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Preclinical Studies to Predict the Disposition of Apo2L/Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand in Humans: Characterization of in Vivo Efficacy, Pharmacokinetics, and Safety

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ABSTRACT

Apo2L/TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is a member of the tumor necrosis factor gene family known to induce apoptosis in a number of cancer cell lines and may have broad-spectrum activity against human malignancies. These studies have evaluated the potency of recombinant soluble human Apo2L/TRAIL in a mouse xenograft model and the disposition and safety of Apo2L/TRAIL in rodents and nonhuman primates. Mice with established COLO205 tumors were given daily i.v. injections of Apo2L/TRAIL (30–120 mg/kg/day). Control tumors doubled in size every 2 to 3 days, while time to tumor doubling in the treatment groups was significantly longer and related to dose (14–21 days). For pharmacokinetic studies, Apo2L/TRAIL was given as an i.v. bolus to mice (10 mg/kg), rats (10 mg/kg), cynomolgus monkeys (1, 5, and 50 mg/kg), and chimpanzees (1 and 5 mg/kg). Apo2L/TRAIL was rapidly eliminated from the serum of all species studied. Half-lives were ~3 to 5 min in rodents and ~23 to 31 min in nonhuman primates. Allometric scaling provided estimates of Apo2L/TRAIL kinetics in humans, suggesting that on a milligram per kilogram basis, doses significantly lower than those used in xenograft studies could be effective in humans. Apo2L/TRAIL clearance was highly correlated with glomerular filtration rate across species, indicating that the kidneys play a critical role in the elimination of this molecule. Safety evaluations in cynomolgus monkeys and chimpanzees revealed no abnormalities associated with Apo2L/TRAIL exposure. In conclusion, these studies have characterized the disposition of Apo2L/TRAIL in rodents and primates and provide information that will be used to predict the pharmacokinetics of Apo2L/TRAIL in humans.

Apo2 ligand, also called tumor necrosis factor (TNF)-related apoptosis-inducing ligand (Apo2L/TRAIL), is a member of the TNF gene superfamily. Native Apo2L/TRAIL is expressed as a type II transmembrane protein that can be cleaved proteolytically to form a soluble homotrimer (Wiley et al., 1995; Pitti et. al., 1996). Apo2L/TRAIL binds five receptors: death receptors (DR) 4 and 5; decoy receptors (DcR) 1 and 2; and osteoprotegerin (OPG) (Ashkenazi and Dixit, 1998, 1999). DR4 and DR5 signal apoptosis, whereas DcR1, DcR2, and OPG can act as decoys that inhibit Apo2L/TRAIL activity.

The therapeutic potential of a recombinant soluble version of human Apo2L/TRAIL that can be produced in *Escherichia coli* and purified as a stable, 60-kDa homotrimer is under evaluation (Ashkenazi et al., 1999; Hymowitz et al., 2000). This optimized preparation of Apo2L/TRAIL selectively induces apoptosis of cancer cells while sparing normal cells. The antitumor activity of Apo2L/TRAIL alone and in combination with chemotherapy after i.p. administration has been demonstrated in several mouse xenograft models of human cancers, including colorectal (Ashkenazi et al., 1999), glioma (Roth et al., 1999), and breast (Walczak et al., 1999). A toxicology study performed in cynomolgus monkeys showed that repeated administration of Apo2L/TRAIL (10 mg/kg/day for 7 days) was well tolerated. Human and cynomolgus monkey Apo2L/TRAIL are 98% homologous in the ectodomains, and the changes are conservative. The receptor ectodomain homology is 91% for DR4, 88% for DR5, 84% for DcR2, and 99% for OPG. Human Apo2L/TRAIL binds to cynomolgus monkey receptors with an affinity comparable to human receptors. Additionally, cynomolgus monkey cells are sensitive in vitro to other constructs of Apo2L/TRAIL (polyhistidine-

ABBREVIATIONS: TNF, tumor necrosis factor; Apo2L/TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DR, death receptor; DcR, decoy receptor; OPG, osteoprotegerin; ELISA, enzyme-linked immunosorbent assay; OD, optical density; AUC, area under the Apo2L/TRAIL serum concentration versus time curve; C_{max}, model-predicted maximum serum Apo2L/TRAIL concentration; Vss, estimated steady-state volume of distribution; GFR, glomular filtration; SBI, Sierra Biomedical, Inc; NIRC, New Iberia Research Center.

and cross-linked flag-tagged variants) but not to the native sequence, recombinant soluble ligand (Lawrence et al., 2001). These observations support the relevance of cynomolgus monkey for characterizing the safety profile of Apo2L/TRAIL, contrary to an earlier report suggesting that studies in nonhuman primates would not be applicable to human patients (Jo et al., 2000). Taken together, the safety and efficacy data generated thus far suggest that Apo2L/TRAIL may be a promising therapeutic candidate against human cancers.

In preparation for studying Apo2L/TRAIL in the clinic, we have evaluated the efficacy of i.v. administration of Apo2L/TRAIL against human colorectal cancer in a xenograft model. The disposition of Apo2L/TRAIL in both rodents and nonhuman primates was also investigated. We have looked for general signs of toxicity in nonhuman primates treated with our optimized preparation of Apo2L/TRAIL. Our results suggest that our optimized preparation of Apo2L/TRAIL is well tolerated by cynomolgus monkeys and chimpanzees.

Materials and Methods

Apo2L/TRAIL. The expression and purification of Apo2L/TRAIL has been described previously (Scholtissek and Grosse, 1988; Ashkenazi et al., 1999). Briefly, an extracellular sequence of native human Apo2L/TRAIL (amino acids 114–281) was subcloned and expressed in *E. coli* strain W3110 in 10- or 100-liter fermenters. Soluble Apo2L/TRAIL was extracted and precipitated by 40% ammonium sulfate and purified to >98% homogeneity by consecutive chromatographic separation steps on hydroxyapatite and Ni-nitrilotriacetic acid agarose columns. Purity was determined by SDS-polyacrylamide gel electrophoresis and silver nitrate or Coomassie blue staining, by amino acid sequence analysis, and by size-exclusion high-performance liquid chromatography. The recombinant protein was >98% homotrimeric, with a zinc content of 1 mol/mol of trimer.

Mouse Xenograft Study. Female nude mice ($n = 70$) were obtained from Charles River Labs (Wilmington, MA). Human colon cancer cells (COLO205; 2×10^6) in log phase were implanted s.c. in the flank of each mouse. Tumor growth was monitored daily, and tumor volume was calculated by the following equation: tumor volume (mm^3) = length $\times$ $width_1$ $\times$ $width_2$ $\times$ 0.5 (Corbert et al., 1997). After 5 days, animals with representative tumors ($n = 50$) were randomized by tumor size into five groups (tumor volume ~280 mm^3). For the next 5 days, mice were given daily i.v. bolus doses of Apo2L/TRAIL (20 mg/ml; 30, 60, 90, or 120 mg/kg/day), or control vehicle via a tail vein. Tumor growth was monitored for 21 to 24 days in the treatment groups.

Mouse Pharmacokinetics. Nude mice were selected because they are currently used for human xenograft studies. Female nude mice ($n = 20$; b.wt. = 26 ± 1.5 g) (Charles River Labs) were housed in micro-isolators throughout the study. Apo2L/TRAIL was administered (10 mg/kg; ~60 μl) as an i.v. bolus via the tail vein. Serial blood samples (~100 μl) were collected predose and between 5 min and 6 h postdose ($n = 4$ mice/time point) via the orbital sinus under Iso-fluorande anesthesia, or via cardiac puncture at sacrifice. Blood was allowed to clot at room temperature and the serum was harvested and stored at −70°C until analyzed by ELISA for total Apo2L/TRAIL concentrations.

Rat Pharmacokinetics. Micro-Renathane polyurethane cannulas (Braintree Scientific Inc., Braintree, MA) were inserted into the femoral (0.84 mm o.d. × 0.36 mm i.d.) and jugular (1.02 mm o.d. × 0.64 mm i.d.) veins of male Sprague-Dawley rats ($n = 4$; b.wt. = 266 ± 13 g) (Charles River Labs) 48 h prior to dosing. A single i.v. bolus dose (10 mg/kg) of Apo2L/TRAIL was given via the femoral vein. Serial blood samples (~200 μl) were taken predose and between 5 min and 6 h postdose from the jugular vein. Fluid volume was replaced with saline or heparinized saline when necessary as judged by the study monitor. Blood was processed to serum and stored at −70°C until analyzed for Apo2L/TRAIL concentration.

Cynomolgus Monkey Pharmacokinetics and Safety. This study was conducted at Sierra Biomedical, Inc. (SBI) (Sparks, NV). Treatment of animals was in accordance with regulations outlined in the USDA Animal Welfare Act and the conditions specified in *The Guide for Care and Use of Laboratory Animals* (National Institutes of Health, 1996).

Male ($n = 6$; b.wt. = 3.8 ± 0.88 kg) and female ($n = 6$; b.wt. = 3.0 ± 0.36 kg) cynomolgus monkeys (*Macaca fascicularis*) from the SBI colony were assigned to one of three treatment groups ($n = 4$/group) based on gender and body weight. The remaining animals ($n = 4$) were treated with vehicle control. Half of the animals in the Apo2L/TRAIL treatment group were naïve while remaining animals had been administered Apo2L/TRAIL 72 h earlier. Prior to receiving an i.v. bolus dose of Apo2L/TRAIL (1, 5, or 50 mg/kg), animals were not sedated but were temporarily restrained within their cages. Serial samples of whole blood (approximately 0.75 ml) were collected by venipuncture from an available peripheral vein into tubes without anticoagulant. Serum (approximately 0.5 ml) was harvested, placed on dry ice, and stored in a freezer maintained at −60 to −80°C until analyzed for Apo2L/TRAIL.

Animals were observed at least twice daily, beginning at least 5 days prior to the day of dosing and continuing through the duration of the study for signs of adverse events associated with Apo2L/TRAIL. Approximately 2 ml of blood was collected at predose, 24 h, 72 h, 1, and 2 weeks postdose for evaluation of clinical pathology. Serum was collected from approximately 1.5 ml of blood for evaluation of serum chemistry. The standard SBI panel (sodium, potassium, chloride, calcium, phosphorous, glucose, total carbon dioxide, total bilirubin, blood urea nitrogen, creatinine, total protein, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, albumin, globulin, albumin/globulin ratio, cholesterol, and triglyceride) was performed on a Beckman Synchron CX7 automated chemistry analyzer (Beckman Instruments, Palo Alto, CA). Hematological analysis was performed on 0.5 ml of whole blood collected in EDTA-containing tubes. The standard SBI hematology panel (red blood cell counts, white blood cell total and differential, hemoglobin concentration, hematocrit, mean cell hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet counts, and blood cell morphology) was performed on an Abbott Cell-Dyn 3500 multiparameter automated hematology analyzer (Abbott Labs, Pomezia, Italy).

Chimpanzee Pharmacokinetics and Safety. The chimpanzee study was conducted at New Iberia Research Center (NIRC) (New Iberia, LA). Male and female chimpanzees were anesthetized and given an i.v. bolus of 1 mg/kg ($n = 3$; b.wt. = 60 ± 8.7 kg) or 5 mg/kg ($n = 4$; b.wt. = 53 ± 8.9 kg) Apo2L/TRAIL. Blood samples (1 ml) were collected for pharmacokinetic analysis between 5 min and 24 h after dosing. The bioactivity of a select number of these samples was evaluated in a cell-based bioassay. Serum was also collected predose and 2 weeks postdose to screen for antibodies to Apo2L/TRAIL. Animals were observed for any abnormal clinical signs for 48 h after dose administration. Blood samples (1 ml) were taken predose and 14 days postdose for evaluation of serum chemistries, hematology, and antibodies to Apo2L/TRAIL. The NIRC standard serum chemistry panel (same as the SBI panel except for triglycerides and cholesterol) was performed on the Roche Molecular Biochemicals/Hitachi 747-100 (Roche Molecular Biochemicals, Summerville, NJ). The standard NIRC hematology panel (including complete blood count, differential, and platelet count) was performed on the Coulter STKS 28 hematology analyzer (Coulter Electronics, Luton, UK).

Apo2L/TRAIL ELISA. An anti-Apo2L/TRAIL monoclonal antibody (clone 2G9.5.7, produced at Genentech, South San Francisco, CA) was coated on ELISA plates (Immuno Plate with MaxiSorp surface, Nunc, Neptune, NJ) overnight at 4°C. After blocking, sample or recombinant Apo2L/TRAIL standard was added. Captured

Apo2L/TRAIL was detected with a biotinylated secondary monoclonal antibody (clone 5C2.8.16, produced at Genentech) followed by streptavidin-horseradish peroxidase (AMDEX, Amersham Pharmacia Biotech, Piscataway, NJ). Color was developed using tetramethyl benzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and the reaction was stopped with 1 M phosphoric acid. Apo2L/TRAIL concentrations in the samples were extrapolated from a four-parameter fit of the Apo2L/TRAIL standard curve. The detection limit of the assay varied slightly depending on the species of serum being evaluated and ranged from 0.1 to 0.65 ng/ml (1/100 minimum dilution). The limit of detection of the assay was set based on two criteria. First, the OD of the lowest point of the standard curve was required to be significantly above the background OD [(OD of standard − 2 S.D.) > (OD of background + 2 S.D.)]. Second, back-calculation of the Apo2L/TRAIL concentration of the lowest point using the four-parameter fit of the same standard curve was required to be within 20% of the actual value. The intra-assay and interassay coefficient of variation of high, mid, and low controls were < 20% (L. DeForge and A. Hebert, unpublished observations).

Apo2L/TRAIL Bioactivity Assays. SK-MES-1 human lung carcinoma cells (HTB-58) (American Type Culture Collection, Rockville, MD) were cultured in 10% fetal bovine serum RPMI medium. Two-fold serial dilutions of standard and sample were performed in 96-well tissue culture plates. SK-MES-1 cells (20,000 cells/well) were added into the 96-well plates and were incubated at 37°C for 24 h. AlamarBlue was added for the last 3 h of the 24-h incubation. Cell killing was determined by fluorescence readings.

Assay for Anti-Apo2L/TRAIL Antibodies. ELISA plates were coated with Apo2L/TRAIL, and serum samples were added to washed plates. After incubation, captured anti-Apo2L/TRAIL antibodies were detected using an horseradish peroxidase-conjugated goat anti-human IgG/IgM antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Color was developed using tetramethyl benzidine, and the reaction was stopped with phosphoric acid.

Pharmacokinetic Analysis. Pharmacokinetic analyses were performed using the WinNonlin Professional Edition computer software version 2.0 (Pharsight Co., Mountain View, CA). A number of models and weighting factors were used to minimize the sum of squares residual value between the observed and model predicted serum drug concentrations. Serum concentration versus time profiles were fit using either a one- or two-compartment model with bolus input and first-order output. Calculation of rate constants and secondary parameters including area under the Apo2L/TRAIL serum concentration versus time curve (AUC), model-predicted maximum serum Apo2L/TRAIL concentration (C_{max}), estimated steady-state volume of distribution (Vss), and half-life have been described previously (see Gibaldi and Perrier, 1982, for an overview).

Statistics. Group mean parameters for rats, cynomolgus monkeys, and chimpanzees were obtained by averaging parameter estimates from individual animals. The effect of Apo2L/TRAIL dose on pharmacokinetic parameters within the same primate species was evaluated using a one-way analysis of variance and Fisher's post hoc test (α = 0.05). Kinetic parameters for mice were calculated by modeling group mean data (see explanation below) and are therefore reported without measures of variance.

Results

Activity of Apo2L/TRAIL in a Mouse Xenograft Study

In an earlier study (Ashkenazi et al., 1999), i.p. administration of a recombinant version of the Apo2L/TRAIL ligand showed significant antitumor activity alone and in combination with chemotherapy. Data from the present study demonstrate that tumor suppression is also possible following i.v. administration. This evaluation is relevant because patterns of exposure will be different following i.v. and i.p. dosing and because Apo2L/TRAIL will probably be given intravenously in the clinic.

The antitumor activity of Apo2L/TRAIL is shown in Fig. 1 and Table 1. Control tumors grew steadily and doubled in size every 2 to 3 days. In contrast, all Apo2L/TRAIL treatment groups showed a marked reduction in tumor size, especially during the treatment period. In the lowest dose group (30 mg/kg/day), tumors returned to baseline levels after ~14 days but the return to baseline was slower (18–21 days) in the higher dose groups. The dose response observed here suggests that the maximal antitumor activity with this regimen is achieved between 60 and 90 mg/kg/day.

Characterization of Apo2L/TRAIL Pharmacokinetics in Rodents and Primates

Group assignment and Apo2L/TRAIL dose levels are given in Table 2. The resulting serum concentration versus time profiles are presented in Fig. 2, and the corresponding kinetic parameters are presented in Table 3.

Rodents. Because of limitations on blood sampling in mice, these data were pooled with each mouse contributing a portion of the total serum versus time profile. Serum concentration versus time data from rats were modeled individually. Apo2L/TRAIL kinetics, similar in mice and rats, were characterized using a one- and a two-compartment model that provided a good fit to the observed data. Results using a two-compartment model are presented. Although including a second compartment gave a better fit to the terminal portion of the curves, most of the data could be fit using a one-compartment model. In fact ~99% of the total AUC was captured in the interval between time-zero and 1 h postdose. Following dosing, Apo2L/TRAIL quickly distributed in a volume similar to serum, suggesting that Apo2L/TRAIL distribution was primarily within the vascular space. Apo2L/TRAIL disappearance from rodents was so rapid that Apo2L/TRAIL was undetectable by 3 h postdose in all animals except one mouse that had detectable serum levels 4 h postdose.

Cynomolgus Monkeys. The disposition of Apo2L/TRAIL in cynomolgus monkeys was studied over a range of doses (1–50 mg/kg). As mentioned earlier, half of the animals used were exposed to Apo2L/TRAIL 72 h prior to this study. The

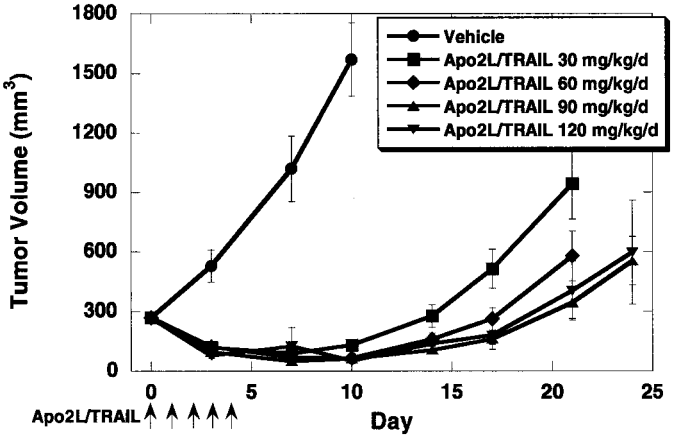

Fig. 1. Nude mice with established COLO205 xenografts were given Apo2L/TRAIL (30, 60, 90, or 120 mg/kg/day) or vehicle as an i.v. bolus for 5 consecutive days (n = 10/group). Results shown are group mean (±S.D.). After day 10 (vehicle) and day 21 (30 and 60 mg/kg/day), animals were euthanized due to large tumor size.

TABLE 1
Group average tumor volumes
Data are group average tumor volumes ± S.E. (n = 10 mice/group).

Dose	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 24
mg/kg/day								
0	270 ± 27	530 ± 82	1000 ± 160	1700 ± 190	N.A.	N.A.	N.A.	N.A.
30	260 ± 27	130 ± 20	90 ± 21	130 ± 23	280 ± 60	520 ± 98	940 ± 180	N.A.
60	270 ± 25	120 ± 9.1	70 ± 10	65 ± 11	160 ± 31	260 ± 56	580 ± 130	N.A.
90	270 ± 25	97 ± 9.0	48 ± 9.2	61 ± 9.0	110 ± 17	160 ± 26	340 ± 82	550 ± 120
120	270 ± 25	77 ± 14	130 ± 93	51 ± 14	140 ± 33	180 ± 71	400 ± 150	600 ± 260

N.A., not available. Mice were euthanized due to large tumor size.

TABLE 2
Pharmacokinetic studies: group assignments and dose levels

Species	No. of Animals	Dose Level	Dose Conc.	Dose Volume	Duration of Injection
		mg/kg	*mg/ml*	*ml/kg*	
Nude mouse	20	10	5	2.0	<5 s
SD rat	4	10	5	1.9	<5 s
Cynomolgus monkey	4	1	0.89	1.12	<30 s
Cynomolgus monkey	4	5	10.1	0.50	<30 s
Cynomolgus monkey	4	50	19.1	2.62	<30 s
Chimpanzee	3	1	5	0.20	50 to 80 s
Chimpanzee	4	5	5	1.0	50 to 80 s

SD, Sprague-Dawley.

disposition of Apo2L/TRAIL in naïve animals was indistinguishable from those dosed previously.

A two-compartment model provided a good fit to observed data but slightly underpredicted serum data starting at the 4-h sampling in the two lowest dose groups. This underprediction did not affect parameter estimates since <1% of the total AUC was associated with samples collected after 4 h. Similarly, only 4% of the total AUC in the 50-mg/kg group was contributed by data collected after 4 h. Apo2L/TRAIL clearance was rapid in all groups and was not significantly affected by dose, suggesting linear kinetics. Calculated AUC and C_{max} increased in a dose-proportional manner over the dose range studied. The steady-state volumes of distribution were approximately equal to serum volume, and the calculated clearance and half-lives were similar among dose groups.

Chimpanzees. Data from individual chimpanzees were modeled using both one- and two-compartment models. When using a two-compartment model, Apo2L/TRAIL elimination is described as biphasic and characterizes a distinct α and β phase. Approximately 99% of the Apo2L/TRAIL elimination was associated with the α phase, indicating that the majority of Apo2L/TRAIL elimination occurred before distribution equilibrium was achieved. Therefore, Apo2L/TRAIL concentration versus time data were fit using a one-compartment model, and parameter estimates were calculated. Kinetic profiles and calculated parameters were similar to those observed for cynomolgus monkeys (Table 3). Additionally, kinetics were linear over the dose range studied and C_{max} and AUC were proportional to dose.

Some samples were spot-checked in a cell-based bioassay to test the integrity of Apo2L/TRAIL after administration to chimpanzees. Apo2L/TRAIL serum concentrations from two chimps given 5 mg/kg were determined in the bioassay and compared with average ELISA-derived concentrations (Fig. 3). These results show good agreement between assays and suggest that Apo2L/TRAIL detected by the ELISA remains bioactive in serum for at least 4 h after i.v. dosing.

Interspecies Scaling

Allometric scaling was used to provide estimates of Apo2L/TRAIL exposure in humans. The relationship between Apo2L/TRAIL kinetic parameters and body weight is shown in Fig. 4, a to d. First, a linear plot was obtained by logarithmic transformation of both axis. The resulting linear relation is described by eq. 1, and parameter (P) was estimated by simple linear regression of the transformed data. In this equation, A is the coefficient (y-axis intercept), B is body weight, and α is the power function (slope) (Ings, 1990).

$$\log P = \log A + \alpha \log B \quad (1)$$

Regression analysis showed that AUC (standardized to a 5-mg/kg dose), Vss, and clearance are strongly correlated with body weight across species. Regression-derived parameter estimates for a 70-kg human given a single i.v. bolus dose of Apo2L/TRAIL (5 mg/kg) are presented in Fig. 4, a to c. Interestingly, Apo2L/TRAIL clearance was highly correlated with literature-reported glomerular filtration rates (Morris and Davies, 1993) (Figs. 4d and 5), suggesting that Apo2L/TRAIL was cleared primarily by kidneys. Additionally, in results from an in vivo tissue distribution study where fully bioactive ^{125}I-Apo2L/TRAIL was given as an i.v. bolus to mice, the kidney had the highest levels of Apo2L/TRAIL localization and degradation, suggesting that it is a major organ of clearance (H. Xiang, C. Nguyen, S. Kelley, J. Fox, D. Xie, K. Totpal, and E. Escandon, unpublished observation).

Apo2L/TRAIL Safety Observations

A preliminary safety profile for Apo2L/TRAIL in nonhuman primates was obtained using clinical observations twice daily for signs of overt toxicity, changes in body weight and food consumption (measured daily), and clinical pathology (prestudy and on days 1, 2, 4, 6, 7, and 13 in the cynomolgus monkeys, and prestudy and on day 15 in chimpanzees). Serum and whole blood samples were analyzed for changes in serum chemistries or hematology. No antibodies against Apo2L/TRAIL were detected in chimpanzee serum collected 14 days postdose.

No overt signs of toxicity or changes in body weight, serum chemistry, or hematology parameters were observed in nonhuman primates that were attributed to Apo2L/TRAIL treatment. In chimpanzees, serum Apo2L/TRAIL concentrations were as high as 138 μg/ml while concentrations in cynomolgus monkeys were as much as 10-fold higher, 1.4 mg/ml.

Discussion

These studies demonstrate the antitumor activity of Apo2L/TRAIL in a xenograft model of colorectal cancer after

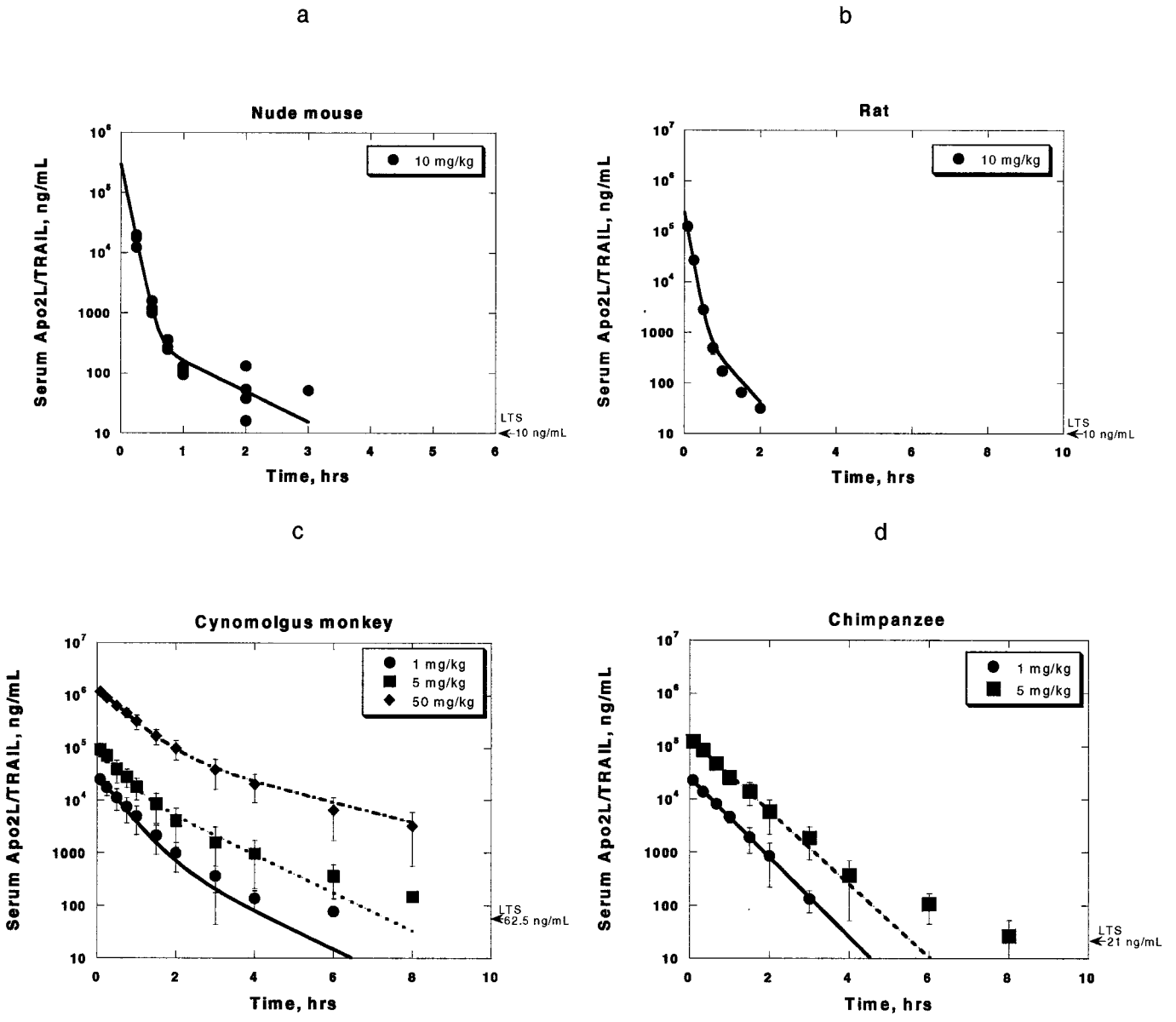

Fig. 2. Serum concentration versus time profiles following single i.v. bolus dosing with Apo2L/TRAIL. a, nude mice (dose = 10 mg/kg); b, Sprague-Dawley rats (dose = 10 mg/kg); c, cynomolgus monkeys (dose = 1, 5, or 50 mg/kg); d, chimpanzee (dose = 1 or 5 mg/kg). LTS, lower limit of assay detection.

i.v. administration, describe the disposition and safety of Apo2L/TRAIL in rodents and primates, and provide estimates of the disposition of Apo2L/TRAIL in humans.

In describing the disposition of Apo2L/TRAIL, we discovered that Apo2L/TRAIL was rapidly eliminated from both rodents and nonhuman primates following i.v. bolus administration. To better understand the reason for this rapid elimination, Apo2L/TRAIL clearance was compared with glomular filtration (GFR). A high correlation between Apo2L/TRAIL clearance and GFR was observed, suggesting that the kidney is a major player in the elimination of Apo2L/TRAIL. This was somewhat surprising since the size of trimeric Apo2L/TRAIL (60 kDa) should impede glomerular filtration. Interestingly, Apo2L/TRAIL clearance seen was similar to the clearance predicted for 20-kDa proteins (Clark et al., 1996), the subunit size of trimeric Apo2L/TRAIL. Dissociation of Apo2L/TRAIL by removal of the coordinating zinc molecule results in the formation of disulfide-linked, dimeric Apo2L/TRAIL that is 10-fold less effective than the trimeric form in our bioassay (K. Totpal, unpublished results). If Apo2L/TRAIL was dissociating in the general circulation, we would expect that serum Apo2L/TRAIL concentrations determined using the bioassay would be lower than those determined using the ELISA. However, both assays returned similar results and suggest that the Apo2L/TRAIL measured in the circulation is trimeric Apo2L/TRAIL. Some of these data are shown in Fig. 3. However, it is possible that Apo2L/TRAIL is dissociating within the kidney, allowing for rapid filtration but limiting the appearance of dimeric or monomeric subunits in the serum.

Several observations were made from these preliminary pharmacokinetic studies. First, our estimated steady-state volumes of distribution indicate that Apo2L/TRAIL may not distribute greatly outside of the vascular space prior to its

TABLE 3
Calculated pharmacokinetic parameters (±S.D.).
Within a primate species, group means in the same column with different numerical superscripts are significantly different (analysis of variance; $\alpha = 0.05$).

Dose	Body Wt.	AUC[a]	C_{max}[a]	Clearance	Vss[a]	Half-Life[b]
mg/kg	*kg*	*μg·h/ml*	*μg/ml*	*ml/h/kg*	*ml/kg*	*min*
Nude mouse						
10	0.026 ± 0.002	26.5	302	377	37.4	3.64
Rat						
10	0.266 ± 0.013	25.6 ± 2.61	240 ± 56	395 ± 40	48.3 ± 13	4.58 ± 0.82
Cynomolgus monkey						
1	3.33 ± 0.65[1]	18.8 ± 8.4[1]	32 ± 6.1[1]	62 ± 26[1]	67 ± 41[1]	24.2 ± 7.8[1]
5	3.35 ± 1.17[1]	67.6 ± 24.0[2]	123 ± 34[2]	83 ± 36[1]	53 ± 9.2[1]	23.0 ± 6.6[1]
50	3.45 ± 0.613[1]	1050 ± 200[3]	1410 ± 180[3]	49 ± 11[1]	47 ± 7.6[1]	31.2 ± 5.58[1]
Chimpanzee						
1	60 ± 8.7[1]	14.7 ± 1.62[1]	25.4 ± 1.89[1]	66.4 ± 4.03[1]	37.9 ± 4.50[1]	23.9 ± 4.12[1]
5	53 ± 8.9[1]	88.7 ± 16.4[2]	138 ± 11.2[2]	58.2 ± 9.29[1]	36.6 ± 4.09[1]	27.0 ± 5.24[1]

[a] AUC is the calculated area under the Apo2L/TRAIL serum concentration vs. time curve; C_{max} is the model-predicted maximum serum concentration of Apo2L/TRAIL; Vss is the estimated volume of distribution of Apo2L/TRAIL at steady-state.
[b] Reported half-life was calculated from the elimination rate constant (K10).

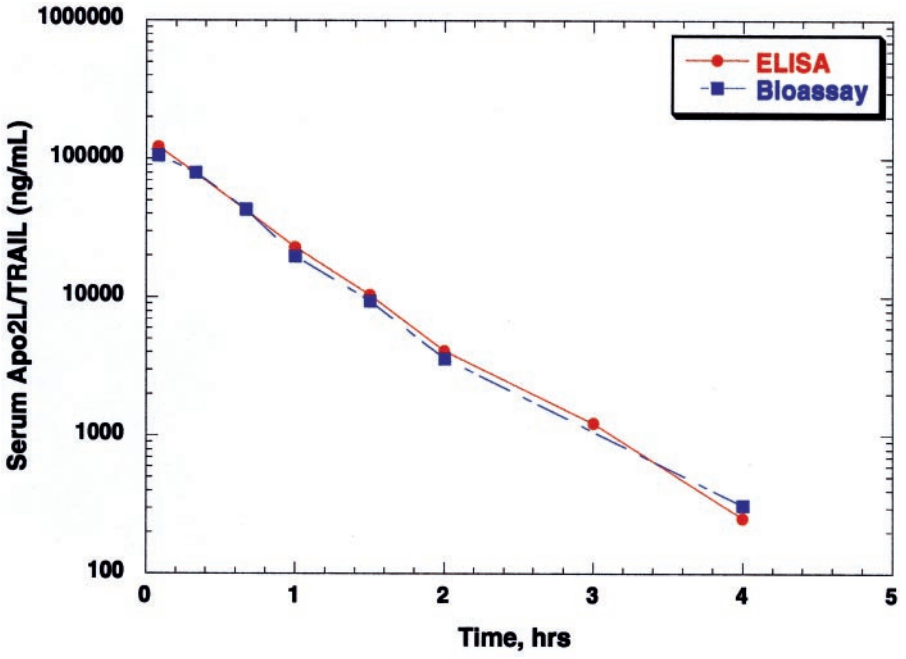

Fig. 3. Apo2L/TRAIL serum concentration versus time plots for select chimp samples. ELISA and bioassay were used to calculate Apo2L/TRAIL concentrations. Results suggest that the integrity/bioactivity of Apo2L/TRAIL detected by ELISA is maintained for at least 4 h after dosing.

elimination from the system. Second, the half-life of Apo2L/TRAIL in mouse was 3 to 5 min. However, Apo2L/TRAIL has demonstrated significant antitumor activity in mice containing human xenografts. We are currently investigating whether administration of Apo2L/TRAIL by i.v. infusion may provide greater drug delivery to the tumor and further enhance the impressive antitumor response observed in these studies.

Pharmacokinetic data from mice, rats, monkeys, and chimpanzees were used to predict the pharmacokinetics of Apo2L/TRAIL in humans. Interspecies scaling provided estimates of Apo2L/TRAIL clearance, Vss, and exposure (AUC) in humans given a single i.v. bolus of 5 mg/kg. These analyses suggest that the disposition of Apo2L/TRAIL in humans might be predicted by body weight and that these kinetic parameters should be similar to those observed in chimpanzees. The ability to scale across such a wide range of species is probably due to the correlation between Apo2L/TRAIL clearance and GFR across species and points toward renal filtration as a common mechanism of elimination across the species studied here. Additionally, Apo2L/TRAIL kinetics were linear across the doses studied, suggesting that receptor-mediated clearance did not contribute significantly to drug clearance.

While other apoptosis-inducing members of the TNF family carried great promise as anticancer agents, severe toxicities toward normal tissues have hampered their use as cancer therapeutics. A lethal inflammatory response resembling septic shock was seen following infusion of TNF to baboons, while administration of agonistic anti-Fas antibodies or recombinant human Fas ligand to rodents results in lethal liver damage (Tracey et al., 1986; Ogasawara et al., 1993; Van Zee et al., 1994; Burress et al., 1996; Tanaka et al., 1997). Additionally, others (Jo et al., 2000) tested a polyhistidine-tagged version of Apo2L/TRAIL in vitro and induced apoptosis in normal human hepatocytes.

Because of that TNFα and Fas ligand experience, and the apoptosis seen in vitro in response to a polyhistidine-tagged version of Apo2L/TRAIL (Jo et al., 2000), the absence of toxicity thus far to high doses of our untagged recombinant soluble preparation of Apo2L/TRAIL in both cynomolgus monkeys (50 mg/kg) and chimpanzees (5 mg/kg) is encourag-

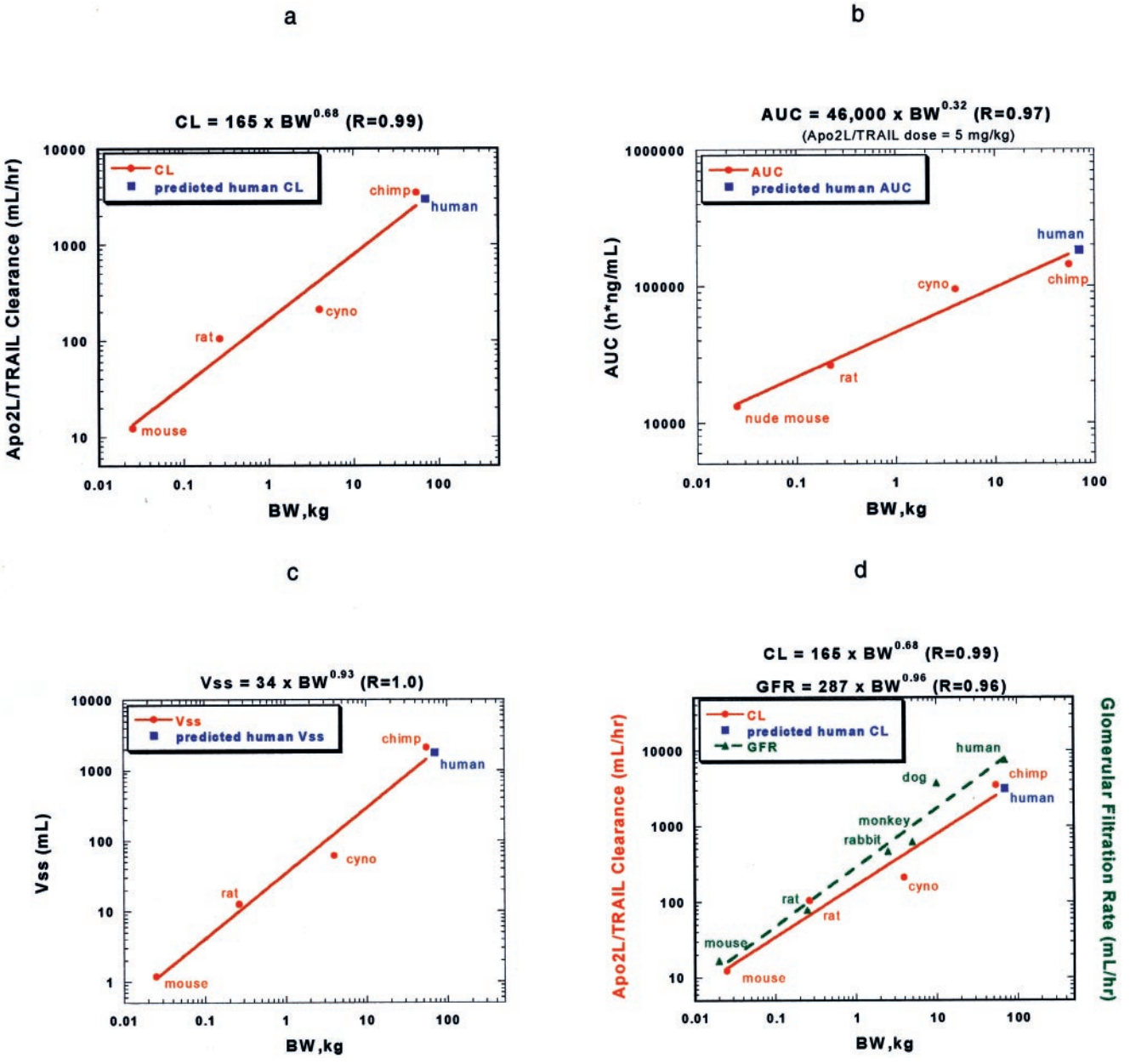

Fig. 4. Interspecies scaling of pharmacokinetic parameters. a, scaling clearance; b, scaling AUC; c, scaling Vss; and d, correlation of Apo2L/TRAIL clearance and GFRs in other species. Apo2L/TRAIL doses used for scaling were 10 mg/kg for rodents and 5 mg/kg for nonhuman primates. Also presented on each plot are estimates of human parameters derived from regression analysis. Human parameter calculations assumed administration of a 5-mg/kg bolus dose to a 70-kg patient. Estimated pharmacokinetic parameters are as follows: AUC = 183 h · μg/ml, Vss = 25.4 ml/kg, and clearance = 42.5 ml/h/kg.

ing. Apo2L/TRAIL doses given to chimpanzees and cynomolgus monkeys were 5- to 500-fold higher than TNFα doses (100 μg/kg) shown to be toxic in baboons. Signs of hepatic compromise were absent in these in vivo studies with serum concentration levels as much as 3500-fold higher than those used with the polyhistidine-tagged version in vitro, supporting the observations of Lawrence et al. (2001) that in vitro toxicity against human hepatocytes was related to the polyhistidine-tagged version of Apo2L/TRAIL used by Jo et al. (2000).

In conclusion, these studies have characterized the disposition of Apo2L/TRAIL in rodents and primates and provide information that will be used to predict the pharmacokinetics of Apo2L/TRAIL in humans. Scaling and the linear kinetics observed suggest that renal filtration appears to be the dominant mechanism of Apo2L/TRAIL and that drug clearance was not significantly affected by a receptor-mediated mechanism. Because large molecular weight compounds often have difficulty penetrating solid tumors, it is unlikely that Apo2L/TRAIL is able to extend beyond the perivascular space before being eliminated. However, Apo2L/TRAIL demonstrates significant in vivo activity in xenograft models of human cancers, suggesting that extensive tumor penetration may not be necessary for activity. We anticipate that the use of interspecies scaling will provide estimates of Apo2L/TRAIL kinetics in humans, and that on a milligram per kilogram basis, doses

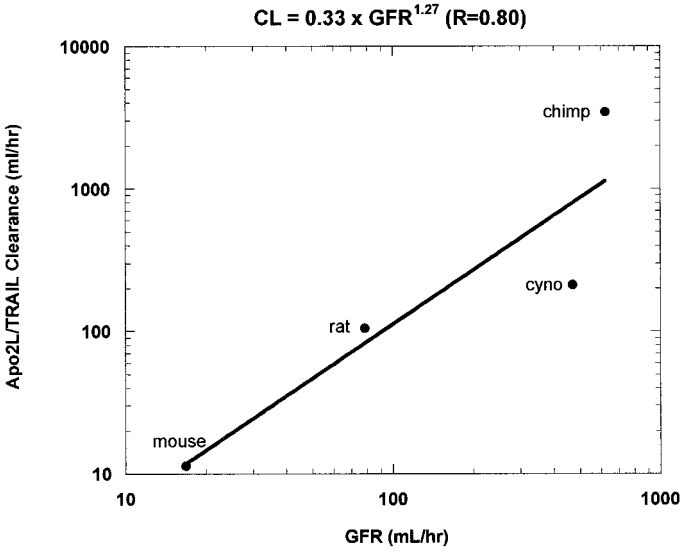

Fig. 5. Correlation of calculated Apo2L/TRAIL clearance and published rates of GFR in mice, rats, monkeys, and chimpanzees. Apo2L/TRAIL clearances are from rodent doses of 10 mg/kg and nonhuman primate doses of 5 mg/kg.

significantly lower than those used in xenograft studies could be effective in humans.

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