

Phase I Trial and Pharmacokinetic Study of Lexatumumab in Pediatric Patients With Solid Tumors

Melinda S. Merchant, James I. Geller, Kristin Baird, Alexander J. Chou, Susana Galli, Ava Charles, Martha Amaoko, Eunice H. Rhee, Anita Price, Leonard H. Wexler, Paul A. Meyers, Brigitte C. Widemann, Maria Tsokos, and Crystal L. Mackall

Melinda S. Merchant, Kristin Baird, Susana Galli, Brigitte C. Widemann, Maria Tsokos, and Crystal L. Mackall, National Cancer Institute; Martha Amaoko and Eunice H. Rhee, National Institutes of Health, Bethesda, MD; Alexander J. Chou, Ava Charles, Anita Price, Leonard H. Wexler, and Paul A. Meyers, Memorial Sloan-Kettering Cancer Center, New York, NY; and James I. Geller, Cincinnati Children's Hospital, Cincinnati, OH.

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Corresponding author: Melinda S. Merchant, MD, PhD, 10 Center Dr, Building 10 CRC, Room 1W-3750, Bethesda, MD 20892-1104; e-mail: merchanm@mail.nih.gov.

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ABSTRACT

Purpose

Lexatumumab is an agonistic, fully human monoclonal antibody against tumor necrosis factor-related apoptosis-inducing ligand receptor 2 with preclinical evidence of activity in pediatric solid tumors.

Patients and Methods

This phase I dose-escalation study examined the safety, tolerability, pharmacokinetics, and immunogenicity of lexatumumab at doses up to, but not exceeding, the adult maximum-tolerated dose (3, 5, 8, and 10 mg/kg), administered once every 2 weeks to patients age ≤ 21 years with recurrent or progressive solid tumors.

Results

Twenty-four patients received a total of 56 cycles of lexatumumab over all four planned dose levels. One patient had grade 2 pericarditis consistent with radiation recall, and one patient developed grade 3 pneumonia with hypoxia during the second cycle. Five patients experienced stable disease for three to 24 cycles. No patients experienced complete or partial response, but several showed evidence of antitumor activity, including one patient with recurrent progressive osteosarcoma who experienced resolution of clinical symptoms and positron emission tomography activity, ongoing more than 1 year off therapy. One patient with hepatoblastoma showed a dramatic biomarker response.

Conclusion

Pediatric patients tolerate 10 mg/kg of lexatumumab administered once every 14 days, the maximum-tolerated dose identified in adults. The drug seems to mediate some clinical activity in pediatric solid tumors and may work with radiation to enhance antitumor effects.

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INTRODUCTION

Tumor necrosis factor (TNF) and TNF-related ligands are cell-associated and -secreted molecules that can induce death in a wide variety of cancer cells. The clinical development of TNF α and Fas agonists, both members of this family, has been hampered by systemic toxicity. TNF-related apoptosis-inducing ligand (TRAIL, Apo2L) is produced by natural killer cells and has been shown to play a role in immunosurveillance.¹ Binding of TRAIL to TRAIL receptor TRAIL-R1 or TRAIL-R2 activates the extrinsic apoptosis pathway. TRAIL-R1 and TRAIL-R2 have been identified in multiple adult and pediatric tumors but have limited expression in normal cells, making them attractive targets for anticancer therapy.²⁻⁸

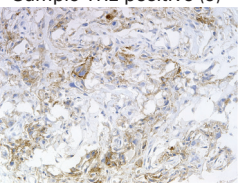
Lexatumumab (HGS-ETR2) is a recombinant, fully human IgG λ monoclonal antibody. Two pre-

vious phase I trials revealed that lexatumumab was well tolerated in adult patients when administered once every 14 or 21 days.^{9,10} Dose-limiting toxicities (DLTs) at 20 mg/kg once every 21 days included transaminitis and asymptomatic elevation of amylase or bilirubin. At the maximum-tolerated dose (MTD) of 10 mg/kg, lexatumumab was well tolerated, and several patients received prolonged therapy, with five patients receiving more than three cycles.^{9,10}

Several pediatric solid tumors have been shown to undergo apoptosis after activation of the extrinsic death pathway by TRAIL receptor agonists.¹¹⁻¹⁴ The extent of apoptosis observed in pediatric tumors such as Ewing sarcoma, rhabdomyosarcoma, osteosarcoma, and neuroblastoma is extensive after TRAIL-R2 agonist binding and, in many cases, results in 100% death of pediatric sarcoma cell lines in vitro.^{3,11,14,15} Preclinical testing has also revealed

	TR1	TR2	Caspase 8	No. of Cycles/Months
Hepatoblastoma	0	0 (rare 2+ cells)	9	1.5
ESFT	0	0	9	1
ESFT (lung met)	0	4	6	5
Osteosarcoma	0	9	9	24
Osteosarcoma	2	9	0	3
Osteosarcoma	0	0 (rare 3+ cells)	3	1
Osteo (lung met)	0	0	6	1
Osteo (lung met)	0	1	9	1
Synovial sarcoma	0	Not evaluable	n.d.	4
Wilms	0	4	6	1

Sample TR2 positive (9)



Sample TR2 negative (0)

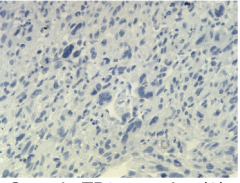


Fig 1. Immunohistochemical analysis of archived tissues. Scores were calculated based on intensity (0, 1, 2, or 3) multiplied by the grade corresponding to the percent of positive cells (grade 0 [0% to 1%], grade 1 [2% to 10%], grade 2 [11% to 50%], grade 3 [51% to 100%]). Maximum score of 9 when 3+ in > 50% of cells, as shown in top example. ESFT, Ewing sarcoma family of tumors; met, metastasis; n.d., not done; osteo, osteosarcoma; TR1, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor 1; TR2, TRAIL receptor 2.

delays in tumor growth after TRAIL-R2 agonist treatment in xenograft models of Ewing sarcoma and osteosarcoma.^{11,16} Although carcinomas frequently display inhibition of the type II or mitochondrial-dependent pathway of death receptor apoptosis,¹⁷ pediatric tumors such as Ewing sarcoma, rhabdomyosarcoma, and osteosarcoma more frequently have intact type I and type II pathways, leading to efficient apoptosis after death ligand binding.^{4,13,18} Such intact apoptosis pathways suggest that TRAIL mimetics such as lexatutumumab may be potent in the clinical treatment of pediatric tumors.

PATIENTS AND METHODS

Patients

The study population consisted of patients age 2 to 21 years with recurrent or progressive solid tumors after standard therapy. Patients must have completed their last dose of irradiation, chemotherapy, or investigational therapy at least 4 weeks before enrollment. Other inclusion criteria included Karnofsky or Lansky score > 50, hemoglobin concentration > 8 g/dL, absolute granulocyte count > 1,000/ μ L, platelet count > 75,000/ μ L, AST and ALT \leq 2.5-fold the upper limit of normal, and bilirubin and serum creatinine within normal limits. Because of hepatotoxicity at > MTD in the first adult phase I study, patients with hepatic metastases were excluded. Patients with primary CNS malignancies or active brain metastases were excluded from the trial because of unknown penetration into the CNS.

Study Design and Treatment

VEG10003 was a multicenter, open-label, nonrandomized phase I study to test the safety and pharmacokinetics of lexatutumumab up to the MTD in the adult studies (NCT00428272). Lexatutumumab (HGS-ETR2; Human Genome Sciences, Rockville, MD) was supplied as a sterile, single-use, lyophilized product stored in the dark at 2°C to 8°C. On reconstitution with 5.0 mL water for injection, lexatutumumab was diluted in normal saline for intravenous administration over 60 minutes. Patients were premedicated with acetaminophen and diphenhydramine. Lexatutumumab was administered once every 14 days. Cycles were 28 days long.

DLTs

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 3). Nonhematologic DLT was defined as any grade 3 or 4 toxicity occurring during cycle one or the failure of any toxicity to recover to grade \leq 1 in 21 days after dosing of lexatutumumab. Because it was anticipated that many patients enrolling onto this trial would have limited marrow reserve, hematologic DLT was defined as grade 4 neutropenia or thrombocytopenia persisting for at least 5 days.

Pharmacokinetics

This trial studied similar doses^{9,10} and schedule¹⁰ and used the same assay method as adult trials.^{9,10} Blood samples (5 mL) were collected from a site other than the infusion line for determination of serum lexatutumumab concentrations on days 1 and 15. Day 1 samples were collected before dosing and at end of infusion, 5 minutes postinfusion, and 6, 24, 48, 96, and 168 hours postdose. On day 15, samples were collected before dosing, at end of infusion, 5 minutes postinfusion, and 6 hours postinfusion completion, and trough samples were collected before subsequent cycles. Blood was processed to obtain serum, and aliquots were frozen at -80°C until assayed. Samples were analyzed for lexatutumumab concentration and immunogenicity by qualified enzyme-linked immunosorbent assays, as previously described.⁹ Lexatutumumab concentration-time data were analyzed using noncompartmental methods. Peak lexatutumumab concentration (C_{max}) and time to peak concentration were determined from each patient's concentration-time plot. Area under the concentration-time curve (AUC) to last measured time point after dose one was calculated with the linear trapezoidal method and extrapolated to

Table 1. Demographics and Clinical Characteristics of Patients Enrolled Onto Trial (N = 24)

Characteristic	No.	%
Age, years		
Median	16	
Range	2-21	
Mean \pm SD	14.5 \pm 5	
Primary tumor type		
Ewing sarcoma	4	17
Osteosarcoma	9	28
Rhabdomyosarcoma	3	13
Synovial sarcoma	2	8
Alveolar soft part sarcoma	1	4
Undifferentiated sarcoma	2	8
Hepatoblastoma	1	4
Nephroblastoma	1	4
SETTLE	1	4
Sex		
Male	15	62
Female	9	38
Lansky/Karnofsky score	60-90	
No. of prior chemotherapy regimens	2-6	

Abbreviations: SETTLE, spindle epithelial tumor with thymus-like differentiation; SD, standard deviation.

Table 2. Pharmacokinetic Parameters for Lexatumumab by Dose Level

Dose Level (mg/kg)	No.	C _{max} (μg/mL)		AUC _{0-24h} (μg × h/mL)		AUC _{0-∞} (μg × h/mL)		Percent Extrapolated		t _{1/2} (days)		CL (mL/d/kg)		Vd _{ss} (mL/kg)		C _{min} (μg/mL)	
		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)		Coefficient of Variation (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3	3	46	37	4,695	64	7,063	87	21	99	6.8	70	20	92	93.7	3.3	6	108
5	3	99	21	13,757	8	21,835	7	37	15	9.9	13	17	116	79.7	12	24	12
8	6	155	26	19,872	24	28,969	22	32	20	8.5	16	6.9	20	74.4	48	31	23
10	9	193	29	26,499	27	37,886	27	30	31	7.9	37	6.8	29	72.5	34	42	19

Abbreviations: AUC, area under the serum concentration-time curve; C_{max}, peak serum concentration; C_{min}, trough serum concentration before second dose; CL, clearance; t_{1/2}, half-life; Vd_{ss}, volume of distribution at steady state.

infinity (AUC_{0-∞}) by adding the last serum concentration after dose one divided by the rate constant, which was derived from the slope of the natural log-transformed concentrations and times on the terminal elimination phase of the concentration-time curve. Half-life was calculated by dividing 0.693 by the terminal rate constant. Clearance (CL) was determined from dose/AUC_{0-∞}. Volume of distribution at steady-state (Vd_{ss}) and mean resident time were calculated from the area under the moment curve. The relationship between CL and age was evaluated by linear regression. Accumulation of lexatumumab was assessed by dividing the trough concentrations after the second and subsequent doses by the trough concentration after the first dose.

Evaluation of Clinical Activity

Baseline imaging was assessed within 14 days of the first lexatumumab dose, after the first two doses (4 weeks), and then every 8 weeks until withdrawal from the study. Baseline imaging included computed tomography (CT) and/or magnetic resonance imaging of target lesions in addition to positron emission tomography (PET)/CT or bone scan when appropriate for the disease. Restaging was planned after the first cycle and then every other cycle after that. Response was evaluated according to the RECIST guidelines.

Immunohistochemistry

Biopsies were not required for study entry, but an attempt was made to receive blocks or unstained slides from previous biopsies or surgeries. Slides were stained for TRAIL-R1, TRAIL-R2, and caspase 8. Priority was given to TR2 staining if fewer slides were available than needed for all stains. Immunohistochemistry (IHC) for TRAIL-R2 was performed as previously described.⁹ Of note, the TRAIL-R2 reagent was selected for greater sensitivity in an IHC assay than the human lexatumumab, but both bind to the extracellular region of TRAIL-R2. IHC for caspase 8 and survivin used mouse monoclonal anti-caspase 8 (clone 1 to 1-37; Upstate; Millipore, Billerica, MA) and rabbit polyclonal antisurvivin (Alpha Diagnostic International, San Antonio, TX). Primary antibodies were applied for 1 hour at room temperature at a concentration of 1:100 for both antibodies, using antibody diluent with background-reducing components (Dako, Carpinteria, CA). Secondary antibodies were performed using EnVision+ System-HRP (DAB) antimouse and antirabbit (Dako) for 30 minutes at room temperature. The peroxidase reaction was developed with 3,3'-diaminobenzidine chromogen solution (Dako) for 5 minutes. Negative controls excluded the primary antibody. Only cytoplasmic immunoreactivity was considered positive for caspase 8 and survivin expression.

Stained specimens were reviewed by two observers and scored for each target antigen according to a standardized method in which both membrane and cytoplasmic staining were captured according to intensity (grades 0, 1, 2, 3) and percentage of tumor cells positive: grade 0 (0% to 1%), grade 1 (2% to 10%), grade 2 (11% to 50%), and grade 3 (51% to 100%). Final score is calculated as described in the legend of Figure 1.

RESULTS

Patient Characteristics

A total of 24 patients were enrolled onto this multicenter trial, and all patients were eligible and evaluable for toxicity. Patient ages ranged from 2 to 21 years, with a median age of 16 years (Table 1). The majority of patients had relapsed pediatric sarcomas, and three patients had a diagnosis of hepatoblastoma, nephroblastoma, and spindle epithelial tumor with thymus-like differentiation, respectively. Three patients were enrolled in each of the first two dose levels. Because of a DLT in a single patient at the 8-mg/kg dose level, six patients were treated at the third dose level, and an expanded cohort of 12 patients was enrolled at the highest dose level. The highest dose tested was the adult MTD of 10 mg/kg. No further escalation was planned based on toxicities identified at 20 mg/kg in adult studies. Patients in the study received a range of one to 24 cycles of lexatumumab treatment.

Toxicity

One patient with synovial sarcoma who received 8 mg/kg of lexatumumab experienced a DLT with hypoxia and pleural effusion associated with a change in pleural-based tumor, probably related to drug administration. No other DLT was observed in patients on this protocol. All 12 patients in the expanded final dose level tolerated 10 mg/kg of lexatumumab without any grade 3 or 4 toxicities. Common non–dose-limiting reactions were: grade 1 to 2 fatigue, grade 1 to 2 pain at tumor site, grade 1 hypokalemia, and grade 1 transaminitis. One patient had grade 2 radiation recall pericarditis, thought to be related to lexatumumab. Two patients had propagation of venous thrombi, judged possibly related to drug administration.

Pharmacokinetics and Immunogenicity

Pharmacokinetic parameters are summarized in Table 2. There was substantial variability in the pharmacokinetic parameters at the first dose level and a disproportionate increase in drug exposure from

the 3- (n = 3) to 5-mg/kg (n = 3) dose level. Less pharmacokinetic variability was observed at the higher dose levels, and a dose-proportionate increase in C_{max} and drug exposure from the 5- to 8- and 10-mg/kg dose levels. The concentration-time profile captured drug exposure incompletely (Appendix Fig A1, online only); approximately 30% of the $AUC_{0-\infty}$ was extrapolated, and the average terminal half-life was 8.3 days. No relationship between age and CL normalized to body weight or body surface area was observed. Drug exposure ($AUC_{0-\infty}$, 30,907 $\mu g \times h/mL$) of the only patient who experienced a DLT at 8 mg/kg was similar to that of other patients treated at the same dose level. The mean trough concentrations for eight patients at the 10-mg/kg dose after the first and second doses were 31.2 and 41.5 $\mu g/mL$, respectively, exceeding levels required for apoptosis in Ewing sarcoma cells in vitro after $1 \times$ dosing and incubation for 72 hours.² The mean accumulation index in patients who had samples obtained for this analysis after the first dose was 1.8 at 3 (n = 2), 1.5 at 5 (n = 2), 1.6 at 8 (n = 3), and 1.5 at 10 mg/kg (n = 8). The mean

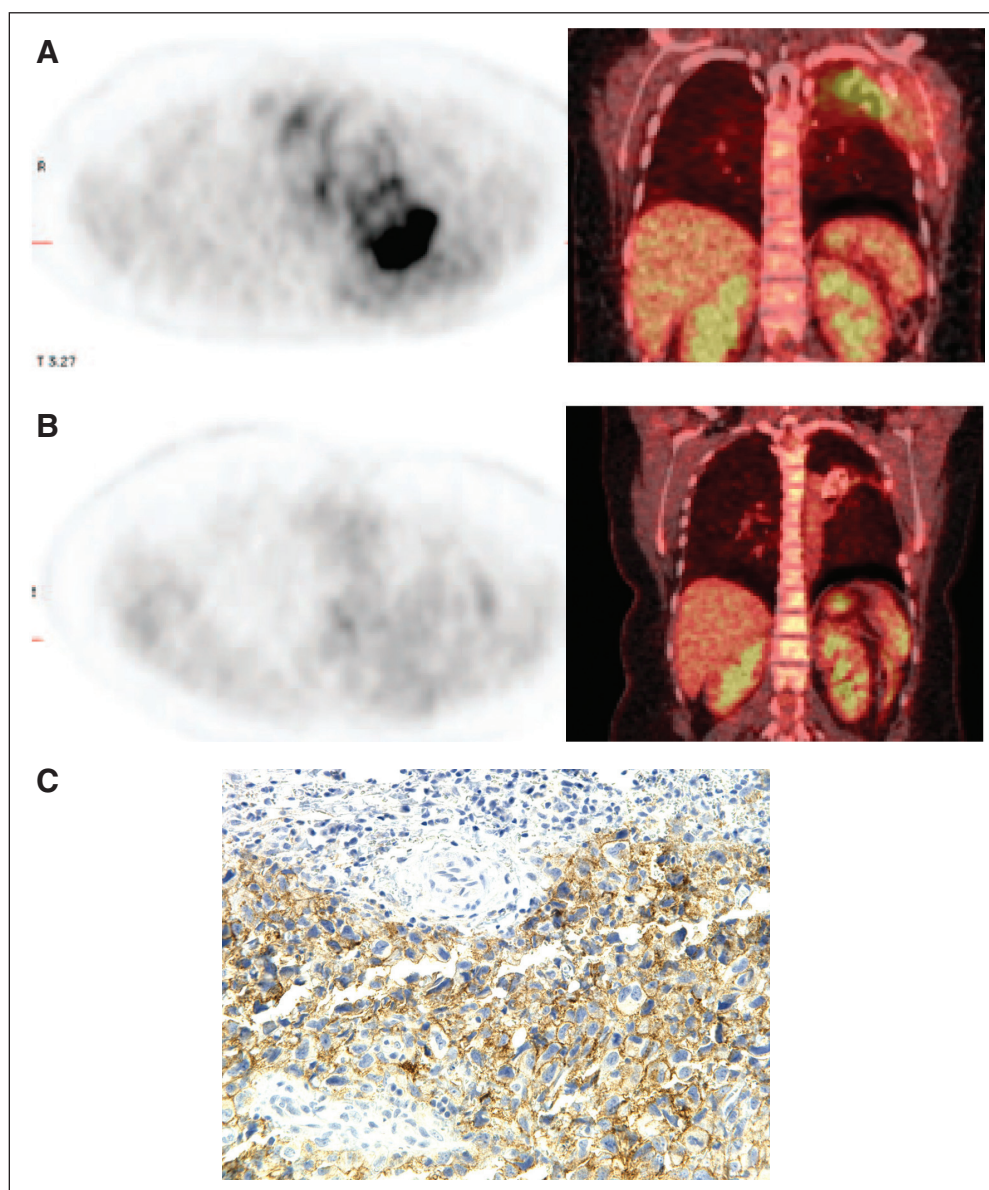


Fig 2. Prolonged clinical benefit after administration of lexatumumab 10 mg/kg in a 16-year-old patient with osteosarcoma of the chest wall. (A) Baseline positron emission tomography (PET)/computed tomography (CT) in patient with cough, shortness of breath, dyspnea on exertion, and chest wall pain. (B) PET/CT 6 months off therapy after 24 months of therapy, with no respiratory complaints or pain. (C) TR2 staining on archived osteosarcoma tissue. TR2, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) receptor 2.

accumulation index after the second dose for eight patients was 2.0 (range, 1.9 to 2.3).

IHC

Fourteen patients had sufficient tissue available to test for TRAIL-R1 and TRAIL-R2, whereas only nine tumors were available to test for caspase 8 expression. Biopsy material was obtained from initial diagnosis in 12 patients and from lung metastases in three patients. TRAIL-R2 was highly expressed in seven samples, whereas five had rare positive cells or < 10% positive, and two tumors had no staining at all (Fig 1). Of the nine tissues stained for caspase 8, seven tissues had high expression, one had low expression, and one had no expression. There was no correlation found between amount of TRAIL-R2 expression and cycles of therapy. However, lack of TRAIL-R2 was associated with shorter time to progression in the clinical trial (Appendix Table A1, online only). TRAIL-R1 was not expressed on any of the malignant tissues except for rare positive TRAIL-R1 cells in two osteosarcomas.

Clinical Antitumor Activity

There were no complete or partial responses by standard RECIST criteria. However, one patient with osteosarcoma enrolled at the 10-mg/kg dose level derived clinical benefit, with resolution of chest wall pain, cough, and shortness of breath interfering with activities of daily living at the time of enrollment. This patient was enrolled onto the study 4 weeks after irradiation, when baseline scanning revealed progressive, metabolically active chest wall and pulmonary disease. She received a total of 24 months of lexatumumab therapy and continues to be asymptomatic, with stable PET-negative imaging more than 1 year off therapy. CT scans showed ossification of the mass in her left chest, and PET scan activity returned to background levels during treatment (Fig 2). One patient age 2 years with hepatoblastoma had dramatic biomarker decrease after a single dose of lexatumumab (Fig 3) but developed seizures revealing CNS metastases within 2 months of starting therapy and was removed from study for CNS radiotherapy. A total of four patients had confirmed stable disease during the trial, and one patient had an unconfirmed stable disease evaluation.

One patient with Ewing sarcoma received radiation therapy 1 month before lexatumumab treatment without clearance of his pericardiac mass. He developed radiation recall pericarditis after lexatumumab administration, which was controlled with a short course of steroids. The bulky disease within the radiation field showed complete regression (Fig 4). Over the course of the next 5 months while receiving lexatumumab, he experienced progression of target lesions outside of the radiation field but no recurrence within the field.

DISCUSSION

Lexatumumab was well tolerated at all dose levels tested, including the adult MTD of 10 mg/kg. This is despite the fact that pediatric patients were more heavily pretreated than patients enrolled onto previous adult studies with this agent. Only one DLT possibly related to drug effect was observed. There were no grade 4 toxicities, and drug-related toxicities \leq grade 3 were readily managed. No cumulative toxicities were observed in the patient who received 2 years of lexatumumab.

The pharmacokinetic profile of lexatumumab in children is similar to that of other monoclonal antibodies, with a small volume of distribution (mean $V_{d_{ss}}$ at MTD, 72.5 mL/kg) and slow elimination (mean CL at MTD, 6.8 mL/d/kg). Variability in the pharmacokinetic

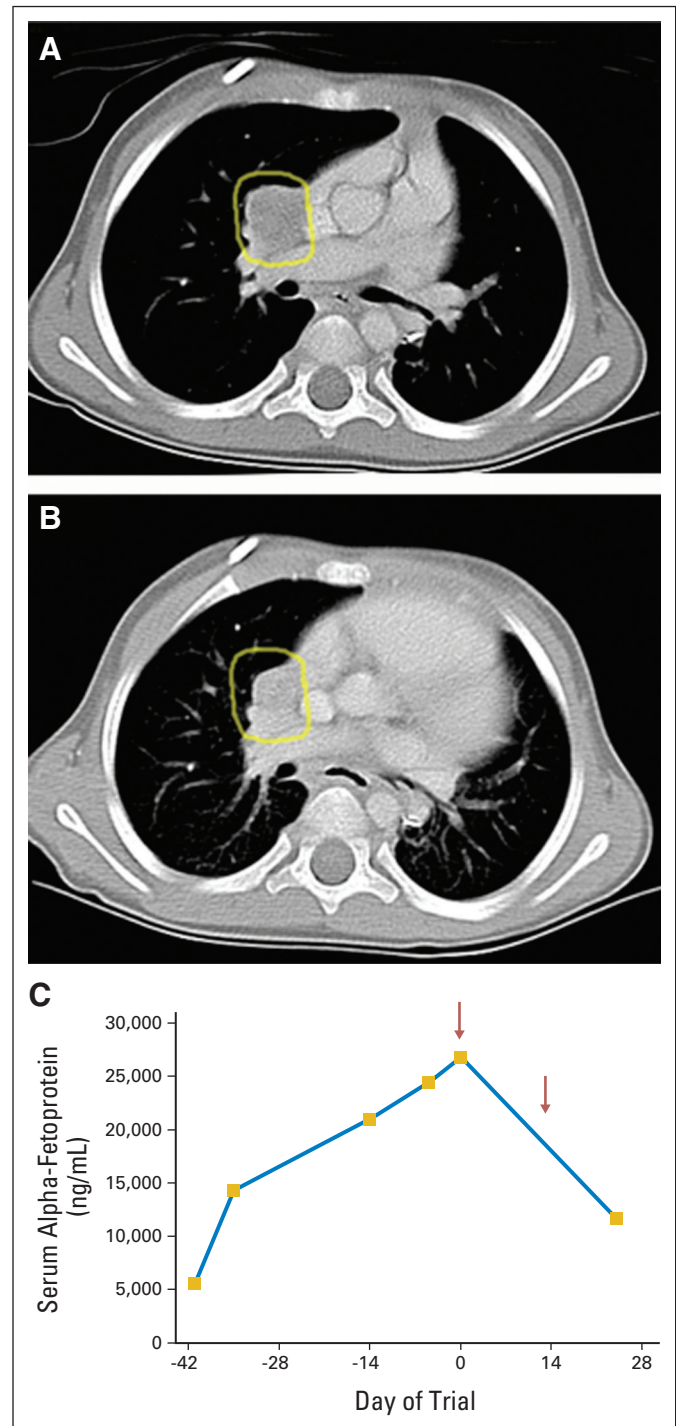


Fig 3. Clinical activity with biomarker response in first cycle in a 2-year-old patient with hepatoblastoma. (A) Computed tomography with metastasis outlined in yellow. (B) Follow-up imaging after two doses, with 24% reduction in longest diameter and 54% reduction in volume by three-dimensional volumetric assessment. (C) Alpha fetoprotein decline over first cycle (arrows indicate lexatumumab administration).

parameters and small sample size ($n = 3$) limit conclusions for the lowest dose level (3 mg/kg). A dose-proportionate increase in drug exposure was observed for the 5-, 8-, and 10-mg/kg dose levels, as described for adult phase I trials of lexatutumumab. Average trough concentrations at the 10-mg/kg dose level after the first and second dose exceeded the 10- to 100-ng/mL concentrations required in vitro for apoptosis of pediatric sarcoma cell lines as well as adult carcinoma lines.^{2,15} A comparison of the pharmacokinetics from the six patients age < 12 years with those of older adolescents in the trial revealed no age dependence in CL. The accumulation indexes of 1.5 after the first dose and 2.0 after the second dose of lexatutumumab at the MTD justifies the dosing schedule of once every 14 days and are consistent with the adult phase I trial using a schedule of once every 14 days. CL, V_{dss} , and C_{max} in children in this study were similar to those of adults treated with lexatutumumab on schedules of once every 21 days and once every 14 days.^{9,10} Mean $AUC_{0-\infty}$ at 10 mg/kg was lower in children ($37,886 \pm 10,179 \mu\text{g} \times \text{h/mL}$ standard deviation [SD]) compared with adults treated with the same dose on schedules of once every 14 days ($56,448 \pm 24,816 \mu\text{g} \times \text{h/mL}$ SD) and once every 21 days ($50,400 \pm 25,488 \mu\text{g} \times \text{h/mL}$ SD), respectively, but within one

SD of the adult drug exposure. The calculation of the terminal half-life in our study was limited by the duration of the sampling interval to 168 hours after the first dose. This may have contributed to the shorter mean half-life observed in children in this trial, which was shorter (7.3 days) compared with those in adult studies (13.7¹⁰ and 16.4 days⁹).

Although it was not the primary aim of this phase I trial, clinical activity was observed in our study. The most striking activity was in a teenager with progressive, unresectable chest wall/lung osteosarcoma who received 2 years of lexatutumumab with resolution of symptoms, ossification of her lesion, and loss of PET activity and has remained symptom free with stable imaging for more than 1 year after cessation of therapy. Furthermore, we saw clear evidence for a biomarker response in hepatoblastoma, but this was associated with progressive disease in the CNS. Previous confirmed partial responses in chondrosarcomas and prolonged stable disease in several sarcomas in adult trials suggest that solid tumors of mesenchymal origin may be responsive to TRAIL-targeting agents and should be targeted in future clinical trials of such agents.^{9,20,21}

With the caveat that few biopsies in this study were from relapsed disease, and receptor expression may evolve during the evolution of

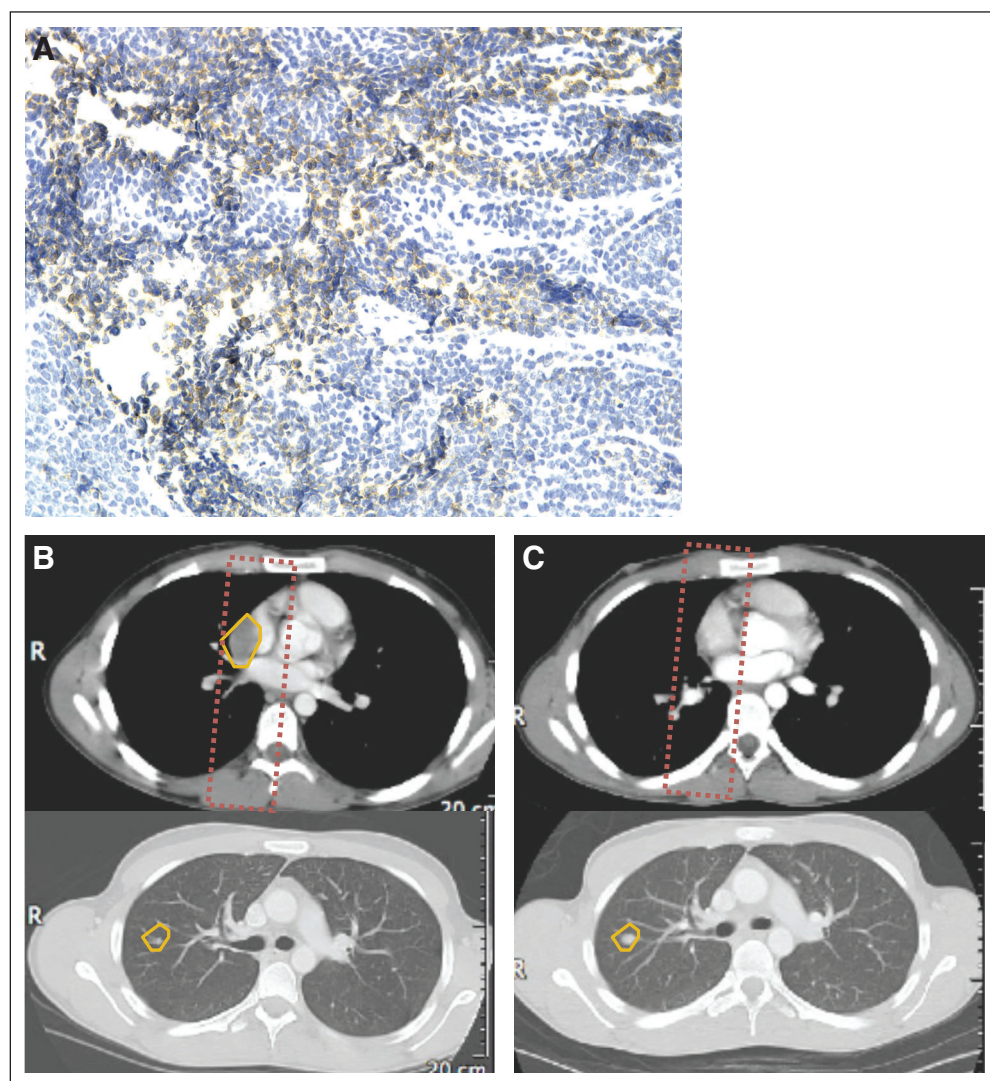


Fig 4. Regression of nontarget lesion in a recently irradiated field, with progressive disease outside the radiation field. (A) TR2 staining on archival tissue from patient age 16 years metastatic recurrent Ewing sarcoma; (B) lung metastasis (outlined in yellow) and prior field of radiation (red dashed line); (C) disappearance of lung metastasis within prior radiation field after three cycles of lexatutumumab but concurrent growth of tumor outside radiation field (lower panel).

cancer in an individual patient, TRAIL-R2 expression seemed to correlate with prolonged stable disease during therapy. Only one patient with rare TR2-positive staining in the tumor received more than three cycles, and all patients with tumors lacking TRAIL-R2 had rapid progression. TRAIL-mediated apoptosis in Ewing sarcoma and rhabdomyosarcoma cells has been shown to be dependent on caspase 8.^{2,22} In our study, caspase 8 also seemed to correlate with clinical activity. Agents that increase TRAIL-R2 and/or caspase 8 expression may improve the efficacy of TRAIL mimetics.

Preclinical studies have revealed that irradiation upregulates TRAIL-R2 on tumor cells without increasing the sensitivity of normal cells to TRAIL receptor agonists.^{6,23-25} There was some suggestion in our clinical trial that irradiation before or after TRAIL receptor agonist therapy may increase antitumor activity. Our findings support the future evaluation of combination trials with TRAIL agonists plus radiation therapy.

In conclusion, this study demonstrates that lexatumumab is well tolerated in heavily pretreated pediatric patients with solid tumors. TRAIL-R2 and caspase 8 are often expressed in pediatric solid tumors, whereas TRAIL-R1 is rarely expressed. Induction of apoptosis by agonists to TRAIL-R2 represents a novel targeting of tumors with intact death receptor pathways. A therapeutic window seems to be attainable, without significant off-target effects. On the basis of preclinical data and activity identified in this trial, the development of lexatumumab or other TRAIL-R2 agonists in combination with

irradiation should be considered. Further study at both the bench and the bedside will be required to determine if concurrent treatment with irradiation and TRAIL-R2 is tolerable or if sequential treatment is best.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Melinda S. Merchant, Eunice H. Rhee, Crystal L. Mackall

Provision of study materials or patients: Melinda S. Merchant, James I. Geller, Kristin Baird, Leonard H. Wexler, Paul A. Meyers, Brigitte C. Widemann, Crystal L. Mackall

Collection and assembly of data: Melinda S. Merchant, James I. Geller, Kristin Baird, Alexander J. Chou, Ava Charles, Anita Price, Leonard H. Wexler, Paul A. Meyers, Brigitte C. Widemann, Maria Tsokos, Crystal L. Mackall

Data analysis and interpretation: Melinda S. Merchant, James I. Geller, Kristin Baird, Susana Galli, Martha Amaoko, Leonard H. Wexler, Paul A. Meyers, Brigitte C. Widemann, Maria Tsokos, Crystal L. Mackall

Manuscript writing: All authors

Final approval of manuscript: All authors

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