[0005] Given that the ligands determine the composition of the receptor dimers, and the intracellular kinase degeneracy of JAK/TYK and RTK enzymes, the number of cytokine and growth factor receptor dimer pairings that occur in nature only represents a small proportion of the total number of signaling-competent receptor pairings theoretically allowed by the system. For example, the human genome encodes for approximately forty different JAK/STAT cytokine receptors. In principle, approximately 1600 unique homo- and hetero-dimeric cytokine receptor pairs could be generated with the potential to signal through different JAK/TYK/STAT combinations. However, the human genome encodes for less than fifty different cytokine ligands, limiting the scope of cytokine receptor dimers to those that can be assembled by the natural ligands. A similar argument can be made for the RTK family of receptors and ligands. Furthermore, given that is has been shown that Death receptors are capable of signaling as dimers or trimers, this concept can also be extended to this family.

[0006] The ability to selectively activate signaling pathways of interest is of great interest. The present invention provides compositions and methods for this purpose.

SUMMARY

[0007] Engineered synthetic signaling molecules, herein termed “synthekines”, are provided. Synthekines are genetically engineered, bi-specific ligands of cell surface receptors, where the synthekine specifically binds at high affinity to the extracellular domain(s) of at least one and frequently two different cell surface receptor polypeptides. The cell surface receptors are characterized by activation of signaling upon multimerization. In some examples, generation of a receptor multimer by binding to a synthekine results in intracellular trans-phosphorylation of the receptor. Synthekines include, without limitation, small organic molecules and polypeptides.

[0007a] In one aspect, the present disclosure refers to a synthekine comprising at least two binding domains, wherein a first binding domain specifically binds to the extracellular domain of a first receptor polypeptide and wherein a second binding domain specifically binds to the extracellular domain of a second receptor polypeptide, wherein said first and second receptor polypeptides are not naturally associated and wherein the first and second binding domains comprise monovalent antibodies.

[0008] Synthekines may also be tri-specific ligands of cell surface receptors, or more, where the synthekine specifically binds at high affinity to the extracellular domain(s) of at least three different cell surface receptor polypeptides. The cell surface receptors are characterized by activation of signaling upon multimerization. In some examples, generation of a receptor multimer by binding to a synthekine results in intracellular trans-phosphorylation of the receptor. Synthekines include, without limitation, small organic molecules and polypeptides.

[0009] In some examples the cell surface receptor polypeptide is one or more of (i) a cytokine receptor that activates the JAK/STAT pathway in the cell; (ii) a receptor tyrosine kinase; or (iii) a TNFR superfamily member. In some examples each of the multimeric receptor polypeptides are naturally expressed in a targeted single cell. In some examples a target cell is engineered to express the one or more of multimeric receptor polypeptides.

[0018] In some examples the linker is a rigid linker, in other examples the linker is a flexible linker. Where the linker is a peptide linker, it may be from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acids in length, and is of sufficient length and amino acid composition to enforce the distance between binding domains. In some examples the linker comprises or consists of one or more glycine and/or serine residues.

[0019] A synthekine can be multimerized, e.g. through an Fc domain, by concatenation, coiled coils, polypeptide zippers, biotin/avidin or streptavidin multimerization, and the like. The synthekine can also be joined to a moiety such as PEG, Fc, etc. as known in the art to enhance stability in vivo.

[0019a] In one example, the present disclosure refers to a synthekine for any of the use as disclosed herein.

[0019b] In one aspect, the present disclosure refers to a nucleic acid sequence encoding the synthekine as disclosed herein.

[0019c] In one aspect, the present disclosure refers to a recombinant vector comprising the nucleic acid sequence as disclosed herein.

[0019d] In one aspect, the present disclosure refers to a recombinant host cell comprising a nucleic acid sequence as disclosed herein.

[0020] In one example, the present disclosure refers to a pharmaceutical composition comprising a synthekine as disclosed herein, and a pharmaceutically acceptable excipient. Compositions of interest include, without limitation, an effective dose of a synthekine in a pharmaceutically acceptable excipient. Compositions may comprise additional agents, *e.g.* adjuvants and the like. Synthekines may be produced synthetically; by various suitable recombinant methods, and the like, as known in the art.

[0020a] In one aspect, the present disclosure refers to use of a synthekine polypeptide that binds to the extracellular domains of two or more receptor polypeptides in a non-natural combination in the manufacture of a medicament for selective activation of a non-natural combination of receptor polypeptides in a cell, wherein the synthekine polypeptide comprises two or more binding domains each of which binding domain comprises a monovalent antibody that binds to the extracellular domain of a subunit of different multimeric receptors and contact of the cell with the synthekine polypeptide causes multimerization of the receptor subunits and activation of signaling and wherein the medicament comprises a therapeutically effective amount of the synthekine useful in the treatment of diseases selected from autoimmune diseases, inflammatory diseases, and cancer.

[0021] In some examples of the disclosure, a method is provided for activating, increasing or enhancing selected JAK/STAT, DD, and/or RTK signaling in a cell. In such methods, a cell expressing cognate receptor polypeptides for a synthekine of interest is contacted with a concentration of a synthekine that is effective to increase signaling, *e.g.* to increase signaling by 25%, 50%, 75%, 90%, 95%, or more, relative to the signaling in the absence of the synthekine. Such signaling activation may induce JAK/STAT or RTK signaling pathways and include, without limitation, modulation of immune responses, growth factor responses, induction of death domain responses, and the like. In some methods, the receptor-expressing cell is contacted *in vitro*. In other examples, the receptor-expressing cell is contacted *in vivo*.

[0022] In some examples of the disclosure, a method is provided for treating or preventing a disease or disorder in a subject in need thereof, the method comprising providing to the subject an effective amount of a synthekine. In particular examples, the subject has an immune disease or dysfunction.

BRIEF DESCRIPTION OF THE DRAWINGS.

[0023] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

What is Claimed is:

1. Use of a synthekine polypeptide that binds to the extracellular domains of two or more receptor polypeptides in a non-natural combination in the manufacture of a medicament for selective activation of a non-natural combination of receptor polypeptides in a cell, wherein the synthekine polypeptide comprises two or more binding domains each of which binding domain comprises a monovalent antibody that binds to the extracellular domain of a subunit of different multimeric receptors and contact of the cell with the synthekine polypeptide causes multimerization of the receptor subunits and activation of signaling and wherein the medicament comprises a therapeutically effective amount of the synthekine useful in the treatment of diseases selected from autoimmune diseases, inflammatory diseases, and cancer.

2. The use of claim 1, wherein the receptor polypeptide(s) are one or more of (i) a cytokine receptor that activates the JAK/STAT pathway in the cell; (ii) a receptor tyrosine kinase; or (iii) a TNFR superfamily member.

3. The use of claim 2, where the cytokine receptors are selected from βc, γc, IL-3Rα, βIL-3R, GM-CSFRα, IL-5Rα, CNTFα, CRLF1, LIFRα, gp130, IL-6Rα, IL-11Rα, OSMRβ, IL-2Rα, IL-2Rβ, IL-2Rγ, IL-4Rα, IL-7Rα, IL-9Rα, IL-13Rα, IL-15Rα, IL-21Rα, IFNAR2, IL-23R, EpoR, IL-12Rβ, IFNAR1, G-CSFR, c-MPLR.

4. The use of claim 3, wherein a synthekine binds to two of such receptors, and activates JAK/STAT signaling.

5. The use of claim 3, wherein a synthekine binds to three of such receptors and activates JAK/STAT signaling.

6. The use of claim 2, where the receptor tyrosine kinase proteins are selected from EGFR, ErbB2, ErbB3, ErbB4, InsR, IGF1R, InsRR, PDGFRα, PDGFRβ, CSF1R/Fms, cKit, Flt-3/Flk2, VEGFR1, VEGFR2, VEGFR3, FGFR1, FGFR2, FGFR3, FGFR4, PTK7/CCK4, TrkA, TrkB, TrkC, Ror1, Ror2, MuSK, Met, Ron, Axl, Mer, Tyro3, Tie1, Tie2, EphA1-8, EphA10, EphB1-4, EphB6, Ret, Ryk, DDR1, DDR2, Ros, LMR1, LMR2, LMR3, ALK, LTK, SuRTK106/STYK1.

7. The use of claim 6, wherein a synthekine binds to two of such receptors, and activates signaling.

8. The use of claim 2, where the TNFR superfamily member is selected from TNFR1 (TNFRSF1A), TNFR2 (TNFRSF1B; TNFRSF2), 41-BB (TNFRSF9); AITR (TNFRSF18); BCMA (TNFRSF17), CD27 (TNFRSF7), CD30 (TNFRSF8), CD40 (TNFRSF5), Death Receptor 1 (TNFRSF10C), Death Receptor-3 (TNFRSF25), Death Receptor 4 (TNFRSF10A), Death Receptor 5 (TNFRSF10B), Death Receptor -6 (TNFRSF21), Decoy Receptor-3 (TNFRSF6B), Decoy Receptor 2 (TNFRSF10D), EDAR, Fas (TNFRSF6), HVEM (TNFRSF14), LTBR (TNFRSF3), OX40 (TNFRSF4), RANK (TNFRSF11A), TACI (TNFRSF13B), Troy (TNFRSF19), XEDAR (TNFRSF27), Osteoprotegerin (TNFRSF11B), TWEAK receptor (TNFRSF12A), BAFF Receptor (TNFRSF13C), NGF receptor (TNFRSF16).

9. The use of claim 8, wherein the synthekine binds to two or more of such receptors and activates signaling.

10. The use of any of claims 1-9, wherein the synthekine comprises binding domains with binding affinity for the ECD of the receptor subunit of less than about 1×10^{-7}M.

11. The use of claim 10, wherein the binding domains are directly joined.

12. The use of claim 10, wherein the binding domains are joined through a linker.

13. The use of any of claims 1-12, wherein the monovalent antibodies are VHHs.

14. The use of any of claims 1-13 wherein the binding domains are joined by a peptide linker comprising from 2-100 amino acids.

15. The use of any of claims 1-13, wherein the binding domains are joined by a non-peptide linker.

16. A synthekine comprising at least two binding domains, wherein a first binding domain specifically binds to the extracellular domain of a first receptor polypeptide and wherein a second binding domain specifically binds to the extracellular domain of a second receptor

polypeptide, wherein said first and second receptor polypeptides are not naturally associated and wherein the first and second binding domains comprise monovalent antibodies.

17. The synthekine of claim 16 wherein the first receptor polypeptide and second receptor polypeptide are selected from of (i) a cytokine receptor that activates the JAK/STAT pathway in the cell; (ii) a receptor tyrosine kinase; or (iii) a TNFR superfamily member.

18. The synthekine of any of claims 17 wherein the cytokine receptor is selected from the group consisting of: βc, γc, IL-3Rα, βIL-3R, GM-CSFRα, IL-5Rα, CNTFα, CRLF1 , LIFRα, gp130, IL-6Rα, IL-11Rα, OSMRβ, IL-2Rα, IL-2Rβ, IL-2Rγ, IL-4Rα, IL-7Rα, IL-9Rα, IL-13Rα, IL-15Rα, IL-21Rα, IFNAR2, IL-23R, EpoR, IL-12Rβ, IFNAR1, G-CSFR, and c-MPLR.

19. The synthekine of any of claims 17 or 18 wherein the receptor tyrosine kinase protein is selected from the group consisting of: EGFR, ErbB2, ErbB3, ErbB4, InsR, IGF1 R, InsRR, PDGFRα, PDGFRp, CSF1 R/Fms, cKit, Flt-3/Flk2, VEGFR1, VEGFR2, VEGFR3, FGFR1 , FGFR2, FGFR3, FGFR4, PTK7/CCK4, TrkA, TrkB, TrkC, Ror1, Ror2, MuSK, Met, Ron, Axl, Mer, Tyro3, Tie1, Tie2, EphA1-8, EphAI O, EphB1-4, EphB6, Ret, Ryk, DDR1, DDR2, Ros, LMR1, LMR2, LMR3, ALK, LTK, SuRTK106/STYK1, Acitivin-R, BMP-R, TGF-beta-R and Noggin-R.

20. The synthekine of any of claims 17 or 18 wherein the TNFR superfamily member is selected from TNFR1 (TNFRSF1A), TNFR2 (TNFRSF1 B; TNFRSF2), 41-BB (TNFRSF9); AITR (TNFRSF18); BCMA (TNFRSF17), CD27 (TNFRSF7), CD30 (TNFRSF8), CD40 (TNFRSF5), Death Receptor 1 (TNFRSF10C), Death Receptor-3 (TNFRSF25), Death Receptor 4 (TNFRSF10A), Death Receptor 5 (TNFRSF10B), Death Receptor-6 (TNFRSF21), Decoy Receptor-3 (TNFRSF6B), Decoy Receptor 2 (TNFRSF10D), EDAR, Fas (TNFRSF6), HVEM (TNFRSF14), LTβR (TNFRSF3), OX40 (TNFRSF4), RANK (TNFRSF1 1A), TACI (TNFRSF13B), Troy (TNFRSF19), XEDAR (TNFRSF27), Osteoprotegerin (TNFRSF11 B), TWEAK receptor (TNFRSF12A), BAFF Receptor (TNFRSF13C), and NGF receptor (TNFRSF16).

21. The synthekine of any of claims 16-20, wherein the at least first and second binding domains are directly joined.

22. The synthekine of any of claims 16-20, wherein the at least first and second binding domains are joined via a linker.

23. The synthekine of any of claims 16 - 22 wherein at least one monovalent antibody is a VHH.

24. A synthekine of any of claims 16 - 23 wherein the synthekine is a polypeptide.

25. A nucleic acid sequence encoding the synthekine of claim 24.

26. A recombinant vector comprising the nucleic acid sequence of claim 25.

27. A recombinant host cell comprising a nucleic acid sequence of claim 25.