evaluable patients remained on protocol therapy for 1–13 cycles. Two patients with relapsed osteosarcoma met criteria for disease control, one of whom remained on treatment for nine cycles before developing progressive disease. The second patient completed all 13 cycles and continued to receive pepinemab through compassionate use. No objective RECIST responses were observed in the 22 patients. With only two patients achieving disease control and no RECIST responders, criteria to open Stage 2 of Part B was not met and the study was terminated.

3.4 | PK and pharmacodynamics

Pepinemab PK were evaluated in 25 patients during cycle 1 and 2. Graphs of the plasma concentration versus time, peak, and trough concentrations are illustrated in Figure 1. The PK parameters for pepinemab are summarized in Table 4 by medians with 25th and 75th percentiles. Patients enrolled in Part A were younger (12.5 years versus 19 years, p value .0738) and had higher peak concentrations (164 versus 119 μg/mL, p value .0257) and a longer half-life (221 versus 146 h, p value .0398) than patients enrolled in Part B. When the data were summarized by age, small differences were observed in the peak concentration; half-life was longest in the adolescents, as compared with children and young adults (305 versus 175 versus 154 h, respectively, p value .1387). Serum clearance was similar among all age groups and between males and females (0.64 and 0.56 mL/h/kg, respectively). With repeated administration, the mean trough concentration increased from 34.5 μg/mL after the first dose to 74.3 μg/mL after the third dose (Figure 1C). All patients had >90% target saturation with mean values ≥95% (Table 5).

3.5 | Antidrug antibodies

Out of 81 total samples, only one positive was detected via ELISA. This one confirmed positive had a negative titer, signifying a possible false positive or very low-level ADAs.

TABLE 3 All toxicities summary (maximum grade).

Toxicity type	Dose level and toxicity grade, no. (%)	
	All dose level ($N = 26$)	
	All	>=Grade 3
Infusion-related reaction	10 (40)	0 (0)
Nausea	8 (32)	0 (0)
Anemia	7 (28)	1 (4)
Fever	7 (28)	0 (0)
Aspartate aminotransferase increased	6 (24)	0 (0)
Fatigue	6 (24)	0 (0)
Hypophosphatemia	6 (24)	0 (0)
Lymphocyte count decreased	6 (24)	1 (4)
White blood cell decreased	6 (24)	0 (0)
Chills	5 (20)	0 (0)
Neutrophil count decreased	5 (20)	1 (4)
Sinus tachycardia	5 (20)	0 (0)
Alanine aminotransferase increased	4 (16)	0 (0)
Alkaline phosphatase increased	4 (16)	0 (0)
Anorexia	4 (16)	0 (0)
Hypokalemia	4 (16)	0 (0)
Headache	3 (12)	0 (0)
Hypoalbuminemia	3 (12)	0 (0)
Hypotension	3 (12)	0 (0)
Platelet count decreased	3 (12)	0 (0)
Arthralgia	2 (8)	1 (4)
Blood bicarbonate decreased	2 (8)	0 (0)
Flushing	2 (8)	0 (0)
Hyperglycemia	2 (8)	0 (0)
Hypertension	2 (8)	0 (0)
Hypocalcemia	2 (8)	0 (0)
Hyponatremia	2 (8)	0 (0)
Investigations—other, bicarbonate low	2 (8)	0 (0)
Myalgia	2 (8)	1 (4)
Vomiting	2 (8)	0 (0)
Acute kidney injury	1 (4)	1 (4)
Blood bilirubin increased	1 (4)	0 (0)
Body odor	1 (4)	0 (0)
Confusion	1 (4)	0 (0)
Cough	1 (4)	0 (0)
Creatinine increased	1 (4)	1 (4)
Diarrhea	1 (4)	0 (0)
Dyspepsia	1 (4)	0 (0)
Eosinophilia	1 (4)	0 (0)
Hyperhidrosis	1 (4)	0 (0)
Hyperkalemia	1 (4)	0 (0)
Hypermagnesemia	1 (4)	0 (0)
Hyperphosphatemia	1 (4)	0 (0)
Investigations—other, hyochloremia	1 (4)	0 (0)
Musculoskeletal and connective tissue disorder—other, muscle aches/pain	1 (4)	0 (0)
Pain in extremity	1 (4)	0 (0)
Pericardial effusion	1 (4)	1 (4)
Peripheral sensory neuropathy	1 (4)	0 (0)
Proteinuria	1 (4)	0 (0)
Pruritus	1 (4)	0 (0)
Rash acneiform	1 (4)	0 (0)
Rash maculo-papular	1 (4)	0 (0)
Sinus bradycardia	1 (4)	0 (0)
Thromboembolic event	1 (4)	0 (0)
Weight loss	1 (4)	0 (0)

3.6 | Total soluble SEMA4D

All patients with submitted samples had detectable levels of SEMA4D in their serum prior to dosing. Levels of total sSEMA4D increased after the second dose, consistent with the expected increase in the half-life of the pepinemab-bound sSEMA4D complex. This pharmacodynamic effect is consistent with other trials involving pepinemab and demonstrates the in vivo binding of pepinemab to the soluble target (data not shown).

3.7 | Tumor expression of SEMA4D

Due to limitations with archival tumor tissue, including a paucity of samples with intact malignant cells, we were unable to determine the expression of SEMA4D or plexin B in tumor samples.

4 | DISCUSSION

To our knowledge, this is the first clinical trial evaluating the role of SEMA4D inhibition in pediatric patients with cancer and specifically osteosarcoma. We achieved our primary goal of demonstrating that inhibition of SEMA4D by pepinemab is safe in children, adolescents, and young adults and results in adequate and sustained target saturation by pepinemab.

The C_{max} (148 μg/mL) and $AUC_{0-168\,h}$ (14,506 μg/mL h) after the first dose in our study were lower than the C_{max} (288 μg/mL) and $AUC_{0-168\,h}$ (26,023 mg/mL h) after the first 20 mg/kg dose of

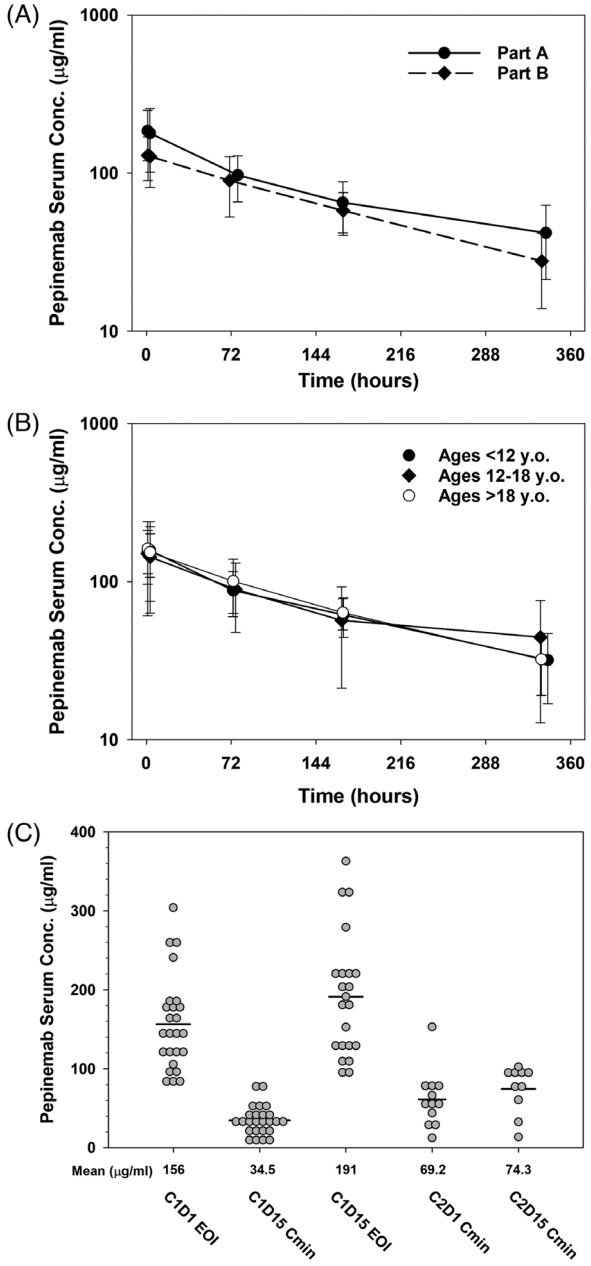

FIGURE 1 Graphs of pepinemab serum concentration versus time data. (A) Mean s (+SD) serum profile for patients treated in Part A and Part B. (B) Mean (+SD) serum profile for patients 18 years C) Peak and trough serum concentration post treatment in Cycle 1 and Cycle 2.

pepinemab in the phase I study for adults with advanced cancer.[12] The C_{max} and $AUC_{0-\infty}$ (23,449 mg/mL h) were also lower than the values reported for a single-dose phase I trial (274 μg/mL and 89,606 mg/mL h, respectively) for adults with multiple sclerosis.[16] These data are consistent with a higher clearance in our study (0.59 mL/h) compared with that reported for the phase I trial in adults with multiple sclerosis (19.5 mL/h). This is of little therapeutic significance given all patients achieved sustained target saturation.

Only two patients achieved our definition of disease control and no patients achieved an objective RECIST response. One possible

TABLE 4 summary of pepinemab pharmacokinetics.

		n	Age (years)	C_{max} (µg/mL)	Half-life (h)	V_z (mL/kg)	CL_{serum} (mL/h/kg)	$AUC_{0-168\,h}$ (µg/mL h)	$AUC_{0-336\,h}$ (µg/mL h)
All participants		25	16 (10, 20)	148 (117, 180)	181 (125, 256)	161 (139, 177)	0.59 (0.5, 0.77)	14,506 (11,164, 18,314)	23,449 (16,538, 27,878)
Study part	Part A	12	12.5 (9.5, 17.5)	164 (143, 250)	221 (175, 301)	149 (135, 177)	0.53 (0.38, 0.58)	17,254 (13,656, 22,903)	25,465 (22,185, 36,497)
	Part B	13	19 (11, 22)	119 (104, 175)	146 (113, 202)	161 (141, 177)	0.77 (0.59, 0.83)	12,372 (10,357, 16,560)	20,829 (16,081, 26,404)
	WRS exact *p* value	.	0.0738	0.0257	0.0398	0.7283	0.0298	0.0768	0.1225
Age group	<12 years	10	9.5 (6, 11)	143 (117, 166)	175 (139, 213)	163 (136, 187)	0.58 (0.54, 0.83)	13,656 (11,802, 18,314)	22,929 (16,538, 26,690)
	12–18 years	5	16 (14, 17)	124 (107, 159)	305 (273, 416)	154 (144, 304)	0.52 (0.27, 0.77)	10,583 (10,238, 22,568)	15,406 (14,667, 37,873)
	>18 years	10	22 (20, 23)	177 (119, 186)	154 (118, 229)	149 (139, 166)	0.6 (0.5, 0.77)	16,220 (12,836, 18,113)	25,968 (21,961, 27,878)
	KW exact *p* value	.	<0.0001	0.6488	0.1387	0.6365	0.6998	0.7994	0.7537
Sex	Female	11	16 (11, 22)	145 (107, 239)	169 (139, 266)	158 (136, 165)	0.56 (0.41, 0.77)	13,515 (10,357, 23,238)	25,890 (19,597, 33,744)
	Male	14	15.5 (7, 20)	154 (117, 180)	192 (113, 229)	163 (139, 187)	0.64 (0.5, 0.83)	15,193 (11,164, 18,113)	22,929 (16,081, 26,404)
	WRS exact *p* value	.	0.3229	0.9786	0.5719	0.6867	0.373	0.8508	0.5357

Data are summarized by medians with 25th and 75th percentiles. PK parameters are compared between study parts and sexes using Wilcoxon rank sum test. The Kruskal–Wallis test compares PK parameters between age groups.

TABLE 5 Percent SEMA4D saturation.

Time	Percent saturation
Cycle 1 Day 1 pretreatment	2.6 ± 5.3 (*N* = 21)
Cycle 1 Day 1 end of Infusion	97.7 ± 3.1 (*N* = 21)
Cycle 1 Day 15 pretreatment	97.8 ± 3.2 (*N* = 18)
Cycle 1 Day 15 end of Infusion	97.6 ± 2.5 (*N* = 15)
Cycle 1 Day 28 pretreatment	95.5 ± 2.9 (*N* = 15)

explanation for this is that this is a heavily pretreated patient population who likely have a degree of ongoing immunosuppression that may dampen their response to pepinemab. Although unlikely, it is also possible that target saturation is not the correct choice for a relevant biologic endpoint and true inhibition of SEMA4D was not achieved. Optimization of the biomarker and biologic endpoint, as well as evaluation of pepinemab in a less heavily pretreated patient population, may result in enhanced activity of this agent in osteosarcoma.

A previous retrospective analysis of outcomes from seven phase II clinical trials for patients with recurrent or refractory osteosarcoma in which agents were not considered efficacious according to traditional response criteria was executed with the goal of establishing baseline EFS outcomes to be used as a comparison for future phase II trials such as ours. For patients entering these clinical trials with measurable disease, the benchmark EFS of >4 months was determined to constitute disease control success. This is now the standard endpoint used in COG clinical trials for patients with relapsed/refractory osteosarcoma. With this, our patient that entered the study with measurable disease, completed all 13 cycles and continued pepinemab through a compassionate use mechanism for two additional years is considered an exceptional responder. This patient remained in a CR at the time of discontinuation of pepinemab.

Our study demonstrates the potential to saturate T-cells via SEMA4D blockade, thus inferring the potential to enhance an immune-mediated antitumor effect. Given this, the increased understanding of the role of SEMA4D in the modulation of the immune TME and with having an exceptional responder on our single agent trial, together with results from preclinical and clinical studies demonstrating that pepinemab combination therapies improve responses, provides rationale for further studies combining pepinemab with other agents that impact the TME and/or the tumor immune response, such as histone deacetylase inhibitors or checkpoint inhibitors.[15,23–25] Although single-agent pepinemab did not have a significant impact on the disease course for osteosarcoma, combining it with such agents has the potential for improved antitumor response and warrants further investigation.

We have demonstrated that pepinemab is well tolerated in children and adolescents at the adult RP2D of 20 mg/kg every 14 days. This dosing leads to sustained target saturation and may contribute to disease control in osteosarcoma. Further studies are warranted to optimize the potential of SEMA4D inhibition in osteosarcoma and other pediatric cancers.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

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