

# Apo2 Ligand/Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Cooperates with Chemotherapy to Inhibit Orthotopic Lung Tumor Growth and Improve Survival

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## ABSTRACT

**Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) is a tumor necrosis factor superfamily member that induces apoptosis through the death receptors DR4 and/or DR5 in various cancer cell types but not in most normal cells. Several lung cancer cell lines express DR4 and DR5 and undergo apoptosis *in vitro* in response to Apo2L/TRAIL. We investigated the efficacy of recombinant soluble human Apo2L/TRAIL and its interaction with chemotherapy in xenograft models based on human NCI-H460 non-small cell lung carcinoma cells. *In vitro*, Taxol enhanced caspase activation and apoptosis induction by Apo2L/TRAIL. *In vivo*, Apo2L/TRAIL or Taxol plus carboplatin chemotherapy partially delayed progression of established subcutaneous tumor xenografts, whereas combined treatment caused tumor regression and a substantially longer growth delay. Apo2L/TRAIL, chemotherapy, or the combination of both inhibited growth of preformed orthotopic lung parenchymal tumors *versus* control by 60%, 57%, or 97%, respectively (all  $P < 0.01$ ;  $n = 8-10$ ). Furthermore, combination treatment improved day-90 survival relative to control (7 of 15 *versus* 1 of 15;  $P = 0.0003$  by Mantel-Cox) as well as to Apo2L/TRAIL (3 of 14;  $P = 0.031$ ) or chemotherapy (3 of 15;  $P = 0.035$ ). These studies provide evidence for *in vivo* activity of Apo2L/TRAIL against lung tumor xenografts and underscore the potential of this ligand for advancing current lung cancer treatment strategies.**

## INTRODUCTION

Lung cancer is the most frequently occurring malignancy worldwide. In the United States, ~170,000 people (80,000 females and 90,000 males) were diagnosed with lung cancer in 2002 (1). It is also the leading cause of cancer-related mortality, accounting for 25% (women) and 31% (men) of such deaths in the United States in 2002 (2). Of the newly diagnosed lung cancers, 75–80% are defined as non-small cell lung cancer (NSCLC). Recent clinical trials demonstrate that the benefit of combination chemotherapy among the fittest patients with advanced NSCLC is marginal (3). Thus, novel treatment strategies are urgently needed to improve the clinical management of this serious disease.

Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) was identified as an apoptosis-inducing member of the tumor necrosis factor gene superfamily (4, 5). Apo2L/TRAIL triggers apoptosis through interaction with the death receptors DR4 and DR5 (6–13). Recombinant soluble human Apo2L/TRAIL is a candidate for clinical investigation in cancer therapy because of the following: (a) it induces apoptosis in a wide variety of human cancer cell lines but not in most normal cells; and (b) it exerts potent antitumor effects without normal tissue toxicity in various cancer xenograft models (14–23). Apo2L/TRAIL induces apoptosis in sev-

eral NSCLC cell lines (15, 24); however, its *in vivo* activity against NSCLC xenografts has not been directly investigated.

In the present study, we tested the effects of Apo2L/TRAIL as a single agent and in combination with chemotherapy on tumor progression and host survival in athymic mouse models based on s.c. or orthotopic xenografts derived from the human NSCLC cell line NCI-H460. Apo2L/TRAIL exerted activity as a single agent and cooperated with clinically established chemotherapy against lung tumors *in vivo*, supporting the potential of this agent to provide a novel, apoptosis-based biological approach for advancing the treatment of lung cancer.

## MATERIALS AND METHODS

**Reagents, Cells, and *in Vitro* Studies.** Recombinant soluble human Apo2L/TRAIL (Apo2L/TRAIL) was produced at Genentech, Inc. (South San Francisco, CA; Refs. 15, 23). Taxol (paclitaxel) and carboplatin (paraplatin) were purchased from Bristol-Myers Squibb Co. (Princeton, NJ). Antibodies for immunoblot analysis were obtained from Cell Signaling Technologies (Beverly, MA; caspase-8), Oncogene Research Products (Boston, MA; caspase-7 and -9), BioMol Research Labs (Plymouth Meeting, PA; caspase-3), and ICN Biomedicals (Aurora, OH; actin).

NCI-H460 NSCLC cells were plated in 96-well dishes (5000 cells/well) and incubated overnight at 37°C in 10% fetal bovine serum RPMI 1640. Next, the medium was replaced, and the cells were incubated with Apo2L/TRAIL, Taxol, or combinations thereof for 24 h, and cell viability was determined by AlamarBlue assay (Trek Diagnostic Systems, Inc.; Ref. 15). Alternatively, cells were lysed 24 h after treatment and analyzed with the Apo-ONE Homogeneous Caspase-3/7 Assay kit (Promega, Madison, WI) or by caspase immunoblot (25).

**Animals.** Female athymic nude mice (The Jackson Laboratory, Bar Harbor, ME) were acclimated to the animal housing facility for at least 1 week before surgery. All of the experimental procedures conformed to the guiding principles of the American Physiology Society and were approved by the Institutional Animal Care and Use Committee of Genentech.

**The s.c. Xenograft Model.** Mice were injected s.c. with human NCI-H460 NSCLC cells (5 million/mouse). Tumor dimensions were measured by a digital caliper, and tumor volumes were calculated as length  $\times$  (width)<sup>2</sup>/2. When tumors reached a volume of ~180 mm<sup>3</sup>, mice were randomized into groups and treated with vehicle, Apo2L/TRAIL (60 mg/kg/day i.p. on days 0–4 and 7–11), chemotherapy (Taxol at 6.25 mg/kg/day s.c. on days 0–4 and 7–11, plus carboplatin at 100 mg/kg i.p. on days 0 and 7), or the combination of these latter Apo2L/TRAIL and chemotherapy regimens.

**Orthotopic Xenograft Model.** Mice were anesthetized with isoflurane. The surgery procedure reported by Doki *et al.* (26) was used with modification. A 5-mm skin incision to the left chest was made ~5 mm (tail side) from the scapula. Fat and muscle were separated from costal bones. On observing left lung motion through the pleura, a 28-gauge needle attached to a 0.1-ml Hamilton syringe was directly inserted through the 6th intercostal space into the lung to a depth of 3 mm. Human NCI-H460 cells (1 million), suspended in 20  $\mu$ l of HBSS containing Matrigel (v/v = 1 of 1), were injected into the lung parenchyma. After injection, a cotton-tipped applicator was pressed on the injection site to stop any bleeding, and the skin incision was closed with a surgical skin clip. A pilot study showed that all of the nude mice ( $n = 12$ ) developed lung cancer at 3 weeks after intrapulmonary injection of the tumor cells.

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Treatment in the orthotopic model was initiated either 4 or 10 days after lung parenchymal injection of tumor cells. After allowing 4 days for tumors to develop, mice were randomized and treated with vehicle, Apo2L/TRAIL (60 mg/kg/day i.p. for 3 weeks), chemotherapy (Taxol at 6.25 mg/kg/day s.c. for 5 days, plus carboplatin at 100 mg/kg, single i.p. dose), or the combination of Apo2L/TRAIL and chemotherapy. On day 26 after study initiation, mice were anesthetized with pentobarbital sodium (60 mg/kg i.p.), and the lungs were removed and fixed in 10% buffered formalin for histological studies. Alternatively, after allowing tumors to develop over 10 days, mice were treated for 2 weeks as described above. On study day 25, the lungs were removed and fixed as described above.

For histology, lungs were embedded in paraffin, step sections were performed, and 5- $\mu$ m thick sections representative of 10 levels of the block were stained with H&E for routine histological evaluation. Quantification of tumor area from histological sections was performed from digital micrographs using a Polaroid Sprintscan 120 and Adobe Photoshop. Data are reported as relative tumor size, defined as the integrated tumor area from 10 sections/lung in test animals compared with vehicle controls. Measurement of tumor areas was carried out in blinded fashion with respect to treatment groups.

**Survival Study in Orthotopic Xenograft Model.** NCI-H460 cells were injected orthotopically. After allowing 10 days for tumors to develop, mice

were randomized into groups and treated with vehicle, Apo2L/TRAIL, chemotherapy, or combination as described above. Survival was followed for 3 months.

**Statistical Analysis.** Results are expressed as mean  $\pm$  SE. To assess differences in tumor size between groups, one-way ANOVA was performed; significant differences were subjected to *post hoc* analysis using Fisher's projected least significant difference method. Survival was compared by log-rank (Mantel-Cox) test. Median survival time was estimated from Kaplan-Meier analysis.

## RESULTS

**In Vitro Activity of Apo2L/TRAIL and Chemotherapy on NCI-H460 Cells.** As a preclinical model for testing activity of Apo2L/TRAIL in lung cancer, we selected the human NSCLC cell line NCI-H460. This is a robust cell line that forms aggressive, rapidly growing tumors when injected s.c. in athymic nude mice (see below) and which we have found previously to be only partially responsive *in vitro* to apoptosis induction by Apo2L/TRAIL (27). Treatment of NCI-H460 cells with Apo2L/TRAIL resulted in a dose-dependent loss

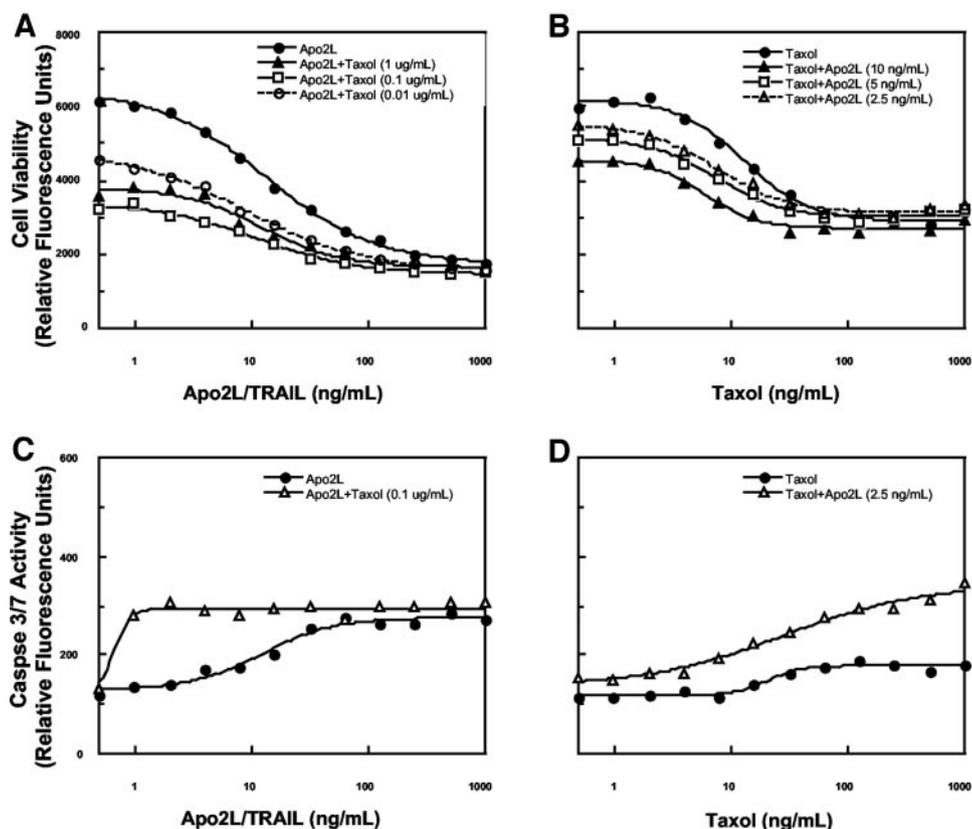
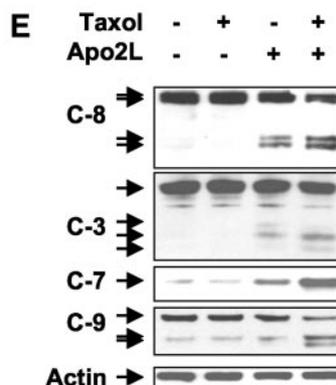


Fig. 1. Interaction of Apo2L ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and Taxol in induction of NCI-H460 cell death *in vitro*. *A*, cells were treated with various Apo2L/TRAIL concentrations in the absence or presence of Taxol concentrations of 0.01, 0.1, or 1  $\mu$ g/ml. After 24 h, cell viability was measured by Alamar Blue assay. *B*, cells were treated with Taxol alone or in combination with Apo2L/TRAIL concentrations of 2.5, 5, or 10 ng/ml and analyzed for viability. *C*, cells were treated with various doses of Apo2L/TRAIL alone or in combination with Taxol at 0.1  $\mu$ g/ml and analyzed by caspase-3/7 activity assay. *D*, cells were treated with various doses of Taxol alone or in combination with Apo2L/TRAIL at 2.5 ng/ml and analyzed by caspase-3/7 activity assay. *E*, cells were treated with buffer control, Taxol (0.1  $\mu$ g/ml), Apo2L/TRAIL (0.1  $\mu$ g/ml), or the combination of both, and caspase processing was analyzed after 24 h by immunoblot. The caspase-8, -3, and -9 antibodies detect both full-length and processed forms of the antigen, whereas the caspase-7 antibody detects only the processed form.



of cell viability (Fig. 1A). Combined treatment with Taxol, a chemotherapeutic agent that is frequently used in first-line treatment of NSCLC, resulted in marked sensitization of NCI-H460 cells to Apo2L/TRAIL-induced cell death (Fig. 1A). Carboplatin, used as well in first-line NSCLC treatment, also enhanced Apo2L/TRAIL-induced apoptosis but did not increase sensitivity beyond the effect of Taxol (data not shown). The augmentation of cell killing was dependent on the dose of Taxol (Fig. 1A) and Apo2L/TRAIL (Fig. 1B). Consistent with apoptosis induction, Apo2L/TRAIL caused a dose-dependent stimulation of effector caspases, as measured by caspase-3/7 enzymatic activity (Fig. 1, C and D); this was substantially augmented by Taxol, which by itself induced less caspase-3/7 activity than Apo2L/TRAIL. Apoptosis was additionally confirmed by detection of sub-diploid DNA (data not shown). Immunoblot analysis revealed that Taxol alone had no detectable effect on caspase processing as compared with control (Fig. 1E). Conversely, Apo2L/TRAIL induced significant processing of caspase-8, an apical caspase that initiates apoptosis downstream of DR4 and DR5 (27), and of the downstream apoptosis effectors caspase-3 and caspase-7, with a minimal increase in processing of caspase-9, which initiates apoptosis downstream of mitochondria. Combined treatment with Taxol and Apo2L/TRAIL led to enhanced processing of all of the caspases examined, particularly caspase-9. These data suggest that the combination of Taxol and Apo2L/TRAIL results in greater caspase activation, leading to a more effective tumor cell kill than that achieved by each agent alone. Taxol did not significantly alter the levels of DR4, DR5, DcR1, DcR2, or Fas-associated death domain (data not shown), indicating that sensitivity to Apo2L/TRAIL was promoted independently of receptor or adaptor modulation.

**Effect of Apo2L/TRAIL and Chemotherapy on Growth of s.c. Tumor Xenografts.** To examine the activity of Apo2L/TRAIL *in vivo*, we assessed the efficacy of the ligand in a xenograft model in which NCI-H460 cells were injected s.c. into athymic nude mice. When the mean tumor volume reached  $\sim 180 \text{ mm}^3$ , mice were randomized into groups and treated over days 0–4 and 7–11 with vehicle, Apo2L/TRAIL, Taxol plus carboplatin (chemotherapy), or the combination of Apo2L/TRAIL and chemotherapy, and tumor volumes were monitored (Fig. 2). Tumors in vehicle-treated animals grew rapidly, although tumors in Apo2L/TRAIL-treated mice grew slower, particularly during both treatment periods, and showed a partial, yet measurable delay in progression. In animals receiving chemotherapy alone, tumor growth was attenuated mainly during the second treatment period (see day 7 and 9 volumes), exhibiting a comparable delay in tumor progression. In contrast, combined treatment with Apo2L/TRAIL and chemotherapy caused a marked tumor regression during the first period of therapy, followed by tumor stasis during the second treatment period and an additional delay in tumor progression up to day 16. Hence, although either Apo2L/TRAIL or chemotherapy alone partially attenuated the aggressive s.c. growth of NCI-H460 tumors, the combination of both treatments caused tumor shrinkage and a more effective delay in tumor progression.

**Effect of Apo2L/TRAIL and Chemotherapy on Growth of Orthotopic Lung Tumor Xenografts.** To investigate the activity of Apo2L/TRAIL against NCI-H460 tumors growing in an orthotopic tissue environment, we used a model in which the cells are surgically introduced into the pulmonary interstitium (see “Materials and Methods”). We conducted two experiments in this model, initiating treatment either 4 or 10 days after intrapulmonary injection of tumor cells. In the first study, starting from day 4, Apo2L/TRAIL injections were given daily over 3 weeks, whereas chemotherapy was given over 5 days (see “Materials and Methods”). On day 26 after study initiation, the lungs were removed and tumor sizes were determined (Fig. 3A). Treatment with Apo2L/TRAIL, chemotherapy, or the combination

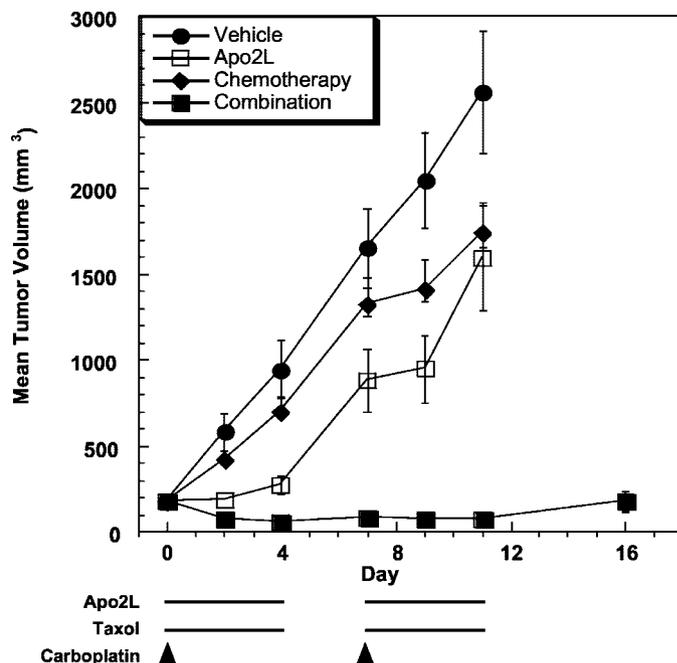


Fig. 2. Effect of Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and/or chemotherapy on growth of established s.c. tumor xenografts. Athymic nude mice carrying NCI-H460 tumors (10/group) received vehicle, Apo2L/TRAIL (60 mg/kg/day i.p. on days 0–4 and 7–11), chemotherapy (Taxol at 6.25 mg/kg/day s.c. on days 0–4 and 7–11, plus carboplatin at 100 mg/kg, i.p. dose on days 0 and 7), or the combination of both Apo2L/TRAIL and chemotherapy regimens; bars,  $\pm$ SD.

reduced the mean tumor size, respectively, by 92% ( $P < 0.0001$ ;  $n = 9$ ), 92% ( $P < 0.0001$ ;  $n = 10$ ), or 99% ( $P < 0.0001$ ;  $n = 9$ ), as compared with the vehicle control ( $n = 8$ ). Thus, both Apo2L/TRAIL and chemotherapy exhibited significant antitumor activity, whereas the combination of both approaches exerted greater tumor inhibition, which was significant not only relative to vehicle control but also in relation to chemotherapy alone ( $P = 0.024$ ). Histological sections from the tumor cell-injected lung of two representative animals from each group are shown in Fig. 3B.

In a subsequent study, we allowed orthotopic lung tumors to grow for a longer period (10 days) before initiation of therapy. Apo2L/TRAIL was dosed over 2 weeks, and chemotherapy was given over 5 days. On day 25 after study initiation, the lungs were removed, and tumor sizes were analyzed (Fig. 4). Treatment with Apo2L/TRAIL, chemotherapy, or the combination reduced the mean tumor size, respectively, by 60% ( $P = 0.0132$ ;  $n = 10$ ), 57% ( $P = 0.0189$ ;  $n = 10$ ), or 97% ( $P = 0.0002$ ;  $n = 10$ ), as compared with vehicle control ( $n = 9$ ). The reduction in tumor size was significantly greater in the combination group than in the chemotherapy alone group ( $P = 0.039$ ). Thus, allowing the tumors to grow additionally before treatment initiation decreased the efficacy of Apo2L/TRAIL or chemotherapy, yet it caused only a minimal reduction in efficacy of the combination treatment.

**Effect of Apo2L/TRAIL and Chemotherapy on Survival of Mice with Orthotopic Lung Tumor Xenografts.** To assess whether the antitumor activity observed in the latter studies translates into a survival benefit, we conducted a third study in the orthotopic model. NCI-H460 tumors were allowed to grow for 10 days after intrapulmonary injection. The mice were then treated with Apo2L/TRAIL over 2 weeks, chemotherapy over 5 days, or the combination of both, and survival was followed for 3 months. Day 90 survival was 1 of 15 (6.7%), 3 of 14 (21.4%), 3 of 15 (20.0%), or 7 of 15 (46.7%), respectively, in the vehicle, Apo2L/TRAIL, chemotherapy, or com-

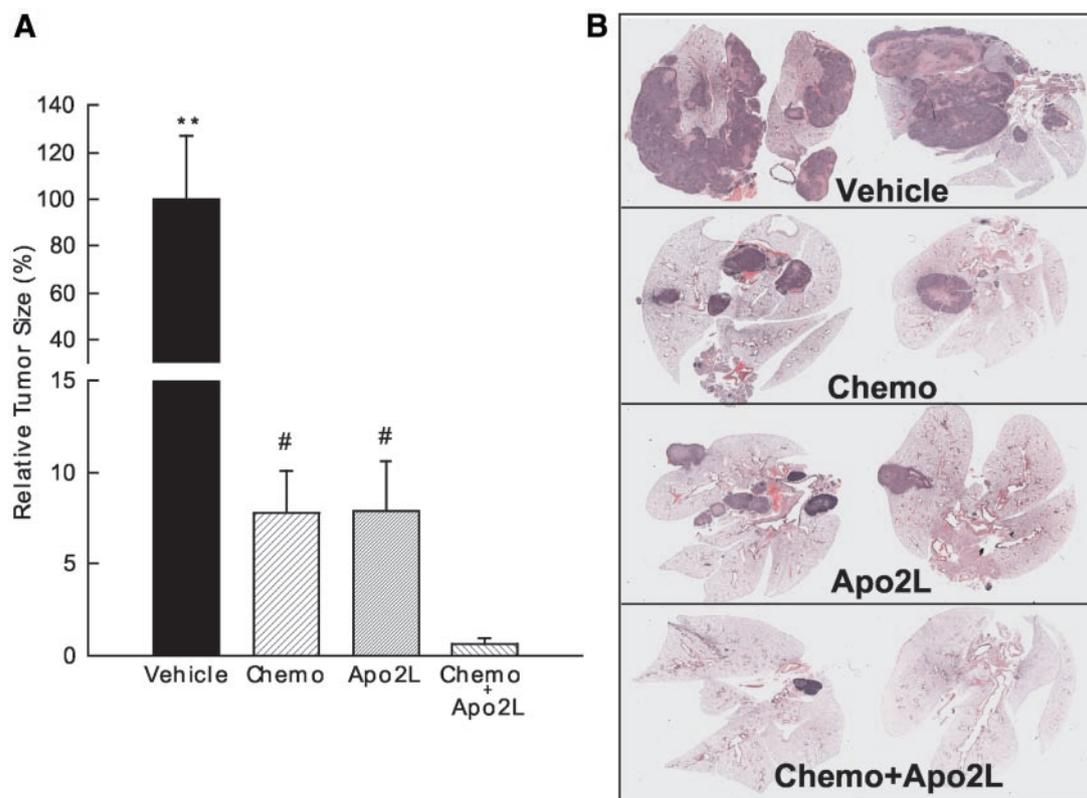


Fig. 3. Effect of day-4-initiated treatment with Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and/or chemotherapy on growth of orthotopic lung tumor xenografts. Four days after intrapulmonary injection of NCI-H460 cells, athymic nude mice received vehicle, Apo2L/TRAIL (60 mg/kg/day i.p. for 3 weeks), chemotherapy (Taxol at 6.25 mg/kg/day s.c. for 5 days, plus carboplatin at 100 mg/kg, single i.p. dose), or the combination of both. *A*, relative tumor size, defined as the integrated tumor area of 10 histological sections/lung in test animals compared with vehicle-treated controls ( $674.5 \pm 185.2 \text{ mm}^2$ ) was determined on day 25.  $**$ ,  $P < 0.0001$  between the vehicle and each of the other 3 groups.  $\#$ ,  $P = 0.024$  between the chemotherapy and combination groups or  $P = 0.025$  between the Apo2L/TRAIL and combination groups ( $n = 8-10$  mice each). *B*, H&E staining of histological sections taken at the median level of sectioning from 2 animals per group. *Chemo*, chemotherapy; *Apo2L*, Apo2L/TRAIL; *Chemo + Apo2L*, combination; bars,  $\pm$ SD.

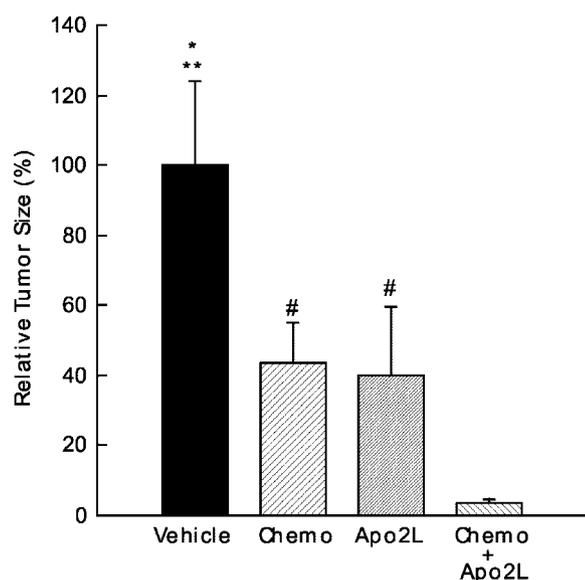


Fig. 4. Effect of day-10-initiated treatment with Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and/or chemotherapy on growth of orthotopic lung tumor xenografts. Ten days after intrapulmonary injection of NCI-H460 tumor cells, athymic nude mice received vehicle, Apo2L/TRAIL (60 mg/kg/day i.p. for 2 weeks), chemotherapy (Taxol at 6.25 mg/kg/day s.c. for 5 days, plus carboplatin at 100 mg/kg, single i.p. dose), or the combination of both. Relative tumor size compared with vehicle-treated controls ( $571.7 \pm 137.6 \text{ mm}^2$ ) was determined on day 26 as described in Fig. 3.  $*$ ,  $P = 0.0189$  between the vehicle and chemotherapy groups or  $P = 0.0132$  between the vehicle and Apo2L/TRAIL groups.  $**$ ,  $P = 0.0002$  between the vehicle and combination groups.  $\#$ ,  $P = 0.0393$  between the chemotherapy and combination groups or  $P = 0.0580$  between the Apo2L/TRAIL and combination groups ( $n = 9-10$  each). *Chemo*, chemotherapy; *Apo2L*, Apo2L/TRAIL; *Chemo + Apo2L*, combination; bars,  $\pm$ SD.

combination treatment group (Fig. 5). Survival benefit from treatment with Apo2L/TRAIL or chemotherapy alone did not reach statistical significance as assessed by log-rank (Mantel-Cox) analysis of the plots, whereas survival benefit from the combined treatment was significant *versus* control ( $P = 0.0003$ ) as well as *versus* Apo2L/TRAIL alone ( $P = 0.031$ ) or chemotherapy alone ( $P = 0.035$ ). The median survival time estimated from Kaplan-Meier analysis was prolonged by 37.5% in the Apo2L/TRAIL group [55 days; 95% confidence interval, 26–68 days]; 20.0% in the chemotherapy group (48 days; 95% confidence interval, 36–68 days); or 122.5% in the combination group (89 days; 95% confidence interval, >49 days), as compared with vehicle control (40 days; 95% confidence interval, 29–46 days). Thus, the positive interaction of Apo2L/TRAIL with chemotherapy was reflected not only by inhibition of tumor growth but also by significantly improved survival.

## DISCUSSION

Apo2L/TRAIL induces apoptosis through DR4 and DR5. As a soluble, zinc-bound trimer, this ligand triggers programmed cell death in a wide variety of cancer cell lines but not in most normal cells (14, 15, 23). Previous studies indicate that Apo2L/TRAIL is capable of inducing apoptosis *in vitro* in various NSCLC cell lines and that its activity frequently can be augmented by combination with chemotherapeutic agents (15, 24). *In vivo* antitumor activity of Apo2L/TRAIL has been demonstrated in xenograft models of colorectal (15–17, 20, 25), breast (16, 19), and prostate cancer (21), as well as glioma (18, 22, 28) and multiple myeloma (29). However, to date Apo2L/TRAIL activity against lung tumor xenografts, growing in a

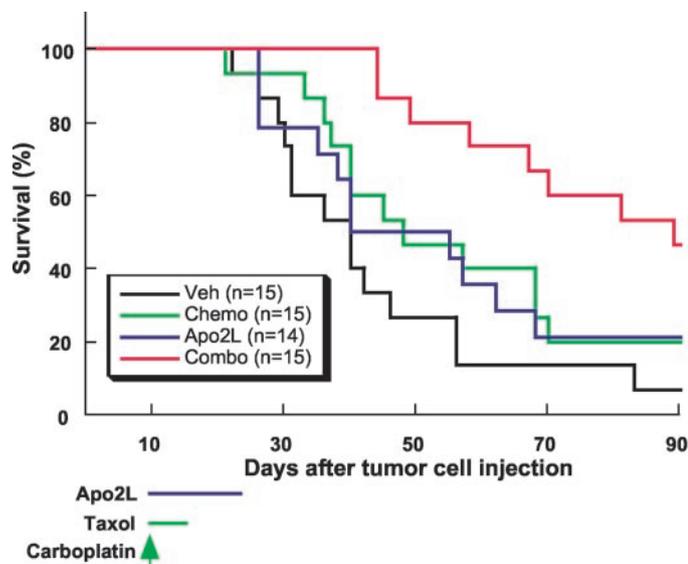


Fig. 5. Effect of day-10-initiated treatment with Apo2 ligand (Apo2L)/tumor necrosis factor-related apoptosis-inducing ligand and/or chemotherapy on survival of mice with orthotopic lung tumor xenografts. Ten days after intrapulmonary injection of NCI-H460 tumor cells, athymic nude mice were treated as in Fig. 4, and survival was followed for 3 months from study initiation. Log-rank (Mantel-Cox) comparison of survival plots indicated that  $P = 0.0003$  between the combination and vehicle groups;  $P = 0.035$  between the combination and chemotherapy groups; and  $P = 0.031$  between the combination and Apo2L/tumor necrosis factor-related apoptosis-inducing ligand groups.

s.c. setting or an orthotopic tissue environment, has not been reported. Our results demonstrate significant Apo2L/TRAIL activity against s.c. as well as orthotopic NCI-H460 lung tumor xenografts and remarkable cooperation of this ligand with a Taxol-carboplatin regimen that is currently used in first-line treatment of NSCLC.

We selected the NCI-H460 NSCLC cell line as a model, because these cells form rapidly growing tumors in xenografted mice and are only partially sensitive *in vitro* to Apo2L/TRAIL; analysis of subdiploid DNA content indicated that at maximum, Apo2L/TRAIL induces apoptosis *in vitro* in ~50% of NCI-H460 cells exposed to 100 ng/ml ligand for 24 h (data not shown). Consistent with this partial sensitivity, single agent activity of Apo2L/TRAIL against NCI-H460 tumor xenografts was relatively limited in comparison to more sensitive cell lines, e.g., Colo205 (15, 30). Efficacy appeared greater against lung-orthotopic as compared with s.c. tumors, possibly because of differences in tumor growth rates and drug accessibility in the two tissue environments. Activity of Apo2L/TRAIL in the orthotopic xenograft model was greater on earlier treatment, perhaps because tumors were larger and less accessible after 10 days *versus* 4 days. It is notable that in both the s.c. and orthotopic settings, the effect of Apo2L/TRAIL on tumor growth was comparable with that of chemotherapy with Taxol and carboplatin. The serum half-life of Apo2L/TRAIL is approximately 3–5 min in mice and approximately 23–31 min in nonhuman primates (30). Hence, it is likely that similar plasma concentrations to those reached in mice with 60 mg/kg Apo2L/TRAIL will be achievable in patients with 5–10-fold lower doses per kg. Furthermore, although we did not investigate this here, other studies show cooperation with chemotherapy at lower doses of Apo2L/TRAIL (15, 20).

Apo2L/TRAIL triggers apoptosis through the cell-extrinsic pathway, which involves direct recruitment and activation of caspase-8 and -10 through DR4 and/or DR5 and the adaptor molecule Fas-associated death domain (27, 31, 32). In contrast, many chemotherapeutic agents initiate apoptosis by engaging the cell-intrinsic pathway, which involves activation of proapoptotic Bcl-2 family members,

leading to the release of cytochrome C and Apaf-1 and, hence, to activation caspase-9 (33). The initiator caspases (*i.e.*, caspase-8, -10, and -9), in turn, activate downstream effectors such as caspase-3 and -7, which execute the apoptotic demise of the cell. Previous work indicates that chemotherapy can sensitize tumor cells to apoptosis induction by Apo2L/TRAIL, in part through death receptor up-regulation, and in part through cross-talk between the intrinsic and extrinsic pathways (14, 25). Taxol substantially increased apoptosis induction by Apo2L/TRAIL in NCI-H460 cells. Although Taxol did not alter receptor or Fas-associated death domain expression, it augmented caspase processing and activation by Apo2L/TRAIL, potentiating apoptosis. Caspase-9 processing was particularly enhanced, suggesting better engagement of the cell-intrinsic pathway.

*In vivo*, combined treatment with Apo2L/TRAIL and Taxol/carboplatin chemotherapy resulted in remarkable antitumor efficacy. In the s.c. xenograft model, this combination caused tumor regression followed by a prolonged delay in tumor growth. In the orthotopic model, treatment with Apo2L/TRAIL plus chemotherapy led to >97% inhibition of tumor growth even when initiated 10 rather than 4 days after tumor cell injection. Moreover, the combined treatment resulted in a significant survival benefit not only compared with the vehicle control but also relative to Apo2L/TRAIL treatment or chemotherapy alone. Together, these results suggest that targeting death receptors in lung cancer with Apo2L/TRAIL may represent a potentially useful new approach to improving the current standard of care for this serious disease.

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## Apo2 Ligand/Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Cooperates with Chemotherapy to Inhibit Orthotopic Lung Tumor Growth and Improve Survival

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