Large Molecule Therapeutics

Immunoglobulin Fc Domain Fusion to TRAIL Significantly Prolongs Its Plasma Half-Life and Enhances Its Antitumor Activity

Haizhen Wang, Jennifer S. Davis, and Xiangwei Wu

Abstract

TRAIL (Apo2L) is a potent inducer of cell death. Interest in TRAIL has increased, following the observation that TRAIL can selectively kill a wide variety of human cancer cells without killing normal cells both in vitro and when grown as xenografts. Therefore, TRAIL has been proposed as a promising anticancer agent and currently is being tested in clinical trials. However, recombinant TRAIL has a very short plasma half-life, which limits its therapeutic potential. To overcome this limitation, we investigated the ability of the human IgG1 fragment crystallizable region (Fc) to enhance TRAIL stability. In this report, we show that Fc-TRAIL chimeric protein displays higher specific activity in vitro and a significantly longer half-life in mice than recombinant human TRAIL (rh-TRAIL). No short-term toxicity, especially liver toxicity, was observed. More importantly, Fc-TRAIL was much more effective in inhibiting tumor growth in a xenograft tumor model compared with rh-TRAIL. Our data suggest that fusion of Fc to TRAIL is able to improve the bioavailability and activity of TRAIL both in vitro and in vivo, and Fc-TRAIL may be explored for future clinical applications in cancer treatment and prevention. Mol Cancer Ther; 13(3); 643–50. ©2014 AACR.

Introduction

The ultimate goal of cancer drug discovery is to identify drugs that will specifically and efficiently kill cancer cells without affecting normal cells. TRAIL (also called Apo2L) seems to fit this description. TRAIL is a membrane-bound TNF family ligand (1, 2) and interacts with an unusually complex receptor system, which in humans comprises the fully functional death receptors 4 and 5 (DR4 and DR5) and the decoy receptors 1 and 2 (DcR1 and DcR2), and osteoprotegerin, which lack functional cytoplasmic signaling domains (3). TRAIL binding to DR4/5 results in receptor aggregation at the membrane and formation of the death-inducing signaling complex by recruiting the adaptor molecule, Fas-associated death domain–containing protein and pro-caspases 8 and 10, leading to the activation of the caspase cascade and apoptosis (4, 5). On the other hand, interactions of TRAIL with DcR1 and DcR2, and osteoprotegerin result in defective death signaling (6). Interest in TRAIL increased following reports that recombinant soluble TRAIL selectively killed a wide variety of transformed human tumor cell lines in vitro and in xenograft models without harming normal cells. TRAIL treatment has not produced toxicity in studies involving cynomolgus monkeys and chimpanzees, and has been well tolerated in phase I clinical testing (7–10). Agonistic anti-DR4 or -DR5 drugs (e.g., mapatumumab and PRO95780), which bind to TRAIL death receptors and trigger cell-death signaling) have produced little or no toxicity in phase I clinical trials (11, 12). However, results from early clinical trials of TRAIL therapy are disappointingly modest overall (13). There are a number of possible reasons for the discouraging outcome in clinic, including loss of cell surface expression of TRAIL receptors, high expression level of antiapoptotic proteins, and development of drug resistance. Agonistic anti-DR4 or -DR5 antibodies each only activate one death receptor; conversely, recombinant forms of human TRAIL can activate both DR4 and DR5, potentially increasing efficacy and broadening application. However, for therapy using recombinant human TRAIL (rh-TRAIL or Dulanermin; Genentech/Amgen), one major limitation is the rapid blood clearance of TRAIL in vivo. It has been shown that the half-life of rh-TRAIL is 3 to 5 minutes in rodents and 23 to 31 minutes in nonhuman primates (8). Therefore, improvement of the serum half-life of rh-TRAIL should be beneficial for its therapeutic application.

Fusion proteins containing the fragment crystallizable region (Fc) of human immunoglobulin have been intensely investigated for their effectiveness to improve biologic and pharmacologic properties of therapeutic proteins, with several notable successes recently coming to market (14). The presence of the Fc domain has markedly increased the plasma half-life of the target proteins, which prolongs...
therapeutic activity, due to its interaction with the neonatal Fc receptor (15), as well as to the slower renal clearance of larger molecules (16). Another significant advantage of Fc fusion proteins lies in the fact that most Fc fusions are expressed as homodimers, thus, increasing the avidity of the therapeutic proteins. Fas ligand, another TNF family member, has previously been fused to Fc yielding mixed structures of both low- and high-molecular weight (17). Interestingly, both low- and high-molecular weight Fc–FasL yielded equivalent cell killing activity. As such, we decided to construct a fusion protein of human Fc and TRAIL testing its activity and toxicity both in vitro and in vivo. Here, we show that Fc fusion enhanced specific activity of TRAIL in vitro and prolonged the serum half-life significantly in mice. We observed no short-term toxicity, including liver toxicity. In addition, we found that in a xenograft tumor model Fc–TRAIL inhibited tumor growth more strongly than rh-TRAIL.

Materials and Methods

Construction of plasmids

The Fc–TRAIL plasmid was constructed via overlap PCR by generating overlapping fragments of human IgG1 Fc and TRAIL cDNA. The cDNA sequence corresponding to amino acids 6–323 of IgG1 Fc were PCR amplified from HEK293T cDNA using a forward primer, including a 5’ exogenous translation start codon. The reverse primer sequence contained the first 10 base pairs of human TRAIL cDNA encoding amino acids 95 to 281. TRAIL cDNA was separately amplified from HCT116 cDNA using a forward primer containing the last 10 base pairs of IgG1 Fc and a reverse primer that extended through the endogenous stop codon of TRAIL. The resulting PCR products were gel purified using the Qia-Quick Gel Extraction Kit (Qiagen), pooled and PCR was repeated with the Fc forward primer and the TRAIL reverse primer. The resulting PCR product was cloned using the TOPO TA Cloning Kit (Invitrogen). The sequence and reading frame were verified, and the Fc–TRAIL coding region was then cloned into pET28a (++; Novagen) for bacterial expression and protein production. The DR5 expression plasmid was described previously (18).

Purification of Fc–TRAIL and FPLC analysis

The Fc–TRAIL fusion–containing plasmid was expressed in BL21 (DE3) E. coli (Invitrogen) and the protein was extracted and purified as previously published (19). rh-TRAIL was also produced in the laboratory (19). For FPLC (fast protein liquid chromatography), 1 mg of rh-TRAIL or 2.5 mg of Fc–TRAIL was loaded onto a Superdex 200 10/300 GL column and separated with the ÄKTA FPLC system (GE Healthcare). Proteins were eluted in TBS buffer (20-mm Tris-HCl, pH 7.4, 150-mm NaCl). All experiments were carried out at 4°C and a flow rate of 0.2 mL/min. Protein content in eluted samples was measured using the absorbance reading at 280 nm. Bio-Rad molecular weight size standards were used as size references.

Cell culture and reagents

NCI-H460 and NCI-H1157 cells were obtained from the American Type Culture Collection, verified yearly by genomic fingerprinting, and maintained in RPMI-1640 media for no more than 20 passages. For in vitro dose–response, cells were incubated with rh-TRAIL or Fc–TRAIL from 0.23 to 29.6 nmol/L. For inhibition of apoptosis, NCI-H460 cells were incubated with the caspase-8 inhibitor Z-IETD–FMK or the pan-caspase inhibitor Z-VAD–FMK (R&D Systems) at 20 μmol/L, with or without 0.23 nmol/L Fc–TRAIL. Cell death was determined using the Annexin V–fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (Sigma), or trypan blue dye exclusion (where indicated). To test specificity of Fc–TRAIL and rh-TRAIL binding to DR5, recombinant DR5–Fc (R&D Systems) was preincubated with equimolar concentrations of either rh-TRAIL or Fc–TRAIL for 30 minutes, then added to NCI-H460 cells. Cells were harvested 24 hours after complex addition and cell death was analyzed using trypan dye exclusion.

Western blotting and immunoprecipitation

Immunoblot analysis was performed using standard SDS–PAGE, transferred to nitrocellulose membranes and detection antibodies used as follows: caspase-8, caspase-3, DR4, DR5, and PARP (Cell Signaling Technology), β-actin (Sigma), and TRAIL (Santa Cruz Biotechnology, Inc.). Immunoprecipitation of Fc–TRAIL–DR5 and rh-TRAIL–DR5 complexes was performed as previously described (20). Briefly, 293T cells were transfected with a DR5-expressing plasmid. Forty-eight hours after transfection, equimolar amounts of either Fc–TRAIL or rh-TRAIL were added to the transfected cells. The cells were collected 15 minutes later and homogenized in lysis buffer [20 mmol/L Tris-HCl, (pH 7.4), 150 mmol/L NaCl, 0.2% Nonidet P40, 10% glycerol, and complete protease inhibitor cocktail (Sigma)]. The extracts were immunoprecipitated with an anti-TRAIL antibody (R&D Systems) and the immunocomplex was analyzed via Western immunoblot.

Animals

All animal experiments were reviewed and approved by the MD Anderson Animal Care and Use Committee. C57BL/6 mice were originally obtained from The Jackson Laboratory and are maintained as part of our breeding colonies. For serum half-life of rh-TRAIL and Fc–TRAIL 7- to 8-week-old male C57BL/6 mice were given 100 μL of rh-TRAIL (0.75 mg/mL) or Fc–TRAIL (1.5 mg/mL) by tail vein injection and blood was collected at the indicated time points. Serum was harvested and TRAIL-specific ELISA (R&D Systems) was performed to determine the TRAIL concentration. For biologic activity of TRAIL in serum, NCI-H460 cells were treated with serum harvested from rh-TRAIL- or Fc–TRAIL–treated mice at the indicated time points and cell death was determined as described above. For xenograft studies, one million NCI-H460 cells were implanted into the flank of 4- to 6-week-old male
athymic nude mice (NCI, Frederick, Maryland). Once the tumor reached approximately 50 mm³, mice were treated with either rh-TRAIL or Fc-TRAIL at 152 nmol/kg (1×) or 760 nmol/kg (5×) by daily intraperitoneal injection and tumors were measured for 10 days. Twelve hours after the last treatment, tumors were collected, processed, and subjected to TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling) analysis using the TACS TdT Fluorescein Kit (R&D Systems). To evaluate potential toxicity of Fc-TRAIL, male C57BL/6J mice were treated beginning at approximately 2 months of age with either PBS, or Fc-TRAIL at 10-fold higher dose than that used in the xenograft studies (1,520 nmol/kg) by intraperitoneal injection every other day for 30 days (15 treatments). Mouse weight and body condition were evaluated at each treatment and organ health was evaluated by histologic examination.

**Statistical analysis**
We compared differences between groups via the Student t test. P values of < 0.05 were considered to be statistically significant.

**Results**

**Construction and production of Fc fusion protein of human TRAIL**
As a 281–amino acid type II transmembrane protein, the amino-terminus of human TRAIL resides in the cytoplasm and its carboxyl-terminus extrudes outside of the cell. A rh-TRAIL of amino acids 114 to 281 (rh-TRAIL, Dulanermin, and AMG951) was shown to be soluble and biologically active. To construct the Fc-TRAIL fusion protein, we fused the Fc portion of human IgG1 to the N-terminus of human TRAIL from amino acids 95 to 281 (Fig. 1A). We included additional amino acids in the N-terminus of TRAIL to minimize the potential interference of the Fc domain on TRAIL interaction with its receptors. The Fc-TRAIL protein was induced in bacteria and purified to homogeneity via an N-terminal His-tag and chromatography (Fig. 1B). Between 5 and 10 mg of purified Fc-TRAIL was obtained from 1 L of bacterial culture.

To analyze the oligomerization status of Fc-TRAIL in our preparation, we performed gel permeation chromatography of recombinant Fc-TRAIL using FPLC. As a control, rh-TRAIL was similarly analyzed. We observed that the vast majority of rh-TRAIL exists as a trimer (peak II in Fig. 1C) with minor amounts in monomer (peak I), hexamer (peak III), and higher oligomer (peak IV) forms. The composition of Fc-TRAIL is more heterogeneous with the various forms of Fc-TRAIL more evenly distributed. We detected dimer (peak a in Fig. 1C), trimer (peak b), hexamer (peak c), and higher oligomer (peak d) forms of Fc-TRAIL. The trimer form seems to be somewhat more prevalent than the others (Fig. 1C). These data demonstrate that a greater portion of Fc-TRAIL exists in higher oligomerization states, which may lead to stronger activity.

**Biologic activity of Fc-TRAIL in vitro**
To test the activity of Fc-TRAIL, we used two non–small cell lung carcinoma cell lines (NCI-H460 and NCI-H157) that express both DR4 and DR5 (Fig. 2A) and are sensitive to rh-TRAIL. In both cell lines, we observed dose-dependent cell killing (Fig. 2B and C). Compared with rh-TRAIL, Fc-TRAIL exhibited higher activity in cell death induction in these two cell lines (Fig. 2B and C). To confirm that Fc-TRAIL induces cell death through the canonical death receptor pathway, cleavage and activation of caspase-8
and -3 were analyzed by Western blot analysis in the Fc-TRAIL–treated cells. As expected, cells treated with rh-TRAIL induced time-dependent caspase-8 and -3 cleavage (Fig. 2D). Similarly, Fc-TRAIL treatment also resulted in cleavage of caspase-8 and -3 (Fig. 2D). Cleavage of PARP was also detected in both rh-TRAIL- and Fc-TRAIL–treated cells (Fig. 2D), indicating that cleaved caspases are active and the treated cells were undergoing apoptosis. Consistent with the results demonstrating that Fc-TRAIL is more active in inducing cell death than rh-TRAIL (Fig. 2B and C), cleavage of caspase-8 and -3 were detected earlier and more abundantly in Fc-TRAIL–treated cells than in rh-TRAIL–treated cells (Fig. 2D). Finally, pan-caspase and caspase-8/10 inhibitors were added to the treated cells to confirm the role of caspase activation in Fc-TRAIL–induced cell killing (Fig. 2E). Both the pan-caspase inhibitor and the caspases 8/10 inhibitor blocked rh-TRAIL–induced apoptosis as expected (data not shown). These inhibitors also blocked Fc-TRAIL–induced cell killing (Fig. 2E). These data indicate that fusion of the Fc domain to TRAIL enhanced its specific activity in vitro and this Fc-TRAIL–induced apoptosis is mediated through the death receptor pathway.

Analysis of Fc-TRAIL-DR5 and rh-TRAIL-DR5 interactions

The observation that Fc-TRAIL displays an increased cell killing activity compared with rh-TRAIL in vitro (Fig. 2) suggests that Fc-TRAIL may bind more strongly to its receptors than rh-TRAIL. To test this, we first explored the antagonistic effect of soluble recombinant DR5-Fc on Fc-TRAIL- and rh-TRAIL–induced apoptosis in NCI-H460 cells (Fig. 3A). Preincubation of either Fc-TRAIL or rh-TRAIL with DR5-Fc inhibited cell death in a dose-dependent manner (Fig. 3A). More importantly, Fc-TRAIL is more sensitive to inhibition by DR5-Fc than rh-TRAIL in this assay (Fig. 3A), suggesting that Fc-TRAIL has stronger affinity to DR5 than rh-TRAIL. To confirm this notion, we evaluated the DR5 complex following either Fc-TRAIL or rh-TRAIL stimulation. We found that Fc-TRAIL binds substantially more DR5 than rh-TRAIL after incubating the ligands with cells overexpressing DR5 (Fig. 3B). Taken together, these results indicate that Fc-TRAIL has stronger affinity to DR5 than rh-TRAIL.

Serum half-life and biologic activity of Fc-TRAIL in mice

To evaluate the effect of Fc on TRAIL stability in vivo, a single dose of Fc-TRAIL was injected into the tail vein of C57BL/6 mice. For comparison, a single dose of rh-TRAIL was also injected into additional C57BL/6 mice. Blood samples were collected at various time points after injection and TRAIL concentrations were determined by ELISA (Fig. 4A). Similar to previous reports that rh-TRAIL has a half-life of 3 to 5 minutes in mice (8), we showed that rh-TRAIL was cleared rapidly in the blood of C57BL/6 mice with half-life of less than 10 minutes (Fig. 4A). In contrast, Fc-TRAIL displayed much slower plasma
clearance rate with an estimated half-life of 100 minutes (Fig. 4A). As a first step to evaluate the biologic activity of Fc-TRAIL in vivo, the corresponding blood samples were analyzed for their cell killing activities in vitro. Consistent with the stability results, cell killing activity declined rapidly in the serum of rh-TRAIL–injected mice, whereas the serum of Fc-TRAIL–injected mice retained cell killing activity for at least 12 hours after Fc-TRAIL injection (Fig. 4B). To demonstrate the antitumor efficacy of Fc-TRAIL, xenograft tumor mice were treated with daily injection of either rh-TRAIL or Fc-TRAIL at two dose levels, 1/2 and 5/2 (1/2 dose is 152 nmol/kg/d, which is equal to 3 mg/kg/d for rh-TRAIL and 7.2 mg/kg/d for Fc-TRAIL). At both doses, Fc-TRAIL displayed significantly stronger tumor growth inhibition compared with rh-TRAIL (Fig. 4C). Consistent with the tumor growth results, cell death analysis performed on the tumors at the end of treatment showed significantly more TUNEL-positive signals in Fc-TRAIL–treated tumors compared with rh-TRAIL (Fig. 4D). In this experiment, 1/2 Fc-TRAIL is equivalent to 5/2 rh-TRAIL in antitumor activity (Fig. 4C and D). These results demonstrate that fusion of the Fc fragment to TRAIL significantly prolongs the serum half-life and greatly enhances the antitumor activity of TRAIL in vivo.

Analysis of toxicity of Fc-TRAIL in mice

Although TRAIL is generally well tolerated and displays little or no toxicity in animals and humans (7, 10, 21), some forms of TRAIL containing either flag or polyhistidine tags have been reported to be toxic to isolated human hepatocytes and brain tissue (9, 22). To evaluate the safety profile of Fc-TRAIL, C57BL/6 mice were given daily doses of 72 mg/kg (10×) of Fc-TRAIL every other day for 30 days (15 treatments). The treated mice did not exhibit any obvious abnormalities and the food uptake was comparable with mice treated with vehicle control (data not shown). There was no significant weight loss during the treatment (Fig. 5A). No gross pathologic abnormality was observed at the end of the Fc-TRAIL treatment and major organs (brain, liver, lung, kidney, intestine, and spleen) show no differences in appearance and size (data not shown). Histologic analysis of brain, kidney (data not shown), and liver (Fig. 5B) sections showed normal structures in the treated animals. These results suggest that Fc-TRAIL, similar to rh-TRAIL, has no obvious toxicity in mice.

Discussion

A number of strategies based on TRAIL and its receptors are currently in development for cancer therapy, including soluble TRAIL protein (Dulanermin) developed by Genentech and Amgen, agonistic TRAIL receptor antibodies developed by several companies, and TRAIL gene delivery in early stages of development (23). In contrast with agonistic antibodies, the soluble TRAIL protein activates both DR4 and DR5, thus in principle should have broader therapeutic applications. However, one of the major drawbacks of soluble TRAIL protein is its poor pharmacokinetic property, specifically rapid clearance from the blood. The data presented in this study show that we can circumvent this deficiency by generating an Fc-TRAIL fusion protein. We demonstrated that not only did the Fc fragment not have a negative impact on TRAIL activity, it actually enhanced the specific activity by approximately 30% compared with rh-TRAIL in two cell lines at different doses in vitro (Fig. 2B and C). This
enhanced activity is likely due to the homodimeric nature of Fc because TRAIL activates its signaling by inducing oligomerization of TRAIL receptors on the cell surface. We have confirmed this notion by showing that more Fc-TRAIL exists as trimer, hexamer, and higher oligomer forms (Fig. 1C). Moreover, Fc-TRAIL displays higher affinity to DR5 than rh-TRAIL (Fig. 3). As we hypothesized, Fc fusion enhanced TRAIL plasma stability in mice by at least 10-fold compared with rh-TRAIL (Fig. 4A). Moreover, the cell killing activity of the sera was sustained for more than 12 hours following a single injection of Fc-TRAIL at 7.2 mg/kg (Fig. 4B), suggesting that Fc-TRAIL did not undergo denaturation in the blood, but remained biologically active. As a result, Fc-TRAIL showed a significantly stronger effect in xenograft tumor growth inhibition as 1× Fc-TRAIL is equivalent to 5× rh-TRAIL in antitumor activity (Fig. 4C and D). Although Fc fusion protein offers several advantages in improving serum half-life and increasing protein dimerization or multimerization, the uniformity of the fusion protein may present a potential concern. We have shown that Fc-TRAIL is heterogeneous in its oligomerization status with dimer, trimer, hexamer, and higher oligomer forms distributed relatively evenly (Fig. 1C). Importantly, despite this heterogeneity, Fc-TRAIL still exhibited 5-fold enhancement in its antitumor activity, which is a significant improvement over rh-TRAIL.

There are a number of reported attempts in the literature to improve TRAIL bioavailability in vivo. One approach involves the attachment of polyethylene glycol (PEG) to TRAIL. Although the authors of this study observed significant increases in serum half-life and greater stability in vitro, the addition of this moiety to TRAIL also led to a decrease in killing activity in vivo. Specifically, the IC_{50} for the PEGylated TRAIL was 4.2-fold higher than that of TRAIL alone (24). The second approach uses nanovector packaging of TRAIL. There are several studies using variations of nanovector or nanoparticle encapsulation of TRAIL (25–27). The most recent study (25), evaluated the ability of nanoparticle-conjugated TRAIL (NP-TRAIL) to induce apoptosis of glioma cells in vitro and in vivo. When directly compared with TRAIL in vitro, the NP-TRAIL offered only modest improvement in apoptotic induction. The in vivo survival curve was similar activity and serum half-life as mammalian cell-produced protein (data not shown).
Fc-TRAIL Conjugation Improves Activity In Vitro and In Vivo

Figure 5. Treatment with high-dose Fc-TRAIL does not induce toxicity in mice. A, mice were treated with either PBS (top graph) or Fc-TRAIL (bottom graph) by intraperitoneal injection for 30 days (15 treatments). Change in body weight is shown as a percentage of beginning weight. Each point is the average of 3 mice. Bars, SD. B, representative liver histology (hematoxylin and eosin stain) following 30 days of treatment with either PBS (left) or Fc-TRAIL (right).

min-conjugated TRAIL had a significantly improved serum half-life and improved antitumor activity, despite the significant reduction of in vitro cell killing activity. However, the large size of the albumin protein, may make it more difficult to purify large quantities of the fusion protein (28). In contrast, the Fc fragment is a smaller moiety and has been successfully used to make fusion proteins that are currently available in clinic (e.g., sTNFR2-Fc, Enbrel). The fusion protein that we have generated is relatively simple to purify (Fig. 1), and exhibits improved cytotoxicity in vitro (Fig. 2) when administered at equimolar concentrations. Fc-TRAIL also demonstrates a significantly improved retention in the serum, and this serum retention corresponds to increased cell killing activity in vitro as well as improved antitumor activity in vivo (Fig. 4C). Not only did Fc-TRAIL exhibit an enhanced antitumor effect over rh-TRAIL at equimolar concentrations, Fc-TRAIL exhibited an additional dose-response, whereas rh-TRAIL did not. This is likely due to the poor pharmacokinetics of rh-TRAIL; because the protein is cleared from the blood in less than 10 minutes (Fig. 4A), the increased concentration is of minimal biologic effect. In contrast, increasing the dose of Fc-TRAIL leads to significant improvement in tumor growth suppression, which is likely due to the enhanced serum stability and biologic activity of this protein (Fig. 4C). Finally, when given 15 doses (72 mg/kg, every other day) of Fc-TRAIL 10-fold higher than the dose used in the xenograft model, mice did not exhibit signs of toxicity (Fig. 5).

In summary, Fc-TRAIL has significantly improved properties over those of rh-TRAIL that make it a promising candidate for targeting cancer in the clinic.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: X. Wu
Development of methodology: H. Wang, X. Wu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Wang, J.S. Davis, X. Wu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Wang, J.S. Davis, X. Wu
Writing, review, and/or revision of the manuscript: H. Wang, J.S. Davis, X. Wu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Wu
Study supervision: X. Wu

Acknowledgments
The authors thank Todd Lin and Gilbert Lee of the Department of Biochemistry and Molecular Biology, The University of Texas MD Anderson Cancer Center for their help in purification of Fc-TRAIL, and Troy Johnson and Paul Leonard for their assistance in FPLC assay.

Grant Support
This work was supported by CPRIT grants RP110107 and RP120280 (to X. Wu). J.S. Davis is supported by the Janice Davis Gordon Memorial Postdoctoral Fellowship in Colorectal Cancer Prevention Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 12, 2013; revised December 6, 2013; accepted December 24, 2013; published OnlineFirst January 15, 2014.
References


Immunoglobulin Fc Domain Fusion to TRAIL Significantly Prolongs Its Plasma Half-Life and Enhances Its Antitumor Activity

Haizhen Wang, Jennifer S. Davis and Xiangwei Wu