Phase 1 and Pharmacokinetic Study of Lexatumumab in Patients with Advanced Cancers

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Abstract

Purpose: To assess the safety and tolerability, pharmacokinetics, and early evidence of antitumor activity of escalating doses of lexatumumab (HGS-ETR2), a fully human agonistic monoclonal antibody which targets and activates the tumor necrosis factor–related apoptosis-inducing ligand receptor 2 (TRAIL-R2) in patients with advanced solid malignancies.

Experimental Design: In this phase 1, open label study, patients with advanced solid malignancies were treated with escalating doses of lexatumumab administered i.v. over 30 to 120 min every 21 days. A cohort of four patients, which could be expanded to six patients, was studied at each dose level. The dose-limiting toxicity (DLT) dose was defined as the dose at which the incidence of DLT in the first two cycles was ≥33%. The maximum tolerated dose was defined as the highest dose at which <33% of subjects experienced DLT. The pharmacokinetics and immunogenicity of lexatumumab were also characterized. Tumor specimens from historical or current biopsies, when available, were stained for TRAIL-R2 using immunohistochemistry techniques.

Results: Thirty-seven patients received 120 cycles of lexatumumab at doses ranging from 0.1 to 20 mg/kg every 21 days as of May 2006. The 20 mg/kg dose was identified as the DLT dose based on DLTs in three of seven patients treated with this dose; DLTs included asymptomatic elevations of serum amylase, transaminases, and bilirubin. The 10 mg/kg dose cohort was expanded to 12 patients and the 10 mg/kg dose was identified as the maximum tolerated dose. The mean (±SD) clearance and apparent terminal half-life values at the 10 mg/kg dose averaged 6.0 (2.9) mL/d/kg and 16.4 (10.9) days, respectively. Twelve patients had durable stable disease that lasted a median of 4.5 months, including three patients with sarcoma having prolonged stable disease (≥6.7 months). Immunohistochemistry for TRAIL-R2 showed specific staining in >10% of tumor cells for 16 of the 20 evaluable specimens submitted (80%).

Conclusions: Lexatumumab was safe and well tolerated at doses up to and including 10 mg/kg every 21 days. Lexatumumab was associated with sustained stable disease in several patients. Pharmacokinetics were linear over the dose range studied, and consistent with a two-compartment model with first-order elimination from the central compartment. Additional evaluation of this novel apoptosis-inducing agent, particularly in combination with chemotherapy agents, is warranted and ongoing.

The ability to directly induce apoptosis in cancer cells is a novel approach to cancer treatment that has only recently begun to be evaluated in clinical studies. Several targeted agents, such as bortezomib and oblimersen sodium, indirectly induce apoptosis in tumor cells by altering the production or expression of apoptotic and antiapoptotic proteins. One emerging area of research is the evaluation of agents which activate the tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) death receptors (TRAIL-R1 and TRAIL-R2), members of the TNF receptor superfamily that, when activated, directly induce programmed cell death in cancer cells.

Lexatumumab (HGS-ETR2) is a fully human high-affinity agonist monoclonal antibody that specifically targets and activates TRAIL-R2 (DR5; ref. 1). Lexatumumab mediates the induction of apoptosis through the activation of the extrinsic apoptosis pathway. The binding of lexatumumab to TRAIL-R2 results in the formation of a death-inducing signaling complex with the adaptor protein FAS-associated death domain. This complex activates caspases 8 or 10, which then activate caspases 3, 6, and 7, leading to the degradation of key cellular signaling and structural components, resulting in programmed cell death (1–3).

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TRAIL-R2 protein is widely expressed by a variety of human tumors, including colon, lung, pancreas, breast, and ovarian carcinomas. TRAIL-R2 is expressed less widely in normal tissues, with staining reported on hepatocytes, myocytes, glial tissue, and bronchial and alveolar epithelium. Tumor cells also seem to be more susceptible than normal cells to the induction of apoptosis via TRAIL-R2 activation (4–8). Preclinical evidence suggests that the presence of TRAIL-R2 is necessary for the activation of apoptosis by lexatumumab, however, the level of receptor expression may not correlate with response, and receptor levels may be up-regulated or down-regulated in response to various stimuli, including chemotherapeutics (9).

Lexatumumab has shown single agent activity in a range of human tumor cell lines and xenograft models, including colorectal, renal, lung, breast, and ovarian tumor cell lines, and in primary tumor cells of hematologic and renal origin (10–13). Furthermore, there is evidence of synergistic or additive activity when lexatumumab is combined with chemotherapeutic regimens, including docetaxel, paclitaxel, and cisplatin (14, 15).

This phase 1 study, the first clinical study of a TRAIL-R2–specific agonist, was undertaken to evaluate the safety and tolerability of escalating doses of lexatumumab in patients with advanced solid malignancies, to evaluate the pharmacokinetics of lexatumumab, and to assess tumor response to lexatumumab. The preliminary results of this study have previously been presented in abstract form (16, 17).

Patients and Methods

Patient selection. Patients gave written informed consent for all clinical and research aspects of the study, which was done according to national and institutional guidelines. The protocol was reviewed by central and institutional review boards.

Patients with relapsed or refractory histologically or cytologically confirmed advanced solid malignancies who had failed standard therapies, for whom no curative therapeutic option existed, or had refused all other therapies, were eligible. Eligible patients had measurable or evaluable disease, adequate hematologic function (platelet count $\geq 100 \times 10^9$/L, hemoglobin $\geq$9.0 g/dL and absolute neutrophil count $\geq 1.5 \times 10^9$/L), adequate hepatic and renal function [aspartate transaminase, alanine transaminases, and alkaline phosphatase $\leq$2.5-fold the upper limit of normal (if alkaline phosphatase was elevated due to bone metastases, $\leq$5-fold the upper limit of normal was acceptable), bilirubin within normal limits, serum creatinine $\leq$1.5-fold the upper limit of normal and APTT $\leq$1.5-fold the upper limit of normal], performance status of 0 to 2 on the Eastern Cooperative Oncology Group Scale, expected survival of at least 3 months, and were $\geq$18 years of age. Exclusion criteria included: chemotherapy, cancer hormonotherapy, immunotherapy, radiotherapy or major surgery within 4 weeks; prior use of any nitrourea or mitomycin-C within 6 weeks; prior use of an investigational agent within 4 weeks; known central nervous system metastases; $\geq$grade 2 neuropathy; previous hematopoietic stem cell transplant; myocardial infarction, cerebrovascular accident or congestive heart failure within 6 months; history of any infection requiring hospitalization or parenteral antibiotics within 2 weeks; coexisting medical illness that would place the subject at undue risk; immunosuppressant therapy; known HIV or hepatitis B or C infection; and pregnancy or nursing mother.

Treatment scheme. The dose levels of lexatumumab evaluated were 0.1, 0.3, 1.0, 3.0, 10.0, and 20.0 mg/kg. Lexatumumab was administered i.v. over 30 min at the 0.1 and 0.3 mg/kg dose and over 2 h at the higher doses. Treatment cycles were repeated every 21 days. Premedication to prevent hypersensitivity reactions was permitted.

Doses were escalated according to a modified three plus three rule. Dose-limiting toxicity (DLT) was defined by the following criteria that were considered possibly, probably, or definitely related to the study agent that occurred during the first two cycles (42 days) of treatment: grade 4 ANC lasting for >5 days, grade 3 febrile neutropenia and/or infection with neutropenia, platelet count $<25 \times 10^9$/L, grade 3 or greater nonhematologic adverse events except alopecia, nausea/vomiting, diarrhea, rash, arthralgia, myalgia, or fatigue unless these symptoms had been treated with an optimal therapeutic regimen, or grade 2 or greater allergic reaction. In summary, lexatumumab was to be administered to four patients at the starting dose. If DLT did not occur in the first four patients, then four patients were enrolled into the next dose level. If, at any dose level, DLT did occur, the cohort was expanded to a total of six patients. If DLT occurred in only one of six patients at a specific dose level, a new cohort could be opened at the next dose. If two or more patients experienced DLT, the next lower dose was expanded to 12 patients. The maximum tolerated dose was defined as the dose level below which <33% of patients experienced DLT.

Lexatumumab (100 mg/vial) was supplied in 10 mL single-use vials by Human Genome Sciences, Inc. Each vial was reconstituted with 5.0 mL of sterile water for injection, with each vial then containing 20 mg/mL of lexatumumab in 1.8% glycine, 1.0% sucrose, 10 mmol/L sodium citrate, and 0.02% (w/v) polysorbate 80 (pH 6.5).

Assessments. Adverse events were graded according to the National Cancer Institutes-Common Terminology Criteria for Adverse Events (version 3.0, April 16, 2003). Treatment-related toxicities were those toxicities considered by the investigators to be possibly, probably, or definitely related to lexatumumab. A physical examination, concurrent medication profile, assessment of performance status, and routine laboratory studies were done prior to treatment and at least weekly after treatment. Routine laboratory studies included a complete blood count, differential WBC count, international normalized ratio, serum electrolytes, aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyl transferase, lactate dehydrogenase, serum creatinine, amylase, total bilirubin, and urinalysis. A medical history, HIV and hepatitis B and C serologic studies, and pregnancy tests were done prior to enrollment. Pretreatment studies also included an electrocardiogram, chest X-ray, and radiologic studies for evaluation of all measurable and evaluable sites of disease. The appropriate tumor markers were assessed. Radiologic studies for disease status were repeated after every two cycles. Patients could continue receiving lexatumumab if they did not develop progressive disease or experience intolerable toxicity. Response was assessed using the Response Evaluation Criteria in Solid Tumors or the Prostate-Specific Working Group Response Guidelines (18, 19).

Pharmacokinetic studies. Venous blood samples (7 mL, no anticoagulant) were collected from patients prior to dosing in cycles 1, 2, 3, and 4; at completion of dosing, as well as at 5 min, 8 h, 7 days, and 14 days post-dose in cycles 1, 2, and 4; at 24 h post-dose in cycles 1 and 4; at 2 and 4 days post-dose in cycle 1; and at 21 and 42 days after the patient's last dose. Blood was also collected prior to dosing in all treatment cycles following the fourth cycle. Blood specimens were collected from a site contralateral to the drug administration site. Blood was processed to obtain serum, and aliquots of serum were pipetted into tubes and stored frozen at -70°C until assayed.

Serum samples were analyzed for lexatumumab by a qualified ELISA that employed TRAIL-R2:FLAG for lexatumumab capture and a horseradish peroxidase–conjugated antihuman IgG antibody for lexatumumab detection. Horseradish peroxidase activity was measured by the colorimetric conversion of the horseradish peroxidase substrate 3,3′,5,5′-tetramethylbenzidine in the presence of hydrogen peroxide and the reaction was stopped with dilute acid. The relative amount of color conversion was measured at A_{450}-A_{570} nm and the concentration of lexatumumab in human serum was interpolated from a standard

Table 1. Patient demographics

Characteristic	No. of patients
Age (y), median (range)	58 (22-76)
Gender, male/female	22/15
Eastern Cooperative Oncology Group performance status 0/1/2	6/24/7
Tumor types (%)	
Soft tissue sarcoma	12 (32)
Osteogenic sarcoma	7 (19)
Colorectal	4 (11)
Liver	2 (5)
Melanoma	2 (5)
Bladder/urothelial	2 (5)
Others (prostate, breast, head and neck, esophageal, thymoma, cholangio, endometrial, skin)	8 (22)

curve. The lower limit of quantitation for lexatumumab was 51.2 ng/mL in neat serum. The coefficient of variation for positive control samples was 20%.

Pharmacokinetic variables were determined by compartmental analysis, using WinNonlin Enterprise (version 5.0.1; Pharsight Corp.). Serum lexatumumab concentration-time profiles for each patient were analyzed individually, using actual times of specimen collection and actual dosing times and amounts. Because concentration-time profiles were multiphasic, two- and three-compartment models with first-order elimination from the central compartment were evaluated. Weightings of 1, $1/p$, $1/p^{0.5}$, and $1/p^2$ (where p is the predicted value for the observation) were assessed for each model. For competing models with the same weighting scheme, the Akaike information criterion was used as the basis for model selection. For competing models with different weighting schemes, model selection was based on the precision of the primary model variables (RSE $\leq$ 100%), randomness of the residuals, and the sum of squared residuals. ANOVA was done to assess dose proportionality over the evaluated dose range.

Detection of human anti-lexatumumab antibodies. Immunogenicity was tested in a two-step procedure. Samples were first assessed in a screening titer assay based on direct binding ELISA on lexatumumab Fab-coated plates. Positive samples were further evaluated in a competitive inhibition of binding assay to confirm the specificity of binding. The direct binding ELISA's sensitivity was determined as a limit of detection of 0.6 μg/mL for anti-lexatumumab antibodies in the absence of any lexatumumab, and 1.0 μg/mL for anti-lexatumumab antibodies in the presence of 50 to 100 μg/mL of lexatumumab in the sample. The limit of detection increases to 2 μg/mL for anti-lexatumumab antibodies in the presence of 200 μg/mL of lexatumumab in the serum sample.

Immunohistochemical staining of tumor tissues. Immunohistochemical staining for TRAIL-R2 was conducted as an exploratory investigation in this phase 1 trial. Tumor tissue was requested from patients with tumors accessible for biopsy. Archival specimens of the tumor for which the patient was enrolled on study were requested if biopsy was not feasible. Tissue specimens were formalin-fixed, paraffin-embedded, and sectioned at 4 to 6 μm onto charged slides. Tissue sections were stained for TRAIL-R2 using prototype pharmDx staining reagents and a standardized method developed with Dako, Inc. Briefly, slides were deparaffinized and hydrated, followed by heat-induced epitope retrieval in a modified citrate buffer (pH 6.1; Dako). Following a 5-min incubation in a dual endogenous enzyme block (Dako), rabbit polyclonal antibody specific for the extracellular portion of TRAIL-R2, or nonspecific rabbit IgG at the same concentration, was applied and incubated for 30 min. Of note, the TRAIL-R2 reagent was selected for greater sensitivity in an immunohistochemistry assay than the fully human lexatumumab monoclonal antibody, but both bind to the extracellular region of TRAIL-R2. Following a series of wash steps in tris-buffered wash solution (pH 7.6; TBST, Dako), primary antibody binding signals were amplified using the horseradish peroxidase–conjugated EnVision polymer system (Dako). After this incubation, slides were washed again, and specific binding was detected by enzymatic conversion of a substrate chromogen (diaminobenzidine). Excess chromagen was rinsed off and hematoxylin was used as a counterstain prior to dehydration and mounting. Each staining run included the staining of cell pellet sections of positive and negative cell line controls for each target antigen. Stained specimens were reviewed by a single observer and scored for each target antigen according to a standardized method in which both membrane and cytoplasmic staining were captured according to intensity and distribution (percentage of tumor cells positive at each intensity level of 0-3+).

Data analysis and compilation. Regular teleconferences were held between the two investigating sites and the sponsoring company during the study to discuss patient safety and study status. All data listings were made available to the investigators for the preparation of this report. The patient demographics, treatment summaries, toxicity listings, and response data were extracted from this verified data set by the lead author, an investigator at one of the clinical sites. Immunohistochemical staining was done and this section of the report was drafted by the sponsor (W. Halpern). Pharmacokinetic analyses were done and the relevant sections drafted by the sponsor (A. Corey), all analyses and raw data were made available to the lead author and chief investigator for review. The first draft of the Introduction and Patients and Methods was written by the sponsor (N.L. Fox), the Results and Discussion were written by the investigators.

Results

General. Thirty-seven patients were enrolled into the study and received at least one dose of lexatumumab. The most common tumor types were soft tissue sarcoma (32%), osteogenic sarcoma (19%), and colorectal cancer (11%).

Table 2. Summary of dose levels explored and number of cycles administered

	Dose (mg/kg)					
	0.1	0.3	1	3	10	20
No. of patients treated	4	4	6	4	12	7
Total no. of cycles administered	7	16	26	14	43	14
Mean no. of cycles (range)	1.8 (1-2)	4.0 (2-10)	4.3 (1-13)*	3.5 (2-6)	4.3 (1-12) †	2.0 (1-4)
No. of patients requiring dose delay	0	0	0	2	2	1

NOTE: Number of cycles as of the database lock in May 2006.
*One subject in the 1 mg/kg cohort received 13 cycles before resection of his tumor. He subsequently received 11 additional cycles outside of the protocol.
† One subject in the 10 mg/kg cohort has continued on study and has received 28 cycles as of January 2007.

Table 3. Summary of the number of patients with the most frequent treatment-emergent adverse events (for ≥10% of subjects) by MedDRA preferred term and severity, regardless of relationship to lexatumumab (n = 37)

Preferred term	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)
Constipation	8 (21.6)	3 (8.1)	—
Nausea	9 (24.3)	1 (2.7)	—
Fatigue	3 (8.1)	5 (13.5)	1 (2.7)
Vomiting	6 (16.2)	2 (5.4)	—
Headache	5 (13.5)	2 (5.4)	—
Lethargy	5 (13.5)	1 (2.7)	1 (2.7)
Pyrexia	3 (8.1)	3 (8.1)	—
Shoulder pain	1 (2.7)	3 (8.1)	2 (5.4)
Arthralgia	2 (5.4)	3 (8.1)	—
Back pain	3 (8.1)	2 (5.4)	—
Diarrhea	4 (10.8)	1 (2.7)	—
Dizziness	5 (13.5)	—	—
Dyspepsia	4 (10.8)	1 (2.7)	—
Dyspnea	4 (10.8)	1 (2.7)	—
Myalgia	3 (8.1)	2 (5.4)	—
Edema peripheral	2 (5.4)	3 (8.1)	—
Pain in extremity	1 (2.7)	1 (2.7)	3 (8.1)
Urinary tract infection	1 (2.7)	3 (8.1)	1 (2.7)
Abdominal pain	2 (5.4)	2 (5.4)	—
Cough	2 (5.4)	2 (5.4)	—
Lower respiratory tract infection	—	2 (5.4)	2 (5.4)

Baseline characteristics are summarized in Table 1. The majority of patients had surgery for their disease (n = 35, 95%), 18 (49%) patients received prior radiotherapy and 32 (87%) patients had previous chemotherapy with a mean of 2.4 (range, 1-5) regimens. The total number of patients treated and the number of cycles at each dose level are summarized in Table 2.

Four patients were treated in each of the 0.1 and 0.3 mg/kg cohorts and experienced no DLT. One patient in the 1.0 mg/kg cohort experienced an asymptomatic grade 3 elevation of serum amylase detected at cycle 1 day 15. The cohort was expanded to six patients, with no additional DLTs observed at that level. No significant toxicity was observed in the 3.0 and 10.0 mg/kg cohorts, which included four patients each. DLTs were experienced by three of the seven patients enrolled in the 20.0 mg/kg dose, so enrollment was suspended in that cohort and the 10.0 mg/kg cohort was expanded to 12 subjects to further explore that dose.

One patient required a dose reduction from 20 to 10 mg/kg for grade 3 elevation of serum amylase levels, and subsequent treatment of this patient at 10 mg/kg was uncomplicated. Five patients required dose delays, none of these being for drug-related adverse events.

Toxicity. Lexatumumab was generally very well tolerated with few grade 3 or 4 adverse events. Hematologic toxicity was rare and mild in severity, with no neutropenia of any grade observed throughout the study. Two (5.4%) patients showed grade 1 thrombocytopenia and six (16.2%) patients experienced an isolated two-grade decrease in lymphocyte count (grade 2 to 4 in one patient only); in all cases, this was brief and uncomplicated. The most frequent treatment-emergent adverse events are summarized in Tables 3 and 4. The most common side effects were constipation, fatigue, and mild nausea with biochemical changes being the predominant laboratory toxicity.

There were four DLTs observed during the study. In two patients, an asymptomatic grade 3 elevation in serum amylase was observed during cycle 1 of treatment (day 15 at 1 mg/kg as above, and day 8 at 20 mg/kg). Both patients were taking ciprofloxacin, which may have contributed to these events. The

Table 4. Number of patients with ≥2-grade shifts from baseline National Cancer Institutes-Common Terminology Criteria for Adverse Events grade for liver and kidney function variables by dose

	0.1 mg/kg, n = 4 (%)	0.3 mg/kg, n = 4 (%)	1 mg/kg, n = 6 (%)	3 mg/kg, n = 4 (%)	10 mg/kg, n = 12 (%)	20 mg/kg, n = 7 (%)	All active, n = 37 (%)
Aspartate transaminase							
Any ≥2-grade shift	—	—	1 (16.7)	1 (25.0)	2 (16.7)	2 (28.6)	6 (16.2)
Alanine transaminase							
Any ≥2-grade shift	—	—	—	2 (50.0)	1 (8.3)	2 (28.6)	5 (13.5)
Alkaline phosphatase							
Any ≥2-grade shift	—	—	1 (16.7)	—	1 (8.3)	—	2 (5.4)
γ-Glutamyl-transferase							
Any ≥2-grade shift	—	—	1 (16.7)	—	—	1 (14.3)	2 (5.4)
Creatinine							
Any ≥2-grade shift	1 (25.0)	—	—	—	—	1 (14.3)	2 (5.4)
Total bilirubin							
Any ≥2-grade shift	—	—	1 (16.7)	—	—	1 (14.3)	2 (5.4)

Table 5. Pharmacokinetic data

Dose (mg/kg)	C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg·d/mL)	$t_{1/2,\alpha}$ (d)	$t_{1/2,\beta}$ (d)	MRT (d)	CL (mL/d/kg)	V_1 (mL/kg)	V_{ss} (mL/kg)
0.1 (n = 3)	1.8 (0.5)	15.2 (6.1)	1.5 (1.5)	18.2 (15.3)	19.0 (11.4)	7.6 (3.6)	60.1 (16.6)	127.5 (62.5)
0.3 (n = 3)	5.2 (2.1)	55.7 (25.7)	1.0 (1.0)	14.7 (2.8)	19.5 (2.0)	6.9 (4.5)	69.4 (39.3)	127.9 (70.2)
1 (n = 5)	24.7 (7.2)	184.4 (38.1)	1.1 (0.7)	11.2 (3.9)	14.5 (5.8)	5.7 (1.4)	42.5 (14.7)	78.1 (24.1)
3 (n = 3)	68.4 (50.9)	503.8 (193.1)	1.2 (0.9)	11.1 (3.2)	14.2 (3.1)	6.7 (2.8)	59.2 (31.9)	99.3 (54.7)
10 (n = 10)	192.6 (29.1)	2,100.7 (1,062.6)	1.7 (1.0)	16.4 (10.9)	20.6 (13.9)	6.0 (2.9)	52.3 (10.0)	94.4 (16.5)
20 (n = 6)	480.0 (112.1)	3,375.5 (1,049.2)	1.1 (0.6)	11.2 (3.5)	13.4 (3.5)	6.4 (1.7)	42.1 (11.0)	83.2 (25.6)
P*	0.4038	0.9317	0.8635	0.8120	0.6935	0.9317	0.3369	0.3557

Abbreviations: C_{max}, maximum serum drug concentration following a single dose; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time zero to infinite time following a single dose; $t_{1/2,\alpha}$, elimination half-life for the initial phase; $t_{1/2,\beta}$, elimination half-life for the terminal phase; MRT, mean residence time; CL, clearance; V_1, volume of distribution for the central compartment; V_{ss}, volume of distribution at steady-state. state.

*From a one-way ANOVA of log-transformed data. C_{max} and $AUC_{0-\infty}$ were dose-normalized prior to analysis.

patient at 20 mg/kg was re-treated at 10 mg/kg for three further cycles with no subsequent changes in amylase levels. Two patients treated at 20 mg/kg developed grade 4 transaminitis (aspartate transaminase and alanine transaminase) during cycle 1, considered in both cases to be drug related. In one patient, this was asymptomatic and resolved by day 23 to baseline (grade 0). The second patient developed sepsis and acute renal failure, and subsequently, grade 3 hyperbilirubinemia and grade 4 transaminitis. This patient died of acute renal failure 25 days after receiving lexatumumab. The acute renal failure was considered to be possibly related to lexatumumab and the transaminitis and hyperbilirubinemia were considered to be probably related to lexatumumab. The 10 mg/kg dose level was, therefore, expanded to 12 patients, and no instances of DLT were observed at this dose level.

Pharmacokinetics. Pharmacokinetic variables for mean serum lexatumumab are summarized by dose group in Table 5, with P values from a one-way ANOVA to assess linearity. C_{max} and $AUC_{0-\infty}$ increased proportionally with dose. There were no significant differences in pharmacokinetics among dose groups, indicating that lexatumumab pharmacokinetics are linear over the dose range studied. At the maximum tolerated dose (10 mg/kg), the mean (SD) clearance and $t_{1/2,\beta}$ were 6.0 (2.9) mL/d/kg and 16.4 (10.9) days, respectively.

The mean V_1 ranged from values similar to the plasma volume up to ~62% greater than the plasma volume, whereas V_{ss} was at least 68% greater than V_1 for each cohort. These results suggest that although the distribution of lexatumumab might initially be restricted to a volume that is similar to or slightly greater than the plasma volume, it does subsequently distribute to tissues.

The disappearance of lexatumumab from serum is biphasic, with mean $t_{1/2,\alpha}$ of 1.0 to 1.7 days and mean $t_{1/2,\beta}$ of 11.1 to 18.2 days. Based on the average $t_{1/2,\beta}$ of 13.8 days, 90% of steady-state would be attained 46 days after the first dose, i.e., prior to the fourth dose on an every 21-day schedule, with a predicted accumulation factor at steady-state of ~1.53.

Mean lexatumumab clearance ranged from 5.7 to 7.6 mL/d/kg among the dose groups, values that are much smaller than the glomerular filtration rate. This indicates that, as expected, there is virtually no renal clearance of this monoclonal antibody. The pharmacokinetic profile of the patient who suffered a fatal adverse event of acute renal failure (described above) was consistent with other patients dosed at that level.

Antitumor activity. Although there were no patients on this phase 1 study who achieved a partial response, 12 patients (32%) had disease stabilization of a median duration of 4.5 months, with three patients continuing on study for >10 cycles of treatment. One patient with an inoperable extraosseous osteosarcoma and documented progressive disease on standard chemotherapy at study entry received 28 cycles of treatment at 10 mg/kg, and remains on lexatumumab without any evidence of cumulative toxicity. Two additional patients with retroperitoneal liposarcoma and chondrosarcoma, with documented progressive disease prior to lexatumumab, received 13 and 10 cycles, respectively. Both patients had prolonged disease stabilization and minimal side effects.

Immunogenicity. Analysis of repeated serum samples for the detection of human anti-lexatumumab antibodies was done in all patients. One sample obtained prior to the first dose of lexatumumab was noted to have a positive anti-lexatumumab antibody evaluation, but no other confirmed positive results were observed after treatment with lexatumumab. Therefore, this result is likely to be a false-positive.

Immunohistochemistry. Twenty of the 37 patients enrolled on this study provided tumor specimens that could be evaluated for TRAIL-R2–specific staining. Of these, one specimen was collected at baseline, and 19 archival specimens had been collected from 6 months to >5 years (mean, 31 months) prior to lexatumumab dose administration.

Of the 20 specimens, 16 had at least 10% of tumor cells demonstrating specific TRAIL-R2 staining. Each of the epithelial-origin tumors had specific TRAIL-R2 staining of both membrane and cytoplasmic compartments, and 7 of the 10 sarcomas had detectable TRAIL-R2. In contrast, 3 of the 10 sarcomas were completely negative for TRAIL-R2, and one sarcoma had <10% of tumor cells stained for TRAIL-R2. The TRAIL-R2 staining results are summarized in Table 6 according to tumor type.

Discussion

With increased understanding of the pathways triggering programmed cell death, there have been attempts to target both the extrinsic route and downstream proteins such as

Table 6. Immunohistochemical staining for TRAIL-R2

Tumor types	No. of specimens evaluated (%)	TRAIL-R2 staining in >50% tumor cells	TRAIL-R2 staining in 10% to 50% of tumor cells	TRAIL-R2 staining in <10% of tumor cells
Soft tissue sarcoma	7 (35)	1	2	4
Osteogenic sarcoma	3 (15)	2	1	0
Colorectal	3 (15)	1	2	0
Bladder/urothelial	2 (10)	0	2	0
Endometrial carcinoma*	1 (5)	1	0	0
Cholangiocellular carcinoma	1 (5)	1	0	0
Prostate adenocarcinoma	1 (5)	0	1	0
Esophageal adenocarcinoma	1 (5)	0	1	0
Thymoma	1 (5)	0	1	0
Total	20	6	10	4

*Histologic diagnosis for the specimen was a malignant mixed müllerian tumor (carcinosarcoma). However, only the carcinomatous component stained and was scored for this tumor.

bcl-2 and inhibitors of apoptosis proteins in cancer cells as potential mechanisms to increase the drive to apoptosis in the cell (20). Initial attempts to target the extrinsic pathway involved the use of its ligand TNF. However, clinical experience with systemic TNFα in the 1980s showed significant problems with toxicity, in terms of inflammatory response and hepatotoxicity, and this agent has not entered general anticancer therapeutic use.

Two of the receptors in this family, TRAIL-R1 (DR4) and TRAIL-R2 (DR5), contain an intact death domain which allows the receptors, when activated, to signal for apoptosis. There has been much interest over recent years in the possibility of targeting the TRAIL receptors as a means of directly activating the extrinsic pathway. These receptors are expressed in a range of tumor cell lines and immunohistochemistry has shown high levels of expression compared with normal tissue in lung, gastrointestinal, and breast cancer samples (21). In general, the intensity and distribution of TRAIL-R2 identified immunohistochemically in specimens provided in this study were consistent with historical data. The majority of specimens evaluated had variable staining both within and between tumors; only four specimens, all sarcomas, had <10% of tumor cell staining.

There was widespread TRAIL-R2 staining of the osteosarcoma specimen from the patient with durable stable disease up to 28 cycles of lexatumumab (Fig. 1). However, TRAIL-R2 staining was low or undetectable for archival liposarcoma and chondrosarcoma specimens from subjects that had stable disease for 13 and 10 cycles, respectively. A relationship between receptor staining and potential for beneficial effect of lexatumumab has not been established, and cannot be determined from this exploratory dose-escalation safety study. It has also been suggested that TRAIL-R2 expression may be an independent and negative prognostic factor in metastatic breast carcinoma (7). Patterns of tumor responses to lexatumumab and receptor expression should continue to be evaluated in future clinical studies, but it would be premature to use TRAIL-R2 expression for screening, especially with archival tissue specimens.

A high-affinity agonist antibody targeting TRAIL-R1, mapatumumab (HGS-ETR1), is also in clinical development. Mapatumumab has been studied in phase 1 trials (22, 23) and as a single-agent in phase 2 trials in non–Hodgkin

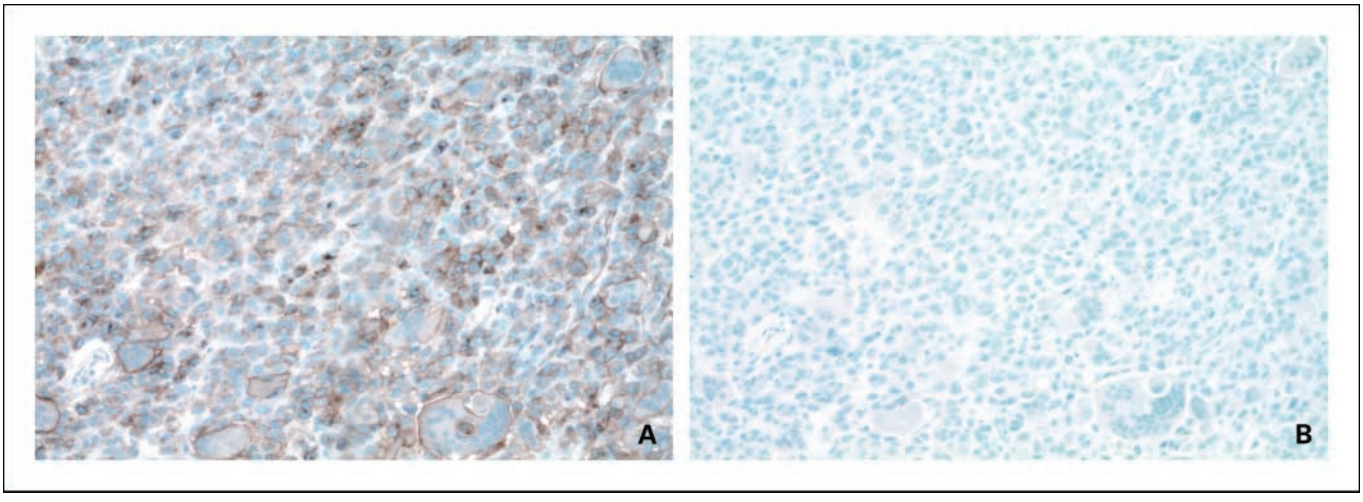

Figure 1. *A,* immunohistochemical staining for TRAIL-R2 in a formalin-fixed, paraffin-embedded extraosseous osteosarcoma specimen from a subject subsequently enrolled on this trial. There is distinct membrane and widespread diffuse cytoplasmic staining with the TRAIL-R2–specific rabbit polyclonal antibody reagent. *B,* there is a lack of staining when nonspecific rabbit IgG at the same concentration is used. Original magnification, ×10.

lymphoma (24), non–small cell lung cancer (25), and colorectal cancer (26). Mapatumumab was well-tolerated and showed single-agent activity in the non–Hodgkin lymphoma trial (24). It is currently being evaluated in combination with cytotoxic chemotherapy at doses as high as 30 mg/kg every 3 weeks (27, 28) and in a randomized phase 2 trial in combination with bortezomib in patients with relapsed/refractory multiple myeloma.

Recombinant human TRAIL, the ligand for this family of receptors which binds to both TRAIL-R1 and TRAIL-R2, as well as the nonsignaling TRAIL decoy receptors, recently entered phase 1 studies. Preliminary data reported thus far indicate that this agent has little toxicity at doses up to 15 mg/kg given daily for 5 days every 3 weeks. A confirmed partial response in a patient with chondrosarcoma was also reported (29). This case is especially noteworthy given the observation of prolonged stable disease in several patients with sarcoma in our study of lexatumumab.

The study reported in this article was designed to establish the recommended dose and toxicity of lexatumumab, which specifically targets and activates TRAIL-R2, in adult patients with advanced solid malignancies. The drug was well tolerated over multiple cycles, with the exception of DLTs of asymptomatic and reversible transaminase and amylase elevation at the 20 mg/kg dose. Accordingly, 10 mg/kg is the dose recommended to be taken forward into further studies. Pharmacokinetic analysis revealed the agent to have a prolonged clearance, with a mean plasma half-life of >2 weeks at the recommended dose, confirming that the intermittent dosing schedule adopted in this phase 1 study was appropriate. A second phase 1 study, evaluating lexatumumab given every 2 weeks, is in progress (30). Similar pharmacokinetic variables have been shown in this study. Interestingly, one DLT of grade 3 hyperamylasemia was observed at the 10 mg/kg dose in a patient who entered the study with grade 2 hyperamylasemia. The hyperamylasemia was considered most likely related to a nutritional supplement, but also possibly related to lexatumumab. In the current study, hyperamylasemia was seen in two patients taking concomitant ciprofloxacin. This possible drug interaction should be monitored in subsequent studies of lexatumumab.

Prolonged disease stabilization was observed in a number of patients, most notably in three patients with metastatic sarcoma, a disease which is largely resistant to systemic treatments. These observations support further trials targeting TRAIL-R2 as a potential therapeutic strategy, particularly in the treatment of sarcomas. Preclinical studies suggest that the TRAIL-targeting agents may be most effective in combination with chemotherapy. Lexatumumab and mapatumumab have shown additive and synergistic antitumor activity with taxane and platinum agents *in vitro* in ovarian cancer cell lines and in xenograft models of non–small cell lung cancer (14, 15, 31).

A phase 1b study evaluating the combination of lexatumumab with a range of cytotoxic chemotherapy regimens was initiated and is nearing completion. The additional phase 1 trial of lexatumumab as a single agent has completed enrollment. Results of these trials are anticipated.

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