

WHAT IS CLAIMED IS:

1. A method of promoting the activation and proliferation of a natural killer cell, the method comprising:

(a) contacting a natural killer cell in a liquid culture medium comprising an effective amount of a first multi-chain chimeric polypeptide for a first period of time under conditions that allow for the differentiation of the natural killer cell; and

(b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) a second multi-chain chimeric polypeptide, and (ii) an IgG1 antibody construct, for a second period of time under conditions that allow for the activation and proliferation of the natural killer cell,

wherein the first multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12; and

wherein the second multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains, and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; the first target-

binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21, and the IgG1 antibody construct comprises at least one antigen-binding domain that binds specifically to the linker domain.

2. The method of claim 1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide in the first multi-chain chimeric polypeptide.

3. The method of claim 1, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

4. The method of any one of claims 1-3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

5. The method of any one of claims 1-3, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

6. The method of any one of claims 1-5, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

7. The method of any one of claims 1-5, wherein the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

8. The method of any one of claims 1-7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

9. The method of claim 8, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

10. The method of any one of claims 1-9, wherein the first multi-chain chimeric polypeptide and the second multi-chain chimeric polypeptide do not stimulate coagulation in a mammal.

11. The method of any one of claims 1-10, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide and/or the second multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

12. The method of claim 11, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

13. The method of any one of claims 1-10, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide and/or the second multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

14. The method of any one of claims 1-13, wherein the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide are each independently a soluble IL-15 or a soluble IL-18.

15. The method of any one of claims 1-14, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18.

16. The method of any one of claims 1-14, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12.

17. The method of claim 16, wherein: the first target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble human IL-18; and
the second target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble human IL-12.

18. The method of claim 1, wherein:
the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to
YFGKLESKLSVIRNLNDQVLFDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKD
SQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGH
DNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTN
TVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTEC

DLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ
PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSS
GKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQKEG
EFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQV
ISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 63); and

the second chimeric polypeptide comprises a sequence that is at least 80% identical to

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
RVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIR
DIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR
EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGG
GGSGGGGSRNLPVATPDPGMFCLHHSQNLLRAVSNMLQKARQTLEFYPTCS
EEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN
FNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPM
SVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTP
SLKCIR (SEQ ID NO: 65).

19. The method of any one of claims 1-18, wherein the first target-binding domain and the linker domain in the first chimeric polypeptide in the second multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

20. The method of any one of claims 1-18, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the linker domain in the first chimeric polypeptide.

21. The method of any one of claims 1-20, wherein the linker domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide in the second multi-chain chimeric polypeptide.

22. The method of any one of claims 1-20, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

23. The method of any one of claims 1-22, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide of the second multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

24. The method of any one of claims 1-22, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

25. The method of any one of claims 1-24, wherein the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide are each independently a soluble IL-7 or a soluble IL-21.

26. The method of any one of claims 1-25, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7.

27. The method of any one of claims 1-25, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21.

28. The method of claim 27, wherein:
the first target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble human IL-7; and

the second target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble human IL-21.

29. The method of claim 1, wherein:

the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTT
NTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTE
CDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLYTLYYWKSSS
SGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEK
GEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ
VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 67); and

the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIIKKLRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLSYRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 69).

30. The method of claim 1, wherein:

the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIIKKLRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDSGGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFS
YPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDE
RTLVRNNTFLSLRDVFGKDLYTLYYWKSSSSGKKTAKTNTNEFLIDVDKG
ENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLI

QSMHIDATLYTESDVHPSCKVTAMKCFLLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:
71); and

the second chimeric polypeptide in the second multi-chain chimeric
polypeptide comprises a sequence that is at least 80% identical to
DCDIEGKDQYQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLQEIKTWCNKMILMGTKEHITCPP
PMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWT
TPSLKCIR (SEQ ID NO: 73).

31. The method of any one of claims 1-30, wherein the IgG1 antibody
construct is a monoclonal IgG1 antibody, where both antigen-binding domains in the
monoclonal IgG1 antibody bind specifically to the linker domain.

32. The method of any one of claims 1-31, wherein the first period of time is
about 15 minutes to about 4 hours, and the second period of time is about 1 day to
about 30 days.

33. The method of any one of claims 1-32, wherein the liquid culture medium
in step (b) comprises the second multi-chain chimeric polypeptide and the IgG1
antibody construct at a molar ratio of about 0.5:1 to about 2:1.

34. The method of any one of claims 1-33, wherein the NK cell was
previously obtained from a subject.

35. The method of claim 34, wherein the NK cell is to be obtained from the
subject prior to step (a).

36. The method of any one of claims 1-35, wherein the NK cell has previously
been genetically modified to express a chimeric antigen receptor or a recombinant T-
cell receptor.

37. The method of any one of claims 1-36, wherein the method further comprises, after step (b), isolating the NK cell.

38. The method of any one of claims 1-37, wherein the NK cell is to be administered to a subject in need thereof, after the contacting step.

39. An activated NK cell produced by the method of any one of claims 1-38.

40. A pharmaceutical composition comprising the activated NK cell of claim 39.

41. A kit comprising a pharmaceutical composition comprising the activated NK cell of claim 39.

42. Use of the activated NK cell of claim 39 or the pharmaceutical composition of claim 40 in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof.

43. Use of the activated NK cell or the activated T cell of claim 39 or the pharmaceutical composition of claim 40 in the manufacture of a medicament for treating a subject in need thereof.

44. A kit comprising:

(a) a first multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first

target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12;

(b) a second multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains, and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; and the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21; and

(c) an IgG1 antibody that comprises at least one antigen-binding domain that binds specifically to the linker domain.