

and inducing IFN γ secretion. However, the IL-12p40 subunit can also exist as a homodimer which has been reported to antagonize IL-12 activity by competing for binding to IL-12 receptor.

[0005] The present invention addresses the short half-life of IL-12 by providing novel IL-12-Fc fusion proteins, as well as novel IL-12 variants with decreased potency.

SUMMARY

[0006] In one aspect, the present disclosure provides a heterodimeric Fc fusion protein comprising: a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain with a first domain linker, wherein said variant IL-12p40 subunit domain comprises the amino acid sequence of SEQ ID NO:363, wherein said first Fc domain comprises the amino acid sequence of SEQ ID NO:42, wherein said first domain linker comprises the amino acid sequence of SEQ ID NO:107; and b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said second Fc domain with a second domain linker, wherein said IL-12p35 subunit domain comprises the amino acid sequence of SEQ ID NO:2, wherein said second Fc domain comprises the amino acid sequence of SEQ ID NO:41, and wherein said second domain linker comprises the amino acid sequence of SEQ ID NO:10, wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains.

In another aspect, the present disclosure provides a heterodimeric Fc fusion protein comprising:

a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain, wherein said first fusion protein comprises the amino acid sequence of SEQ ID NO:382 further comprising the amino acid substitutions M428L/N434S; and b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said Fc domain, wherein said second fusion protein comprises the amino acid sequence of SEQ ID NO:404 further comprising the amino acid substitutions M428L/N434S, wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains. In some examples, the present disclosure provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[0007] In some examples, the present disclosure provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E,

WHAT IS CLAIMED IS:

1. A heterodimeric Fc fusion protein comprising:

a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain with a first domain linker, wherein said variant IL-12p40 subunit domain comprises the amino acid sequence of SEQ ID NO:363, wherein said first Fc domain comprises the amino acid sequence of SEQ ID NO:42, wherein said first domain linker comprises the amino acid sequence of SEQ ID NO:107; and

b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said second Fc domain with a second domain linker, wherein said IL-12p35 subunit domain comprises the amino acid sequence of SEQ ID NO:2, wherein said second Fc domain comprises the amino acid sequence of SEQ ID NO:41, and wherein said second domain linker comprises the amino acid sequence of SEQ ID NO:10, wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains.

2. A heterodimeric Fc fusion protein comprising:

a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain, wherein said first fusion protein comprises the amino acid sequence of SEQ ID NO:382 further comprising the amino acid substitutions M428L/N434S; and

b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said Fc domain, wherein said second fusion protein comprises the amino acid sequence of SEQ ID NO:404 further comprising the amino acid substitutions

M428L/N434S, wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains.

3. A composition comprising a heterodimeric Fc fusion protein according to claim 1 or claim 2 for use in therapy.
4. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to claim 1 or claim 2.
5. A host cell comprising said one or more nucleic acids of claim 4.
6. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 5 under conditions whereby said heterodimeric Fc fusion protein is produced.
7. A method of purifying a heterodimeric Fc fusion protein according to claim 1 or claim 2, said method comprising:
 - a) providing a composition comprising the heterodimeric Fc fusion protein;
 - b) loading said composition onto an ion exchange column; and
 - c) collecting a fraction containing said heterodimeric Fc fusion protein.
8. Use of a heterodimeric Fc fusion protein according to claim 1 or claim 2 in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein said protein is to be administered to said patient.
9. The use according to claim 8, wherein a checkpoint blockade antibody is to be further administered.
10. The use according to claim 9, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.

11. The use according to claim 10, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.
12. The use according to claim 10, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.
13. The use according to any one of claims 8-12, wherein the patient exhibits an increase in lymphocytes following administration.
14. The use according to any one of claims 8-13, wherein the patient exhibits an increase in peripheral CD8+ T cells following administration.
15. Use of a heterodimeric Fc fusion protein according to claim 1 or claim 2 in the manufacture of a medicament for inducing T cell expansion in a patient in need thereof, wherein said protein is to be administered to said patient.
16. The use according to claim 15, wherein a checkpoint blockade antibody is to be further administered.
17. The use according to claim 16, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.
18. The use according to claim 17, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.
19. The use according to claim 17, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.
20. The use according to any one of claims 15 to 19, wherein the T cell expansion is at least a 2-fold increase in T cells.

21. The use according to any one of claims 9 to 14 and 16 to 20 wherein the heterodimeric Fc fusion protein is to be administered before the checkpoint blockade antibody to increase checkpoint expression prior to treatment.

22. The heterodimeric Fc fusion protein according to claim 1, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N434S.