

MULTI-CHAIN CHIMERIC POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to U.S. Provisional Patent Application Serial No. 62/864,996, filed on June 21, 2019; the entire contents of which are herein incorporated by reference.

TECHNICAL FIELD

 The present disclosure relates to the field of biotechnology, and more
10 specifically, to the use of a combination of two multi-chain chimeric polypeptides, each having a linker domain positioned between two target-binding domains.

BACKGROUND

 Adoptive immunotherapy or cellular therapy requires the culture of immune cells obtained from a subject *in vivo* (and optionally genetic manipulation of the
15 immune cells to express a T-cell or a chimeric antigen receptor) before administration back into the subject. A sufficient number of immune cells is necessary in order to provide a therapeutic effect in the subject. In many examples, immune cells obtained from a subject need to be cultured for three or more weeks before a therapeutically effective number of immune cells can be obtained. In addition, many methods of
20 culturing immune cells obtained from a subject *in vitro* require a layer of feeder cells, which requires subsequent purification or isolation of the immune cells before administration back to the subject.

SUMMARY

 The present ~~invention~~disclosure is based on the discovery that a specific two-
25 step method of contacting NK cells results in a significant expansion of NK cells *in vitro* and the expanded NK cells are highly activated with a long persistence and a high degree of cytotoxicity against diseased cells in a recipient host.

 In one ~~aspect~~example, provided herein is a method of promoting the activation and proliferation of a natural killer cell, the method includes: (a) contacting a natural
30 killer cell in a liquid culture medium that includes 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 that includes 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 includes: a first chimeric polypeptide that includes 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 that includes 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 includes: a first chimeric polypeptide that includes a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and a second chimeric polypeptide that includes 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 includes at least one antigen-binding domain that binds specifically to the linker domain.

In one aspect, the present disclosure refers to 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.

In some embodimentexamples, 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. In some embodimentexamples, the first chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some embodimentexamples, 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. In some [embodimentexamples](#), the first chimeric
 polypeptide in the first multi-chain chimeric polypeptide further includes a linker
 sequence between the soluble tissue factor domain and the first domain of the pair
 5 of affinity domains in the first chimeric polypeptide. In some
[embodimentexamples](#), 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. In some [embodimentexamples](#), the second chimeric polypeptide in
 10 the first multi-chain chimeric polypeptide further includes a linker sequence
 between the second domain of the pair of affinity domains and the second target-
 binding domain in the second chimeric polypeptide. In some
[embodimentexamples](#), the soluble tissue factor domain is a soluble human tissue
 factor domain. In some [embodimentexamples](#), the soluble human tissue factor
 15 domain includes a sequence that is at least 80% identical to SEQ ID NO: 5. In
 some [embodimentexamples](#), the soluble human tissue factor domain includes a
 sequence that is at least 90% identical to SEQ ID NO: 5. In some
[embodimentexamples](#), the soluble human tissue factor domain includes a sequence
 that is at least 95% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the
 20 soluble human tissue factor domain includes SEQ ID NO: 5. In some
[embodimentexamples](#), the soluble human tissue factor domain includes a
 sequence that is at least 80% identical to SEQ ID NO: 9. In some
[embodimentexamples](#), the soluble human tissue factor domain includes a sequence
 that is at least 90% identical to SEQ ID NO: 9. In some [embodimentexamples](#), the
 25 soluble human tissue factor domain includes a sequence that is at least 95%
 identical to SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human
 tissue factor domain includes SEQ ID NO: 9. In some [embodimentexamples](#), the
 soluble human tissue factor domain includes a sequence that is at least 80%
 identical to SEQ ID NO: 10. In some [embodimentexamples](#), the soluble human
 30 tissue factor domain includes a sequence that is at least 90% identical to SEQ ID
 NO: 10. In some [embodimentexamples](#), the soluble human tissue factor domain
 includes a sequence that is at least 95% identical to SEQ ID NO: 10. In some
[embodimentexamples](#), the soluble human tissue factor domain includes SEQ ID
 NO: 10. In some [embodimentexamples](#), the soluble human tissue factor domain
 35 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. In some embodimentexamples, the soluble human tissue factor domain does not comprise any 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. In some embodimentexamples, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodimentexamples, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodimentexamples, the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some embodimentexamples, the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a signal sequence at its N-terminal end. In some embodimentexamples, the signal sequence includes SEQ ID NO: 28. In some embodimentexamples, the signal sequence is SEQ ID NO: 28. In some embodimentexamples, the pair of affinity domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some embodimentexamples, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodimentexamples, the soluble IL-15 includes a sequence that is 80% identical to SEQ ID NO: 26. In some embodimentexamples,

the soluble IL-15 includes a sequence that is 90% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 includes a sequence that is 95% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 includes SEQ ID NO: 26. In some [embodimentexamples](#), the sushi domain of IL15R α includes a sushi domain from human IL15R α . In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 80% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 90% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 95% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α is a mature full-length IL15R α .

In some [embodimentexamples](#), the pair of affinity domains in the first 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the first target-binding domain in the first multi-chain chimeric polypeptide includes a soluble IL-18. In some [embodimentexamples](#), the soluble IL-18 is a soluble human IL-18. In some [embodimentexamples](#), the soluble human IL-18 includes a sequence at least 80% identical to SEQ ID NO: 20. In some [embodimentexamples](#), the soluble human IL-18 includes a sequence at least 90% identical to SEQ ID NO: 20. In some [embodimentexamples](#), the soluble human IL-18 includes a sequence at least 95% identical to SEQ ID NO: 20. In some [embodimentexamples](#), the soluble human IL-18 includes a sequence of SEQ ID NO: 20. In some [embodimentexamples](#), the second target-binding domain in the first multi-chain chimeric

polypeptide includes a soluble IL-12. In some [embodimentexamples](#), the soluble IL-12 is a soluble human IL-12. In some [embodimentexamples](#), the soluble human IL-12 includes a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35). In some [embodimentexamples](#), the soluble human IL-15 further includes a linker sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35). In some [embodimentexamples](#), the linker sequence includes SEQ ID NO: 11. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 80% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 90% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 95% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) includes SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 80% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 90% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 95% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) includes SEQ ID NO: 16. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes a sequence that is at least 80% identical to

YFGKLESKLSVIRNLNDQVLFDIQGNRPLFEDMTDSDCRDNAPRTIFIISMY
 KDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRS
 VPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQN
 EDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSK
 CFYTTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
 FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKD
 LIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRK
 STDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
 SCKVTAMKCFLELQVISLES GDASIHTVENLILANNSLSSNGNVTESGC
 KECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 63). In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric

polypeptide in the first multi-chain chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first

5 multi-chain chimeric polypeptide includes

MKWVTFISLLFLFSSAYSIFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD
SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPD
NIKDTKSDIIFQORSVPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDEL
GDRSIMFTVQNEDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQIS
10 TKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGE
PLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLR
DVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESD
VHPSCKVTAMKCFLELQVISLESGDASIHDTVENLILANNSLSSNGNVTESG
15 CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 64). In some

[embodimentexamples](#), the second chimeric polypeptide includes a sequence that is at least 80% identical to

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
20 KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
RVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIR
DIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR
EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGG
GGSGGGGSRNLPVATPDPGMFCLHHSQNLLRAVSNMLQKARQTLEFYPCTS
25 EEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN
FNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPM
SVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTP
SLKCIR (SEQ ID NO: 65). In some [embodimentexamples](#), the second chimeric

30 polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric polypeptide includes SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric

polypeptide includes

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEED
GITWTL DQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKKE
DGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRG
5 SSDPQGVTCGAATLSAERV RGDNKEYEYSVEQCEDSACPAAEESLPIEVMVD
AVHKLKYENYTSSFFIRDI IKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPH
SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS
WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC LHHSQNLLRAV
SNMLQKARQTLEFY PCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRET
10 SFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFL
DQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNASITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTPSLKCIR (SEQ ID NO: 66). In some

embodiment examples, the first target-binding domain and the linker domain in the
15 first chimeric polypeptide in the second multi-chain chimeric polypeptide directly abut
each other in the first chimeric polypeptide. In some embodiment examples, the first
chimeric polypeptide in the second multi-chain chimeric polypeptide further includes a
linker sequence between the first target-binding domain and the linker domain in the first
chimeric polypeptide. In some embodiment examples, the linker domain and the first
20 domain of the pair of affinity domains directly abut each other in the first chimeric
polypeptide in the second multi-chain chimeric polypeptide. In some
embodiment examples, the first chimeric polypeptide in the second multi-chain chimeric
polypeptide further includes a linker sequence between the linker domain and the first
domain of the pair of affinity domains in the first chimeric polypeptide. In some
25 embodiment examples, 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. In
some embodiment examples, the second chimeric polypeptide in the second multi-chain
chimeric polypeptide further includes a linker sequence between the second domain of the
30 pair of affinity domains and the second target-binding domain in the second chimeric
polypeptide. In some embodiment examples, the linker domain is a soluble tissue factor
domain. In some embodiment examples, the soluble tissue factor domain is a soluble
human tissue factor domain. In some embodiment examples, the soluble human tissue
factor domain includes a sequence that is at least 80%

identical to SEQ ID NO: 5. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodimentexamples, the soluble human tissue factor domain includes SEQ ID NO: 5. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain includes SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodimentexamples, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 10. In some embodimentexamples, the soluble human tissue factor domain includes SEQ ID NO: 10.

In some embodimentexamples, 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. In some embodimentexamples, the soluble human tissue factor domain does not comprise any 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. In some [embodimentexamples](#), the soluble tissue factor domain is not capable of binding to Factor VIIa. In some [embodimentexamples](#), the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some [embodimentexamples](#), the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some [embodimentexamples](#), the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further includes a signal sequence at its N-terminal end. In some [embodimentexamples](#), the signal sequence includes SEQ ID NO: 28. In some [embodimentexamples](#), the signal sequence is SEQ ID NO: 28. In some [embodimentexamples](#), the pair of affinity domains in 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. In some [embodimentexamples](#), the soluble IL-15 has a D8N or D8A amino acid substitution. In some [embodimentexamples](#), the soluble IL-15 includes a sequence that is 80% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 includes a sequence that is 90% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 includes a sequence that is 95% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 includes SEQ ID NO: 26. In some [embodimentexamples](#), the sushi domain of IL15R α includes a sushi domain from human IL15R α . In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 80% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 90% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes a sequence that is 95% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α includes SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α is a mature full-length IL15R α . In some [embodimentexamples](#), the pair of affinity domains in 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the first target-binding domain in the second multi-chain chimeric polypeptide includes a soluble IL-21. In some [embodimentexamples](#), the soluble IL-21 is a soluble human IL-21. In some [embodimentexamples](#), the soluble human IL-21 includes a sequence at least 80% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the soluble human IL-21 includes a sequence at least 90% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the soluble human IL-21 includes a sequence at least 95% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the soluble human IL-21 includes a sequence of SEQ ID NO: 22. In some [embodimentexamples](#), the second target-binding domain includes a soluble IL-7. In some [embodimentexamples](#), the soluble IL-7 is a soluble human IL-7. In some [embodimentexamples](#), the soluble human IL-7 includes a sequence at least 80% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 includes a sequence at least 90% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 includes a sequence at least 95% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 includes a sequence of SEQ ID NO: 23.

In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 80% identical to DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLEIKTCWNKILMGTKEHSGTT NTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTE

CDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETN
LGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVE
CMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA
5 MKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEL
EEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 67). In some

[embodimentexamples](#), the first chimeric polypeptide in the second multi-chain
chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID
NO: 67. In some [embodimentexamples](#), the first chimeric polypeptide in the
10 second multi-chain chimeric polypeptide includes a sequence that is at least 95%
identical to SEQ ID NO: 67. In some [embodimentexamples](#), the first chimeric
polypeptide in the second multi-chain chimeric polypeptide includes SEQ ID NO:
67. In some [embodimentexamples](#), the first chimeric polypeptide in the second
multi-chain chimeric polypeptide includes

15 MKWVTFISLLFLFSSAYSDCDIEGKDQKQYESVLMVSIDQLLDSMKEIGSN
CLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVS
EGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKR
LLQEIKTCWNKILMGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVN
QVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAG
20 NVESTGSAGEPLYENSPEFTPYLETN LGQPTIQSFEQVGTKVNVTVEDERT
LVRRNNTFLSLRDVFGKDLYTLYYWKSSSSGKKTAKTNTNEFLIDVDKG
ENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIE
DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVE
NLIILANNSLSSNGNVTESGCKECEEL
25 EEKNIKEFLQSFVHIVQMFINTS

(SEQ ID NO: 68). In some [embodimentexamples](#), the second chimeric
polypeptide in the second multi-chain chimeric polypeptide includes a sequence
that is at least 80% identical to

QGGDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEK
30 KPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYS
LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID
NO: 69). In some [embodimentexamples](#), the second chimeric polypeptide in the
second multi-chain chimeric polypeptide includes a sequence that is at least 90%
identical to SEQ ID NO: 69. In some [embodimentexamples](#), the second chimeric
35 polypeptide in the second multi-chain chimeric polypeptide includes a sequence
that is at least 95% identical to SEQ ID NO:

69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide includes SEQ ID NO: 69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide includes

5 MKWVTFISLLFLFSSAYSQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQK
HRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSV
EHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSL
KCIR (SEQ ID NO: 70). In some [embodimentexamples](#), the first chimeric

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

QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIIKKLKRKPPSTNAGRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQ
15 VYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVEST
GSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLS
LRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNGNVTESGCKECE

20 ELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 71). In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 71. In some [embodimentexamples](#),
25 the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes

MGVKVLFALICIAVAEAQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQKHRLT
30 CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTW
KSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQ
TYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNV
TVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVD
KGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEF

RENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLEELQVIS
LESGDASIHTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHI
VQMFINTS (SEQ ID NO: 72). In some [embodimentexamples](#), the second chimeric

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

DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPP
PMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWT

TPSLKCIR (SEQ ID NO: 73). In some [embodimentexamples](#), the second chimeric
polypeptide in the second multi-chain chimeric polypeptide includes a sequence that
is at least 90% identical to SEQ ID NO: 73. In some [embodimentexamples](#), the
second chimeric polypeptide in the second multi-chain chimeric polypeptide includes
a sequence that is at least 95% identical to SEQ ID NO: 73. In some

[embodimentexamples](#), the second chimeric polypeptide in the second multi-chain
chimeric polypeptide includes SEQ ID NO: 73. In some [embodimentexamples](#), the
second chimeric polypeptide in the second multi-chain chimeric polypeptide includes
MGVKVLFALICIAVAEADCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCL
NNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTT
ILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKT
CWNKILMGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSS
LTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 74). In some

[embodimentexamples](#), 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. In some [embodimentexamples](#), the IgG1 antibody
construct is a bispecific IgG1 antibody, where one of the two antigen-binding
domains in the bispecific IgG1 antibody binds specifically to the linker domain. In
some [embodimentexamples](#), the first period of time is about 15 minutes to about 4
hours. In some [embodimentexamples](#), the first period of time is about 30 minutes to
about 3.5 hours. In some [embodimentexamples](#), the first period of time is about 1
hour to about 3.5 hours. In some [embodimentexamples](#), the first period of time is
about 2 hours to about 3.5 hours. In some [embodimentexamples](#), the liquid culture
medium in step (a) and/or step (b) is a serum-containing liquid culture medium.

In some [embodimentexamples](#), the NK cells are in the liquid culture medium in step (a) at a density of about 4.0×10^7 cells/mL to about 1.5×10^8 cells/mL. In some [embodimentexamples](#), the NK cells are in the liquid culture medium in step (a) at a density of about 5.0×10^7 cells/mL to about 2.0×10^8 cells/mL. In some

5 [embodimentexamples](#), the second period of time is about 1 day to about 30 days. In some [embodimentexamples](#), the second period of time is about 7 days to about 30 days. In some [embodimentexamples](#), the second period of time is about 14 days to about 21 days. In some [embodimentexamples](#), the liquid culture medium in step (b) includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio

10 of about 0.5:1 to about 2:1. In some [embodimentexamples](#), the liquid culture medium in step (b) includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.8:1 to about 1.2:1. In some [embodimentexamples](#), the NK cells are in the liquid culture medium in step (b) are maintained at a density of about 0.5×10^6 cells/mL to about 2.0×10^6 cells/mL. In some [embodimentexamples](#), the

15 NK cells are in the liquid culture medium in step (b) are maintained at a density of about 1.0×10^6 cells/mL to about 2.0×10^6 cells/mL. In some [embodimentexamples](#), the NK cell was previously obtained from a subject. In some [embodimentexamples](#), the NK cell was previously obtained from umbilical cord blood or induced pluripotent stem cells. In some [embodimentexamples](#), the method further includes obtaining the NK cell from the

20 subject prior to step (a). In some [embodimentexamples](#), the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. In some [embodimentexamples](#), the method further includes, between step (a) and step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some [embodimentexamples](#), the method

25 further includes, prior to step (a), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some [embodimentexamples](#), the method further includes, during step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some [embodimentexamples](#), the method further includes, after step (b),

30 isolating the NK cell. In some [embodimentexamples](#), after step (b), the NK cell has an increased level of expression or secretion of one or more proteins selected from the group consisting of: TNF- α , IFN- γ , granzyme A, granzyme B, perforin, 2B4, CD8, CD11a, CD16, CD25, CD27, CD48, CD49d, CD54, CD56, CD58, CD62L, CD69, CD70, CD94, CD137, CD158a, CD158b, CD158e, CD178,

CD226, CD253, NKG2C, NKG2D, LIR-1, LILR-B1, KIR2DL1, KIR3DL1, KIR2DL2, KIR2DL3, CXCR3, NKp30, NKp44, NKp46, NKG2D, DNAM-1, TRAIL, FasL, CXCR3, CXCR4, LTB, MX1, BAX, TNF- α , and IFN- γ as compared to the level of expression or secretion of the one or more proteins prior to step (a). In

5 some embodimentexamples, the method further includes, after the contacting step, administering the NK cell to a subject in need thereof. In some

embodimentexamples, the subject has been identified or diagnosed as having an age-related disease or condition. In some embodimentexamples, the age-related disease or

10 condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound

15 healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some embodimentexamples, the subject has been identified or diagnosed

20 as having a cancer. In some embodimentexamples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia

25 (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma,

30 endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodimentexamples, the subject has been diagnosed or identified as having an infectious disease. In some embodimentexamples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus,

rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is an activated NK cell produced by the method of any of the above embodimentexamples. Also provided is a pharmaceutical composition comprising the activated NK cell of the above embodimentexample. Also provided is a kit that includes a pharmaceutical composition that includes the activated NK cell of any of the above embodimentexamples. In another aspectexample, provided herein is a method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method includes administering to the subject a therapeutically effective amount of the activated NK cell of any of the above embodimentexamples or the pharmaceutical composition of any of the above embodimentexamples. In some embodimentexamples, the subject has been identified or diagnosed as having a cancer. In some embodimentexamples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodimentexamples, the subject has been identified or diagnosed as having an aging-related disease or condition. In some embodimentexamples, the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis,

NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some embodimentexamples, the subject has been diagnosed or identified as having an infectious disease. In some embodimentexamples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

In one aspect, the present disclosure refers to an activated NK cell produced by the method disclosed herein

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the activated NK cell disclosed herein.

In one aspect, the present disclosure refers to a kit comprising a pharmaceutical composition comprising the activated NK cell disclosed herein.

In one aspect, the present disclosure refers to use of the activated NK cell disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof.

In some embodimentexamples, provided herein is a method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the activated NK cell or the activated T cell of any of the above embodimentexamples or the pharmaceutical composition of any of the above embodimentexample. In some embodimentexamples, the subject has been identified or diagnosed as having a cancer. In some embodimentexamples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodimentexamples, the subject has been identified or diagnosed as having an aging-related disease or condition. In some embodimentexamples, the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular

degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some embodimentexamples, the subject has been diagnosed or identified as having an infectious disease. In some

5 embodimentexamples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

10 In one aspect, the present disclosure refers to use of the activated NK cell or the activated T cell disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a subject in need thereof.

In another aspectexample, provided herein is 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
15 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
20 multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12; 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
25 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
30 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. In some embodimentexamples, the first target-binding domain and the soluble tissue factor domain directly abut each other in the
35 first chimeric polypeptide in the first multi-chain chimeric polypeptide. In some embodimentexamples, 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the soluble tissue factor domain is a soluble human tissue factor domain. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human tissue factor domain comprises SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 10. In some [embodimentexamples](#), the soluble human

tissue factor domain comprises SEQ ID NO: 10. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the soluble human tissue factor domain does not comprise any 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. In some [embodimentexamples](#), the soluble tissue factor domain is not capable of binding to Factor VIIa. In some [embodimentexamples](#), the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some [embodimentexamples](#), the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some [embodimentexamples](#), the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end. In some [embodimentexamples](#), the signal sequence comprises SEQ ID NO: 28. In some [embodimentexamples](#), the signal sequence is SEQ ID NO: 28. In some [embodimentexamples](#), the pair of affinity

domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some

embodimentexamples, the soluble IL-15 has a D8N or D8A amino acid substitution.

In some embodimentexamples, the soluble IL-15 comprises a sequence that is 80%

5 identical to SEQ ID NO: 26. In some embodimentexamples, the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26. In some

embodimentexamples, the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26. In some embodimentexamples, the soluble IL-15 comprises SEQ ID

10 NO: 26. In some embodimentexamples, the sushi domain of IL15R α comprises a sushi domain from human IL15R α . In some embodimentexamples, the sushi domain from

human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24. In some

embodimentexamples, the sushi domain from human IL15R α comprises a sequence that

is 90% identical to SEQ ID NO: 24. In some embodimentexamples, the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24. In

15 some embodimentexamples, the sushi domain from human IL15R α comprises SEQ ID NO: 24. In some embodimentexamples, the sushi domain from human IL15R α is a

mature full-length IL15R α . In some embodimentexamples, the pair of affinity domains in the first 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

20 mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some embodimentexamples,

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. In

some embodimentexamples, the first target-binding domain in the first multi-chain

25 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. In some embodimentexamples, 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

30 specifically to a receptor for IL-12. In some embodimentexamples, the first target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-18.

In some embodimentexamples, the soluble IL-18 is a soluble human IL-18. In some

embodimentexamples, the soluble human IL-18 comprises a sequence at least 80%

identical to SEQ ID NO: 20. In some embodimentexamples, the soluble human IL-18

comprises a sequence at least 90% identical to SEQ ID NO: 20. In some [embodimentexamples](#), the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 20. In some [embodimentexamples](#), the soluble human IL-18 comprises a sequence of SEQ ID NO: 20. In some [embodimentexamples](#), the second target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-12. In some [embodimentexamples](#), the soluble IL-12 is a soluble human IL-12. In some [embodimentexamples](#), the soluble human IL-12 comprises a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35). In some [embodimentexamples](#), the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35). In some [embodimentexamples](#), the linker sequence comprises SEQ ID NO: 11. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 β (p40) comprises SEQ ID NO: 14. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 16. In some [embodimentexamples](#), the sequence of soluble human IL-12 α (p35) comprises SEQ ID NO: 16. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 63. In some [embodimentexamples](#), the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 64. In some [embodimentexamples](#), the second chimeric polypeptide comprises a

sequence that is at least 80% identical to SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 65. In some

5 [embodimentexamples](#), the second chimeric polypeptide comprises SEQ ID NO: 65. In some [embodimentexamples](#), the second chimeric polypeptide comprises SEQ ID NO: 66. In some [embodimentexamples](#), the first target-binding domain and the linker domain in the first chimeric polypeptide in the second multi-chain chimeric polypeptide directly

10 abut each other in the first chimeric polypeptide. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some

15 [embodimentexamples](#), 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. In some [embodimentexamples](#), 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

20 chimeric polypeptide directly abut each other in the second chimeric polypeptide. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the linker domain is a soluble tissue factor

25 domain. In some [embodimentexamples](#), the soluble tissue factor domain is a soluble human tissue factor domain. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some [embodimentexamples](#),

30 the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises SEQ ID NO: 5. In some [embodimentexamples](#), the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9. In some [embodimentexamples](#), the soluble human tissue factor domain

comprises a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodimentexamples, the soluble human tissue factor domain comprises SEQ ID NO: 9. In some

- 5 embodimentexamples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodimentexamples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodimentexamples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.
- 10 In some embodimentexamples, the soluble human tissue factor domain comprises SEQ ID NO: 10. In some embodimentexamples, 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
- 15 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
- 20 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. In some embodimentexamples, the soluble human tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino
- 25 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
- 30 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. In some [embodimentexamples](#), the soluble tissue factor domain is not capable of binding to Factor VIIa. In some [embodimentexamples](#), the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some [embodimentexamples](#), the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some [embodimentexamples](#), the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end. In some [embodimentexamples](#), the signal sequence comprises SEQ ID NO: 28. In some [embodimentexamples](#), the signal sequence is SEQ ID NO: 28. In some [embodimentexamples](#), the pair of affinity domains in 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. In some [embodimentexamples](#), the soluble IL-15 has a D8N or D8A amino acid substitution. In some [embodimentexamples](#), the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26. In some [embodimentexamples](#), the soluble IL-15 comprises SEQ ID NO: 26. In some [embodimentexamples](#), the sushi domain of IL15R α comprises a sushi domain from human IL15R α . In some [embodimentexamples](#), the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α comprises SEQ ID NO: 24. In some [embodimentexamples](#), the sushi domain from human IL15R α is a mature full-length IL15R α . In some [embodimentexamples](#), the pair of affinity domains in 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the first target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble IL-21. In some [embodimentexamples](#), the soluble IL-21 is a soluble human IL-21. In some [embodimentexamples](#), the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 22. In some [embodimentexamples](#), the second target-binding domain comprises a soluble IL-7. 323. In some [embodimentexamples](#), the soluble IL-7 is a soluble human IL-7. In some [embodimentexamples](#), the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 23. In some [embodimentexamples](#), the soluble human IL-7 comprises a sequence of SEQ ID NO: 23. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 67. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 67. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 67. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 67. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 68. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ

ID NO: 69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 69. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 70. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 71. In some [embodimentexamples](#), the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 72. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 73. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 73. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 73. In some [embodimentexamples](#), the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 74. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In one aspect, the present disclosure refers to 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.

As used herein, the term “chimeric” refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two

different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., a scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some embodiment examples, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

A “multi-chain chimeric polypeptide” means a complex of two or more chimeric polypeptides.

An “antigen-binding domain” is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some embodiment examples, the antigen-binding domain can be an antibody or a fragment thereof. In some embodiment examples, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art.

A “soluble tissue factor domain” refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

The term “soluble interleukin protein” is used herein to refer to a mature and secreted interleukin protein or a biologically active fragment thereof. In some examples, a soluble interleukin protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble interleukin proteins are described herein.

The term “soluble cytokine protein” is used herein to refer to a mature and secreted cytokine protein or a biologically active fragment thereof. In some

examples, a soluble cytokine protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble cytokine proteins are described herein.

The term “soluble interleukin receptor” is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble interleukin receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype interleukin receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble interleukin receptors are described herein.

The term “soluble cytokine receptor” is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble cytokine receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype cytokine receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble cytokine receptors are described herein.

The term “ligand of a co-stimulatory molecule” is used herein in the broadest sense to refer to a polypeptide that is capable of binding and activating a co-stimulatory receptor molecule on an immune cell (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a ligand of a co-stimulatory molecule can include a sequence that is at least 70%

identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to a ligand of a co-stimulatory molecule that retains its ability to specifically bind to one or more of its natural receptors. Non-limiting examples of ligands of co-stimulatory molecules are described herein.

The term “antibody” is used herein in its broadest sense and includes certain types of immunoglobulin molecules that include one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes, e.g., intact antibodies (e.g., intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

“Affinity” refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, “affinity” refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (KD). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity for an antigen-binding domain and its corresponding antigen or epitope are known in the art.

The term “pair of affinity domains” is two different protein domain(s) that bind specifically to each other with a KD of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some embodiment examples, a pair of affinity domains can be a pair of synthetic proteins. Non-limiting examples of pairs of affinity domains are described herein.

The term “epitope” means a portion of an antigen that specifically binds to an antigen-binding domain. Epitopes can, e.g., consist of surface-accessible amino acid

residues and/or sugar side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

An “immune effector cell” refers to a cell of the immune system of a mammal that is capable, directly or indirectly, of recognizing and/or causing cytostasis or cell death of a pathogenic cell (e.g., a cancer cell) in the mammal. Non-limiting examples of immune effector cells include macrophages, natural killer cells, T-lymphocytes (e.g., cytotoxic T-lymphocytes and T-helper cells), neutrophils, monocytes, and eosinophils. Additional examples of immune effector cells are known in the art.

The term “treatment” means to ameliorate at least one symptom of a disorder. In some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in need of such treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this inventiondisclosure belongs. Methods and materials are described herein for use in the present inventiondisclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the inventiondisclosure will be apparent from the following detailed description and figures, and from the claims.