

Accordingly, there is a need in the art for novel TRAIL receptor agonists that exhibit high biological activity, high stability, low toxicity, and allow for efficient recombinant manufacturing.

5 Summary

The present disclosure provides specific TRAIL receptor agonist proteins that exhibit low proteolytic degradation, long half-life, and low TRAIL receptor superclustering *in vivo* (along with concomitant toxicity).

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The TRAIL receptor agonist proteins of the instant disclosure generally comprise: (i) a first soluble TRAIL cytokine domain; (ii) a first peptide linker; (iii) a second soluble TRAIL domain; (iv) a second peptide linker; (v) a third soluble TRAIL domain; and (vi) an antibody Fc fragment.

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Disclosed is a single-chain fusion polypeptide comprising: (i) a first soluble TRAIL domain, (ii) a first peptide linker, (iii) a second soluble TRAIL domain, (iv) a second peptide linker, (v) a third soluble TRAIL domain, and (vi) an antibody Fc fragment. In one embodiment, the antibody Fc fragment (vi) is located N terminal to the first TRAIL domain (i) and/or C-terminal to the third TRAIL domain (v). In another embodiment the antibody Fc fragment is located C-terminally to the third TRAIL domain (v). In one embodiment, the polypeptide is substantially non aggregating. In another embodiment, the second and/or third soluble TRAIL domain is an N-terminally shortened domain which optionally comprises amino acid sequence mutations.

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In one embodiment, at least one of the soluble TRAIL domains, particularly at least one of the soluble TRAIL domains (iii) and (v), is a soluble TRAIL domain with an N-terminal sequence which starts between amino acid Gln120 and Val122 of human TRAIL and wherein Arg121 may be replaced by a neutral amino acid, e.g., Ser or Gly. In another embodiment, at least one of the soluble TRAIL domains, particularly at least one of the soluble TRAIL domains (iii) and (v), is a soluble TRAIL domain with an N-terminal sequence selected from (a) Arg121 – Val122 – Ala123 and (b)

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(Gly/Ser)121 – Val122 – Ala123. In one embodiment, the soluble TRAIL domain ends with amino acid Gly281 of human TRAIL and/or optionally comprises a mutation at positions R130, G160, H168, R170, H177, Y189, R191, Q193, E195, N199, K201, Y213, T214, S215, H264, I266, D267 or D269 or at two or more of said positions. In one embodiment, the soluble TRAIL domain (i) consists of amino acids Gln120 – Gly281 of human TRAIL according to SEQ ID NO: 1 and the soluble TRAIL domains (iii) and (v) consist of amino acids Arg121 – Gly281 of human TRAIL according to SEQ ID NO: 1.

In one embodiment, the first and second peptide linkers (ii) and (iv) independently have a length of 3-8 amino acids, particularly a length of 3, 4, 5, 6, 7, or 8 amino acids, and preferably are glycine/serine linkers, optionally comprising an asparagine residue which may be glycosylated. In one embodiment, the first and the second peptide linkers (ii) and (iv) consist of the amino acid sequence according to SEQ ID NO: 2. In another embodiment, the polypeptide additionally comprises an N-terminal signal peptide domain, *e.g.*, of SEQ ID NO: 12, which may comprise a protease cleavage site, and/or which additionally comprises a C-terminal element which may comprise and/or connect to a recognition/purification domain, *e.g.*, a Strep-tag according to SEQ ID NO: 13.

In one embodiment, the antibody Fc fragment (vi) is fused to the soluble TRAIL domain (i) and/or (v) via a hinge-linker, preferably of SEQ ID NO: 11. In another embodiment, the antibody Fc fragment (vi) consists of the amino acid sequence as shown in SEQ ID NO: 10 or 17. In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO: 14, 15 or 18.

Disclosed is a TRAIL receptor agonist protein comprising a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19, 20 or 21. In one aspect, there is provided a TRAIL receptor agonist protein comprising a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19.

Disclosed is a TRAIL receptor agonist protein comprising a polypeptide having the amino acid sequence set forth in SEQ ID NO: 26, 27, 28, 29, or 30.

In another aspect, there is provided a TRAIL receptor agonist protein comprising a dimer of two polypeptides having the amino acid sequence set forth in SEQ ID NO: 19. In one embodiment, the two polypeptides are covalently linked through three interchain disulfide bonds formed between cysteine residues 513, 519, and 522 of each polypeptide.

In one embodiment, one or more of the asparagine residues at positions 168 and 337 of the polypeptide(s) are N-glycosylated. In another embodiment, the asparagine residues at positions 168 and 337 of the polypeptide(s) are both N-glycosylated.

In another embodiment, the polypeptide(s) are further post-translationally modified. In another embodiment, the post-translational modification comprises the N-terminal glutamine modified to pyroglutamate.

In another aspect, there is provided a pharmaceutical composition comprising a TRAIL receptor agonist protein disclosed herein and one or more pharmaceutically acceptable carriers, diluents, excipients, and/or adjuvants. In another aspect, there is provided a nucleic acid molecule encoding the TRAIL receptor agonist protein disclosed herein. In another aspect, there is provided an expression vector comprising the nucleic acid molecule disclosed herein. In another aspect, there is provided a cell comprising the nucleic acid molecule disclosed herein. In a further embodiment, the cell is a eukaryotic cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is a Chinese Hamster Ovary (CHO) cell. In other embodiments, the cell is selected from the group consisting of CHO-DBX11, CHO-DG44, CHO-S, and CHO-K1 cells. In other embodiments, the cell is selected from the group consisting of Vero, BHK, HeLa, COS, MDCK, HEK-293, NIH-3T3, W138, BT483, Hs578T, HTB2, BT20, T47D, NS0, CRL7030, HsS78Bst, PER.C6, SP2/0-Ag14, and hybridoma cells.

Disclosed is a method of treating a subject having a TRAIL-associated disease or disorder, the method comprising administering to the subject an effective amount of the TRAIL receptor agonist protein. In one

intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal administration.

In one embodiment, the TRAIL receptor agonist protein is administered as a single bolus. In another embodiment, TRAIL receptor agonist protein may be administered over several divided doses. The TRAIL receptor agonist protein can be administered at about 0.1-100 mg/kg. In one embodiment, the TRAIL receptor agonist protein can be administered at a dosage selected from the group consisting of: about 0.1-0.5, 0.1-1, 0.1-10, 0.1-20, 0.1-50, 0.1-75, 1-10, 1-15, 1-7.5, 1.25-15, 1.25-7.5, 2.5-7.5, 2.5-15, 5-15, 5-7.5, 1-20, 1-50, 7-75, 1-100, 5-10, 5-15, 5-20, 5-25, 5-50, 5-75, 10-20, 10-50, 10-75, and 10-100 mg/kg. In other embodiments, the TRAIL receptor agonist protein is present in pharmaceutical compositions at about 0.1-100 mg/ml. In one embodiment, the TRAIL receptor agonist protein is present in pharmaceutical compositions at an amount selected from the group consisting of: about 0.1-0.5, 0.1-1, 0.1-10, 0.1-20, 0.1-50, 0.1-75, 1-10, 1-20, 1-50, 1-75, 1-100, 5-10, 5-15, 5-20, 5-25, 5-50, 5-75, 10-20, 10-50, 10-75, or 10-100 mg/ml. In other embodiments, a therapeutically effective amount of TRAIL receptor agonist protein is administered to a subject. In another embodiment, a prophylactically effective amount of TRAIL receptor agonist protein is administered to a subject.

In another aspect, there is provided use of an effective amount of the TRAIL receptor agonist protein as disclosed herein in the manufacture of a medicament for treating a subject having a TRAIL-associated disease or disorder, wherein the TRAIL-associated disease or disorder is a cancer.

Description of the Figures

Figure 1 Domain structure of a single-chain fusion polypeptide comprising three TRAIL domains. I., II., III. Soluble TRAIL domains.

Figure 2 Schematic picture representing the general structure of TRAIL.
 ■ ■ ■ Cell membrane, N-terminus located within the cell,
 1. anti-parallel β -fold of receptor-binding domain (RBD),
 2. interface of RBD and cell membrane,
 3. protease cleavage site.

Claims

1. A TRAIL receptor agonist protein comprising a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19.
2. A TRAIL receptor agonist protein comprising a dimer of two polypeptides having the amino acid sequence set forth in SEQ ID NO: 19.
3. The TRAIL receptor agonist protein of claim 2, wherein the two polypeptides are covalently linked through three interchain disulfide bonds formed between cysteine residues 513, 519, and 522 of each polypeptide.
4. The TRAIL receptor agonist protein of claim 1, 2 or 3, wherein one or more of the asparagine residues at positions 168 and 337 of the polypeptide(s) are N-glycosylated.
5. The TRAIL receptor agonist protein of claim 1, 2 or 3, wherein the asparagine residues at positions 168 and 337 of the polypeptide(s) are both N-glycosylated.
6. The TRAIL receptor agonist protein of claims 1-5, wherein the polypeptide(s) are further post-translationally modified.
7. The TRAIL receptor agonist protein of claim 6, wherein the post-translational modification comprises modification of the N-terminal glutamine to pyroglutamate.
8. A pharmaceutical composition comprising the TRAIL receptor agonist protein of any one of claims 1-7 and one or more pharmaceutically acceptable carriers, diluents, excipients, and/or adjuvants.
9. A nucleic acid molecule encoding the TRAIL receptor agonist protein of claim 1.
10. An expression vector comprising the nucleic acid molecule of claim 9.

11. A cell comprising the nucleic acid molecule of claim 9.
12. The cell of claim 11, which is a eukaryotic cell.
13. The cell of claim 11, wherein the cell is a mammalian cell.
14. The cell of claim 11, wherein the cell is a Chinese Hamster Ovary (CHO) cell.
15. Use of an effective amount of the TRAIL receptor agonist protein of any one of claims 1-7 in the manufacture of a medicament for treating a subject having a TRAIL-associated disease or disorder, wherein the TRAIL-associated disease or disorder is a cancer.
16. The use of claim 15, wherein the cancer is a tumor.
17. The use of claim 16, wherein the tumor is a solid tumor.
18. The use of claim 16, wherein the tumor is a lymphatic tumor.
19. The use of claim 17, wherein the solid tumor is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), pancreatic cancer, sarcoma, esophageal cancer, gastric cancer, breast cancer, and head and neck cancer.
20. The use of claim 18, wherein the lymphatic tumor is acute myeloid leukemia (AML).
21. The use of claim 19, wherein the sarcoma is Ewing's sarcoma.