

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. The heterodimeric Fc fusion protein according to claim 1, 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.~~

~~3. The heterodimeric Fc fusion protein according to claims 1 or 2, 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, E59K/K99E/K264E,~~
~~D18K/E59K/K99E/K264E, D18K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E,~~
~~E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D,~~
~~N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D,~~
~~N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D,~~
~~N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q,~~
~~N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q,~~
~~N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,~~
~~E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E,~~
~~E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E,~~
~~E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E,~~
~~E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E,~~
~~E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,~~
~~E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,~~
~~E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and~~
~~E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.~~

4. The heterodimeric Fc fusion protein according to any one of claims 1-3, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70

~~(IL 12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL 12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL 12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL 12p40(D161N)), xviii) SEQ ID NO:74 (IL 12p40(E73Q)), xix) SEQ ID NO:75 (IL 12p40(Q144E)), xx) SEQ ID NO:76 (IL 12p40(E262Q)), xxi) SEQ ID NO:77 (IL 12p40(E100Q)), xxii) SEQ ID NO:78 (IL 12p40(D18N)), xxiii) SEQ ID NO:79 (IL 12p40(E33Q)), xxiv) SEQ ID NO:80 (IL 12p40(Q229E)), xxv) SEQ ID NO:81 (IL 12p40(E235Q)), xxvi) SEQ ID NO:82 (IL 12p40(Q256N)), xxvii) SEQ ID NO:83 (IL 12p40(E299Q)), xxviii) SEQ ID NO:84 (IL 12p40(D87N)), xxix) IL 12p40(E32Q), xxx) IL 12p40(D34N), xxxi) IL 12p40(S43E), xxxii) IL 12p40(S43K), xxxiii) SEQ ID NO:XXX (IL 12p40(E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL 12p40(E59K)), xxxv) IL 12p40(K99E), xxxvi) IL 12p40(K163E), xxxvii) IL 12p40(E187Q), xxxviii) IL 12p40(K258E), xxxix) IL 12p40(K260E), xl) SEQ ID NO:206 (IL 12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL 12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL 12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL 12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL 12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL 12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL 12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL 12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL 12p40(E59Q/K260E)), xlix) SEQ ID NO: 326 (IL 12p40 (D34N/E59K)), l) SEQ ID NO: 325 (IL 12p40(E59K/K99E)), li) SEQ ID NO: 339 (IL 12p40(D18K/E59K/K99E)), lii) SEQ ID NO: 343 (IL 12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: 336 (IL 12p40 (E59K/K99Y)), liv) SEQ ID NO: 335 (IL 12p40 (E59Y/K99E)), lv) SEQ ID NO: 338 (IL 12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: 340 (IL 12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: 341 (IL 12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: 342 (IL 12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: 343 (IL 12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: 344 (IL 12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: 360 (IL 12p40 (C252S)), lxii) SEQ ID NO: 361 (IL 12p40 (D18K/E59K/K99E/C252S)), lxiii) SEQ ID NO: 362 (IL 12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: 363 (IL 12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: 364 (IL 12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: 365 (IL 12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: 254 (IL 12p40 (N103D/N113D)), lxviii) SEQ ID NO: 255 (IL 12p40 (N103D/N200D)), lxix) SEQ ID NO: 256 (IL 12p40 (N103D/N281D)), lxx) SEQ ID NO: 257 (IL 12p40 (N113D/N200D)), lxxi) SEQ ID NO: 258 (IL 12p40 (N113D/N281D)), lxxii) SEQ ID NO: 259 (IL 12p40 (N200D/N281D)), lxxiii) SEQ ID NO: 260 (IL 12p40 (N103D/N113D/N200D)),~~

~~lxxiv) SEQ ID NO: 261 (IL 12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: 262 (IL 12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: 263 (IL 12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: 264 (IL 12p40 (N103Q)), lxxviii) SEQ ID NO: 265 (IL 12p40 (N113Q)), lxxix) SEQ ID NO: 266 (IL 12p40 (N200Q)), lxxx) SEQ ID NO: 267 (IL 12p40 (N281Q)), lxxxi) SEQ ID NO: 268 (IL 12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: 269 (IL 12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: 270 (IL 12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: 271 (IL 12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: 272 (IL 12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: 273 (IL 12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: 274 (IL 12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: 275 (IL 12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: 276 (IL 12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: 277 (IL 12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO: 278 (IL 12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO: 327 (IL 12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO: 328 (IL 12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO: 329 (IL 12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO: 331 (IL 12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: 337 (IL 12p40 (E59Y/K99Y)), xcvii) SEQ ID NO: 366 (IL 12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO: 367 (IL 12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO: 368 (IL 12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO: 369 (IL 12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO: 370 (IL 12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO: 371 (IL 12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO: 372 (IL 12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO: 373 (IL 12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO: 374 (IL 12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO: 375 (IL 12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO: 376 (IL 12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO: 377 (IL 12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO: 378 (IL 12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).~~

~~5. The heterodimeric Fc fusion protein according to any one of claims 1-4, wherein said variant IL-12p40 subunit has at least 90% identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).~~

~~6. The heterodimeric Fc fusion protein according to any one of claims 1-5, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rβ1), IL-12 receptor subunit beta-2 (IL-12Rβ2), and/or IL-12 receptor complex.~~

~~7. The heterodimeric Fc fusion according to any one of claims 1-6, wherein said heterodimeric Fc fusion protein is selected from the group consisting of: XENP31251 (SEQ ID NOS:302 and 452), XENP31254 (SEQ ID NOS:305 and 455), XENP31258 (SEQ ID NOS:309 and 460), XENP32186 (SEQ ID NOS:317 and 461), XENP32187 (SEQ ID NOS:380 and 402), XENP32188 (SEQ ID NOS:381 and 403), XENP32189 (SEQ ID NOS:382 and 404), XENP32190 (SEQ ID NOS:425 and 405), and XENP32191 (SEQ ID NOS:384 and 406).~~

~~8. The heterodimeric Fc fusion protein according to any one of claims 1-7, further comprising one or more modifications to the IL-12p40 subunit selected from the group consisting of: N103Q, N113Q, N200Q, and N281Q.~~

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.

93. A composition comprising a heterodimeric Fc fusion protein according to ~~any one of claims 1-8~~ or claim 2 for use in therapy.

104. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to ~~any one of claims 1~~ or claim 2-8.

115. A host cell comprising said one or more nucleic acids of claim 104.

126. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 115 under conditions whereby said heterodimeric Fc fusion protein is produced.

137. A method of purifying a heterodimeric Fc fusion protein according to ~~any one of claims 1 or claim 2-8~~, 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.

148. Use of a heterodimeric Fc fusion protein according to claims ~~1~~ or claim 2-8 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.

159. The use according to claim 148, wherein a checkpoint blockade antibody is to be further administered.

[1610](#). The use according to claim [159](#), 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.

[1711](#). The use according to claim [1610](#), wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

[1812](#). The use according to claim [1610](#), wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

[1913](#). The use according to any one of claims [14 to 188-12](#), wherein the patient exhibits an increase in lymphocytes following administration.

[2014](#). The use according to any one of claims [14 to 198-13](#), wherein the patient exhibits an increase in peripheral CD8+ T cells following administration.

[2115](#). Use of a heterodimeric Fc fusion protein according to claims [1-81](#) 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.

[2216](#). The use according to claim [2115](#), wherein a checkpoint blockade antibody is to be further administered.

[2317](#). The use according to claim [2216](#), 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.

[2418](#). The use according to claim [2317](#), wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

[2519](#). The use according to claim [2317](#), wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

~~26~~20. The use according to any one of claims ~~24~~15 to ~~25~~19, wherein the T cell expansion is at least a 2-fold increase in T cells.

~~27~~21. The use according to any one of claims ~~15~~9 to ~~20~~14 and ~~22~~16 to ~~26~~20 wherein the heterodimeric Fc fusion protein is to be administered before the checkpoint blockade antibody to increase checkpoint expression prior to treatment.

~~28~~22. The heterodimeric Fc fusion protein according to ~~any one of claims~~ ~~11~~8, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N43~~2~~4S.

~~29. The heterodimeric fusion protein according to claim 1 wherein said first fusion protein comprises the amino acid sequence of SEQ ID NO:382 further comprising the amino acid substitutions M428L/N434S, and wherein said second fusion protein comprises the amino acid sequence of SEQ ID NO:404 further comprising the amino acid substitutions M428L/N434S.~~