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.

Summary

In a first aspect, there is provided a nucleic acid molecule comprising SEQ ID NO:16. In a second aspect, there is provided a nucleic acid molecule comprising nucleotides 19 to 2298 of SEQ ID NO:16. In a third aspect, there is provided a nucleic acid molecule comprising nucleotides 79 to 2298 of SEQ ID NO:16.

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).

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.

In one embodiment, the present disclosure provides 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.

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)

(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.

In another embodiment, the present disclosure provides a TRAIL receptor agonist protein comprising a polypeptide having the amino acid sequence set forth in SEQ ID NO: 19, 20 or 21.

In another embodiment, the present disclosure provides 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 embodiment, the present disclosure provides a TRAIL receptor agonist protein comprising 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 embodiment, the present disclosure provides 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 embodiment, the present disclosure provides a nucleic acid molecule encoding the TRAIL receptor agonist protein. In another embodiment, the present disclosure provides an expression vector comprising the nucleic acid molecule. In a fourth aspect, there is provided an expression vector comprising the nucleic acid molecule according to the second or third aspect. In another embodiment, the present disclosure provides a cell comprising the nucleic acid molecule. In a fifth aspect, there is provided a cell comprising the nucleic acid molecule according to the second or third aspect. 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-Agl4, and hybridoma cells.

In another embodiment, the present disclosure provides 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

Claims

1. A nucleic acid molecule comprising SEQ ID NO:16.

2. A nucleic acid molecule comprising nucleotides 19 to 2298 of SEQ ID NO:16.

3. A nucleic acid molecule comprising nucleotides 79 to 2298 of SEQ ID NO:16.

4. An expression vector comprising the nucleic acid molecule according to Claims 2 or 3.

5. A cell comprising the nucleic acid molecule according to Claims 2 or 3.

6. The cell according to Claim 5, which is a eukaryotic cell.

7. The cell according to Claim 6, wherein the cell is a mammalian cell.

8. The cell according to Claim 7, wherein the cell is a Chinese Hamster Ovary (CHO) cell.