

MULTI-FUNCTIONAL AND MULTI-VALENT INTERLEUKIN-TGF-BETA RECEPTOR FUSION POLYPEPTIDES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/094,277 filed on October 20, 2020, which application is incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 15, 2021, is named 57949-701_601_SL.txt and is 82,739 bytes in size.

BACKGROUND

[0003] Tumorigenesis that drives and leads to the development of cancer can be generally hallmarked by aberrant cellular processes that lead to, for example, sustained proliferative signaling, a reduced response to growth suppressors, increased resistance to cell death, replicative immortality, and increased angiogenesis. These processes are thought to promote tumor invasion and metastasis. Accordingly, the ability to target molecules associated with these processes may be important for the development of cancer therapeutics.

[0004] Transforming growth factor- β (TGF- β) family or proteins are pleiotropic cytokines that promotes angiogenesis and/or immunosuppression in tumorigenic environments. TGF- β proteins were originally named for their ability to transform normal fibroblasts to cells capable of anchorage-independent growth. Produced primarily by hematopoietic and tumor cells, TGF- β proteins can regulate (e.g., stimulate or inhibit) the growth and differentiation of cells from a diversity of normal or neoplastic tissue origins. Notably, TGF- β proteins are known to be involved in many proliferative and non-proliferative cellular processes such as, e.g., cell proliferation and differentiation, embryonic development, extracellular matrix formation, bone development, wound healing, hematopoiesis, and immune and inflammatory responses. Increased levels of TGF- β expression and activity are involved in a large number of pathologic conditions, including, but not limited to, tumorigenesis, wherein members of the TGF- β protein family are known to have a number of biological activities related to tumorigenic processes (e.g., angiogenesis and immunosuppression) and metastasis.

[0005] Interleukin-2 (IL-2) is a potent cytokine that acts on the immune system to generate primarily a cell-mediated immune response. Generally, IL-2 is locally produced by immune cells at high concentrations near the site of an antigen to supply the necessary co-stimulatory signals for generating an immune system response to the antigen. IL-2 is therefore an immune-activating molecule and may hold the potential to promote immunotherapeutic responses in treating tumors. For example, in addition to stimulating T cells, IL-2 has also been shown to stimulate lymphocytes (T cells, B cells, NK cells, NKT cells). However, High dose of IL-2 based therapies have previously been associated with toxicity observed with IL-2 systemic administration. Such deleterious effects present hurdles for the successful utilization of IL-2 as an effective therapeutic.

SUMMARY

[0006] Provided herein are fusion proteins that function to activate an immune response and inhibit pro-tumorigenic immunosuppressive signaling. As described herein, immune activation and the inhibition of immune suppression is generally achieved by fusion proteins that comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF- β activity (e.g., two or more polypeptides that bind to a TGF- β protein). The compositions provided herein are also useful for or in the inhibition of tumorigenesis (e.g., angiogenesis or metastasis). Accordingly, also disclosed herein are methods of inhibiting and/or treating cancer using the fusion proteins disclosed herein.

[0007] Provided herein are fusion polypeptides, comprising: an interleukin (IL) polypeptide; a first TGF β superfamily receptor polypeptide; and a second TGF β superfamily receptor polypeptide. Also provided are fusion polypeptides comprising: an IL polypeptide that binds to and agonizes an IL receptor; a first TGF β superfamily receptor polypeptide that binds to and sequesters soluble TGF β ; and a second TGF β superfamily receptor polypeptide that binds to and sequesters soluble TGF β .

[0008] In some embodiments, the IL polypeptide comprises an IL-2 polypeptide or IL-15. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide. In some embodiments, the IL-2 polypeptide comprises SEQ ID NO: 2. In some embodiments, the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL-2 polypeptide consists of SEQ ID NO: 2. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide or IL-15. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide. In some embodiments, the

IL-2 polypeptide comprises SEQ ID NO: 3. In some embodiments, the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In some embodiments, the IL-2 polypeptide consists of SEQ ID NO: 3. In some embodiments, the IL polypeptide comprises an IL-15 polypeptide. In some embodiments, the IL-15 polypeptide comprises SEQ ID NO: 5. In some embodiments, the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5. In some embodiments, the IL-15 polypeptide consists of SEQ ID NO: 5.

[0009] In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises an activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, or a TGF β receptor II polypeptide or fragment thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a TGF β receptor II (TBR II) polypeptide or fragment thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBR II) polypeptide or fragment thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBR II) polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGF β superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In some embodiments, the soluble TGF β receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF- β 1 polypeptide, a TGF- β 2 polypeptide, a TGF- β 3 polypeptide, a activin β A polypeptide, a activin β B polypeptide, a activin β C polypeptide, a activin β E polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal

polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an arteman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide.

[0010] In some embodiments, the truncated TGF β superfamily receptor polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof. In some embodiments, the truncated TGF β superfamily receptor polypeptide is a truncated soluble TGF β receptor II (sT β RII) polypeptide. In some embodiments, the truncation comprises greater than 2, 5, 7, 10, 15, 20, 25, or 30 amino acids. In some embodiments, the IL polypeptide comprises a truncated IL polypeptide. In some embodiments, the truncated IL polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof. In some embodiments, the truncated IL polypeptide comprises a truncated IL-2 polypeptide or IL-15 polypeptide. In some embodiments, the fusion polypeptides comprise a linker polypeptide or a linker molecule attaching the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide. In some embodiments, the fusion polypeptides comprise a linker polypeptide fusing the IL polypeptide and the first TGF β superfamily receptor polypeptide. In some embodiments, the fusion polypeptides comprise a pharmacokinetic (PK) modulator.

[0011] In some embodiments, the pharmacokinetic modulator comprises an immunoglobulin constant (Fc) region polypeptide. In some embodiments, the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide. In some embodiments, the immunoglobulin Fc region is an IgG Fc region. In some embodiments, the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments, the PK modulator comprises an albumin polypeptide. In some embodiments, the albumin polypeptide is a human albumin polypeptide.

[0012] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10-25. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an

amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25. In some embodiments, the fusion polypeptide consists of any one of SEQ ID NOs: 10-25. In some embodiments, the fusion polypeptide activates target cells expressing an IL-2 or IL-15 receptor. In some embodiments, the target cell is an immune cell. In some embodiments, the immune cell is a T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells. Further provided are pharmaceuticals composition comprising a fusion polypeptide, comprising a pharmaceutically acceptable carrier or excipient.

[0013] Also provided herein are methods of inhibiting or reducing tumor growth and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGF β superfamily receptor polypeptide; and a second TGF β superfamily receptor polypeptide, thereby inhibiting or reducing tumor growth and/or progression in the individual. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2.

[0014] In some embodiments, the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cells within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer a cancer comprising a solid tumor. Provided are methods of neutralizing or killing tumor cells in an individual having cancer,

comprising administering to the individual a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGF β superfamily receptor polypeptide; and a second TGF β superfamily receptor polypeptide; thereby neutralizing or killing tumor cells in the individual. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer comprises a solid tumor.

[0015] Additionally provided are methods of treating or ameliorating cancer in a subject having a cancer, comprising administering to the subject a fusion polypeptide comprising an interleukin (IL) polypeptide; a first TGF β superfamily receptor polypeptide; and a second TGF β superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the subject. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof.

[0016] Further provided are methods of activating a cell expressing an IL receptor comprising contacting a cell with a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGF β superfamily receptor polypeptide; and a second TGF β superfamily receptor polypeptide. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell comprises T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells. In some embodiments, the immune cells are contacted in vivo. In some embodiments, the immune cells are contacted ex

vivo. In some embodiments, a leukopak or leukapheresis product comprise the immune cells. In some embodiments, the immune cells are derived from an apheresis sample. In some embodiments, the immune cells are subsequently used for a cell-based therapy. In some embodiments, IL polypeptide is IL-2 or IL-15. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBR II) polypeptide or fragment. A host cell, comprising a nucleic acid molecule comprising a nucleic acid sequence encoding the recombinant fusion polypeptide as described herein.

INCORPORATION BY REFERENCE

[0017] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0019] **FIGs. 1A and 1B** show data depicting the quantification of IFN-gamma production by human T cells previously stimulated by human monovalent FIST (a fusion protein comprising an interleukin-2 (IL-2) polypeptide, a single sTBR II receptor polypeptide) or bivalent FIST (also referred to as bFIST - a fusion polypeptide comprising an interleukin-2 (IL-2) polypeptide, a first sTBR II receptor polypeptide, and a second sTBR II receptor polypeptide), and CXCL10 production by human NK cells stimulated with human FIST (monovalent FIST) or, human bivalent FIST.

[0020] **FIG. 2** shows data depicting the quantification of active TGF β 1 detected in solution after 30 minutes incubation period with 5-20 nanomoles of human monovalent FIST (monovalent FIST), bivalent FIST (bFIST), or sTBR II.

[0021] **FIG. 3** shows a schematic representation of exemplary bivalent FIST (bFIST) fusion polypeptides.

[0022] FIGs. 4A, 4B, and 4C show comparison between bivalent FIST protein and monovalent FIST in the blockage of TGF β isoforms.

[0023] FIGs. 5A, 5B, 5C, 5D, 5E, and 5F show inhibition of TGF β 1-mediated suppression of cytotoxic T cell proliferation.

[0024] FIGs. 6A, 6B, 6C, 6D, 6E, and 6F show proliferation of effector memory T cells and terminal differentiated effector memory cells, and the quantification of IFN γ production by stimulated T cells with the bivalent FIST, monovalent FIST (monovalent FIST), and controls.

[0025] FIGs. 7A, 7B, 7C, 7D, and 7E show bivalent FIST inhibition and/or reduction of TGF β 1-mediated suppression of primary NK cell proliferation and IFN γ and CXCL10 production.

[0026] FIGs. 8A and 8B show that treatment of NK cells with bivalent FIST increases NK cell cytotoxicity and activation and cytotoxicity against cancer cells.

[0027] FIGs. 9A, 9B, 9C, and 9D show that treatment of B cells with bivalent FIST increases maturation, proliferation and IFN γ production.

[0028] FIGs. 10A, 10B, and 10C show that treatment of lung cancer cells with bivalent FIST inhibits the upregulation of TGF β 1-induced Epithelial to Mesenchymal transition (EMT) markers on lung cancer cells.

DETAILED DESCRIPTION

[0029] Provided herein are multi-functional fusion proteins that are capable of, e.g., activating an immune response, inhibiting and/or reducing immunosuppressive signaling, and/or suppressing or inhibiting angiogenic or invasive signaling. In the context of cancer, immune activation is thought to be the physiological function that promotes the recognition and neutralization of transformed cells before they grow into tumors, or to kill (e.g., neutralize) tumors after they are formed. The specific recognition of antigens based on foreignness, molecular weight, complexity, level of expression, and degradability is thought to give rise to the capacities of the immune system to limit tumor expansion. In opposition, tumor cells are thought to possess the capacity to limit responsiveness through various mechanisms, for example, via production of immunosuppressive factors or molecules (e.g., immune suppressive cytokines).

[0030] In order to activate an immune response and inhibit immunosuppressive signaling, the fusion proteins disclosed herein generally comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF β activity (e.g., two or more polypeptides that bind a TGF β protein). The balance between activating and inhibitory signals is an important factor in

maintaining immune homeostasis. During tumorigenesis, immunostimulatory (e.g., immune activating) signals (e.g., proinflammatory cytokines) are antagonized by tumor-derived immunosuppressive signaling molecules (e.g., immunosuppressive cytokines). Among the group of immunosuppressive cytokines thought to be important in driving tumorigenesis are members of the TGF superfamily of proteins (e.g., TGF β).

[0031] The Transforming Growth Factor Beta (TGF β) superfamily includes the TGF β proteins, Bone Morphogenetic Proteins (BMPs), Growth Differentiation Factors (GDFs), Glial-derived Neurotrophic Factors (GDNFs), Activins, Inhibins, Nodal, Lefty, and Müllerian Inhibiting Substance (MIS). Generally, ligands of the TGF β superfamily form dimers that bind to heterodimeric receptor complexes consisting of type I and type II receptor subunits with serine/threonine kinase domains.

[0032] Transforming Growth Factor β (TGF β) superfamily of proteins are cytokines involved in essential cellular functions such as proliferation, differentiation, apoptosis, tissue remodeling, angiogenesis, immune response, and cell adhesion (see, for example, Massagué, J. TGF β signaling in context. *Nat Rev Mol Cell Biol* 13, 616–630 (2012)). TGF β superfamily proteins are also important factors in the pathophysiology of disease states such as chronic inflammatory conditions and cancer. The members of this family include the three isoforms of TGF β s, β 1, β 2, β 3; bone morphogenetic proteins (BMPs) and activins. In addition to immunosuppression, TGF β proteins can act as a prometastatic and proangiogenic factor in late-stage cancer by constitutively inducing epithelial to mesenchymal transition (EMT) and tumor associated angiogenesis.

[0033] Blocking tumor-derived active TGF β for therapeutic purposes has been extensively explored. Many therapeutic approaches target TGF β pathway for the treatment of invasive cancers such as breast cancer and melanoma. For example, antagonists of TGF β binding to its heteromeric receptor have shown reduction of tumor cell motility, intravasation, and metastases in three experimental models of breast cancer. However, these treatment strategies in general do not affect cellular proliferation, indicating that TGF β blocking agents are not sufficient to promote the neutralization or killing of tumor cells.

[0034] The utilization of an interleukin polypeptide (e.g., interleukin-2 or interleukin-15) in the fusion proteins disclosed herein is useful for activating an immune response in addition to the inhibition of TGF β . The interleukin family of proteins generally promote the activation, development, and differentiation of immune cells (e.g., T cells, NK cells, and other lymphocytes), and play essential roles in both innate and adaptive immunity. Accordingly, immune cell

activation, development, and differentiation can be achieved by the fusion proteins disclosed herein.

[0035] Proinflammatory or immune-stimulating interleukins (e.g., interleukin-2 or interleukin-15) constitute useful adjuvants for activating an immune response. For example, interleukin-2 (IL-2) is a factor for lymphocyte activation and clonal expansion, promoting the activation, development, and differentiation of cytotoxic T cells. IL-2 and IL-15 also stimulate NK cell proliferation and cytotoxicity. However, IL-2 can operate as both an immunostimulatory (e.g., immune-activating) and immunosuppressive agent. As immunosuppressor, IL-2 maintains peripheral tolerance by inducing the generation of regulatory cells. For these reasons, IL-2 is considered a double-edge sword. The versatility of interleukin function is influenced by the environment and the interaction with signaling agents.

[0036] Accordingly, the fusion proteins disclosed herein are multi-functional in that the fusion proteins are useful for immune activation, the inhibition of immunosuppression, and/or the suppression or inhibition of angiogenic or invasive signaling. In some embodiments, immune activation, the inhibition of immunosuppression, and/or the suppression or inhibition of angiogenic or invasive signaling can be used to treat a tumor. In some embodiments, activating an immune (e.g., activating cells expressing an IL-2 receptor), inhibiting of immunosuppression of immune cells (e.g., blocking or inhibiting an interaction between a cell and TGF β a), and/or the suppression or inhibition of angiogenic or invasive signaling in a subject can be used to treat a tumor or cancer. As disclosed, the activation of an immune response and inhibition of an immunosuppressive response is, at least in part, achieved by the interleukin polypeptide. In turn, the inhibition or reduction of an immunosuppressive signaling or signaling molecules and/or inhibition of angiogenic or invasive signaling or signaling molecules is achieved, at least in part, through the use of one or more polypeptides that inhibit activity of TGF β proteins.

[0037] As used herein, the term “fusion protein” generally refers to a protein that includes polypeptide components derived from more than one parental protein or polypeptide. Generally, a fusion protein is expressed from a fusion gene in which a nucleotide sequence encoding a polypeptide sequence from one protein is appended in frame with, and optionally separated by a linker from, a nucleotide sequence encoding a polypeptide sequence from a different protein. The fusion gene can then be expressed by a recombinant host cell as a single protein.

[0038] A “domain” of a protein, as used herein, generally is any portion of the entire protein, up to and including the complete protein, but typically comprising less than the complete protein. A domain can, but need not, fold independently of the rest of the protein chain and/or be correlated

with a particular biological, biochemical, or structural function or location (e.g., a ligand binding domain, or a cytosolic, transmembrane or extracellular domain).

[0039] The term “recombinant” indicates that the material (e.g., a nucleic acid or a polypeptide) has been artificially or synthetically (e.g., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a “recombinant nucleic acid” is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling, or other well-known molecular biological procedures. A “recombinant DNA molecule,” is comprised of segments of DNA joined together by means of such molecular biological techniques. The term “recombinant protein” or “recombinant polypeptide” as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule. A “recombinant host cell” is a cell that contains and/or expresses a recombinant nucleic acid.

[0040] A “polynucleotide sequence” or “nucleotide sequence” or “nucleic acid sequence,” as used interchangeably herein, is a polymer of nucleotides, including an oligonucleotide, a DNA, and RNA, a nucleic acid, or a character string representing a nucleotide polymer, depending on context. From any specified polynucleotide sequence, either the given nucleic acid or the complementary polynucleotide sequence can be determined. Included are DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

[0041] Polynucleotide and polypeptide sequences of the current disclosure can be defined in terms of particular identity and/or similarity with certain polynucleotides and polypeptides described herein. In some embodiments, sequence identity will typically be greater than 60%, greater than 75%, greater than 80%, greater than 90%, and/or greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence disclosed herein. Optimal alignment can be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAST, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, BLAST searches are performed with the BLAST program, score=100, wordlength=12, to obtain sequences with the desired percent sequence identity. In some

embodiments, to obtain gapped alignments for comparison purposes, Gapped BLAST. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. In some embodiments, Clustal Omega is used.

[0042] As used herein, the terms “nucleic acid molecule encoding,” “DNA sequence encoding,” “nucleic acid sequence encoding” or “DNA encoding” refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of ribonucleotides along the mRNA chain, and also determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the RNA sequence and for the amino acid sequence.

[0043] “Expression of a gene” or “expression of a nucleic acid” means transcription of DNA into RNA (optionally including modification of the RNA, e.g., splicing), translation of RNA into a polypeptide (possibly including subsequent post-translational modification of the polypeptide), or both transcription and translation, as indicated by the context.

[0044] The term “gene” is used broadly to refer to any nucleic acid associated with a biological function. Genes typically include coding sequences and/or the regulatory sequences required for expression of such coding sequences. The term “gene” applies to a specific genomic or recombinant sequence, as well as to a cDNA or mRNA encoded by that sequence. A “fusion gene” contains a coding region that encodes a transgene. Genes also include non-expressed nucleic acid segments that, for example, form recognition sequences for other proteins. Non-expressed regulatory sequences including transcriptional control elements to which regulatory proteins, such as transcription factors, bind, resulting in transcription of adjacent or nearby sequences.

[0045] As used herein the term “coding region” or “coding sequence” when used in reference to a structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of an mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet “ATG” which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (e.g., TAA, TAG, TGA). Transcriptional control signals in eukaryotes comprise “promoter” and “enhancer” elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect, and mammalian cells and viruses (analogous control elements, e.g., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein

of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types.

[0046] The term “expression vector” refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the expression vector, operably linked to the coding sequence for the inventive toxin peptide analog, so that the expressed toxin peptide analog can be secreted by the recombinant host cell, for more facile isolation of the toxin peptide analog from the cell, if desired. Such techniques are well known in the art.

[0047] As used herein, individual is synonymous with patient and/or subject and includes and/or refers to a human and may be a human that has been diagnosed as needing to treat a disease or condition as disclosed herein. However, examples are not limited to humans and include, chimpanzees, marmosets, cows, horses, sheep, goats, pigs, rabbits, dogs, cats, rats, mice, guinea pigs, and the like. The individual is typically a human and may be a human that has been diagnosed as needing to treat a disease or condition as disclosed herein.

[0048] As used herein, the term “inhibition” or “inhibiting” includes and/or refers to the reduction or suppression of a given condition, symptom, disorder, or disease, and/or a decrease in the baseline activity of a biological activity or process.

[0049] As used herein, the term “treating” or “treatment” of includes and/or refers to ameliorating the disease or disorder or symptoms thereof (e.g., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In certain embodiments, “treating” or “treatment” also includes and/or refers to alleviating or ameliorating at least one physical and/or biological parameters including those which may not be discernible by the patient. In certain embodiments, “treating” or “treatment” includes and/or refers to modulating a disease, disorder, or biological process either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical and/or biological parameter), or both. In certain embodiments, “treating” or “treatment” includes and/or refers to preventing or delaying the onset or development or progression of the disease or disorder. In certain embodiments, “treating” or “treatment” includes and/or refers to preventing or delaying or inhibiting the

deterioration of (i) a healthy physiological state or (ii) a baseline physiological state (e.g., the progression of a disease or disorder).

[0050] As used herein, “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification includes and/or refers to “one” and also consistent with the meaning of “one or more”, “at least one”, and “one or more than one”. Similarly, the word “another” may mean at least a second or more.

[0051] As used herein, the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “include” and “includes”) or “containing” (and any form of containing, such as “contain” and “contains”), are inclusive or open-ended and do not exclude additional, unrecited elements or process steps.

[0052] As used herein, the term “about” in the context of a given value or range includes and/or refers to a value or range that is within 20%, within 10%, and/or within 5% of the given value or range.

[0053] As used herein, the term “and/or” is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each were set out individually herein.

[0054] As used herein, a “sample” includes and/or refers to any fluid or liquid sample which is being analyzed in order to detect and/or quantify an analyte. In some embodiments, a sample is a biological sample. Examples of samples include without limitation a bodily fluid, an extract, a solution containing proteins and/or DNA, a cell extract, a cell lysate, or a tissue lysate. Non-limiting examples of bodily fluids include urine, saliva, blood, serum, plasma, cerebrospinal fluid, tears, semen, sweat, pleural effusion, liquified fecal matter, and lacrimal gland secretion.

[0055] As used herein, in any instance or embodiment described herein, “comprising” may be replaced with “consisting essentially of” and/or “consisting of”. used herein, in any instance or embodiment described herein, “comprises” may be replaced with “consists essentially of” and/or “consists of”.

Interleukins

[0056] The fusion proteins provided herein generally comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF β activity (e.g., two or more polypeptides that bind a TGF β protein). Interleukins (IL) are a class of cytokines first thought to

be expressed by leukocytes alone but have later been found to be produced by many other body cells. Interleukins play important roles in, e.g., the activation and differentiation of immune cells, as well as cell proliferation, maturation, migration, and adhesion. The function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses. Accordingly, the interleukin polypeptide of the fusion protein is useful for the regulation (e.g., activation) of an immune response.

[0057] Interleukin-2 (IL-2) is an interleukin useful for activating the immune response in the context of a fusion protein comprising an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF β activity. Interleukin-2 (IL-2) is a pleiotropic cytokine that is induced by antigen stimulation and thus plays a significant role in regulating the immune response (see, for example, Spolski, R., Li, P. & Leonard, W.J. Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nat Rev Immunol* 18, 648–659 (2018)). For example, can promote an innate antitumor response due to its effectiveness at inducing locoregional tumor rejection, acting as autocrine factor for T cells and supporting the development of cytotoxic T cells, stimulating NK cells proliferation and cytolytic activity

[0058] IL-2 is a small, 15.5-kDa four α -helical bundle cytokine. It is produced predominately by antigen-stimulated CD4⁺ T cells, while it can also be produced by CD8⁺ cells, natural killer (NK) cells, and activated dendritic cells (DC). IL-2 is an important factor for the maintenance of CD4⁺ regulatory T cells and plays a critical role in the differentiation of CD4⁺ T cells into a variety of T cell subsets. It can promote CD8⁺ T-cell and NK cell cytotoxicity activity, and modulate T-cell differentiation programs in response to antigen, promoting naive CD4⁺ T-cell differentiation into T helper-1 (Th1) and T helper-2 (Th2) cells while inhibiting T helper-17 (Th17) differentiation. Notably, IL-2 was one of the first FDA-approved immunotherapy drugs for metastatic melanoma and renal cell cancer. However, IL-2 immunotherapy has not been widely applied due to its short half-life in vivo and severe toxicity at the therapeutic dosage.

[0059] The term “interleukin-2” or “IL-2” refers to any native IL-2 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. IL-2 includes unprocessed IL-2 as well as any form of IL-2 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-2 is shown in SEQ ID NO: 1. Unprocessed human IL-2 additionally comprises an N-terminal 20 amino acid signal peptide, which is absent in the mature IL-2 molecule. IL-2 also includes “wild-type IL-2” or a naturally occurring IL-2. The sequence of a native human IL-2 molecule is shown in SEQ ID NO:

1. For the purpose of the present disclosure, the term wild-type also encompasses forms of IL-2 comprising one or more amino acid mutations. For example, the fusion proteins described herein can comprise an IL-2 polypeptide having greater than 80 %, 85 %, 90 %, 95 %, 96 %, 97 %, 98 %, 99 % sequence identity to the amino acid sequence of SEQ ID NO: 1.

[0060] In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

[0061] The IL-2 polypeptide can comprise a full-length native sequence or truncations thereof. In some embodiments, the IL-2 polypeptide is a truncated IL-2 polypeptide. The truncated IL-2 polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about

10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 polypeptide having an amino acid sequence of SEQ ID NO: 2. In some embodiments, the fusion protein comprises an IL-2 polypeptide having an amino acid sequence of SEQ ID NO: 3. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

[0062] In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino

acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

[0063] In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

[0064] Interleukin-15 (IL-15) is also an interleukin useful for activating the immune response in the context of a fusion protein comprising an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF β activity. Interleukin 15 (IL-15) is a cytokine of about 12–14 kilodaltons comprising a four- α -helix structure. IL-15 belongs to the family of cytokines consisting of interleukins IL-2, IL-4, IL-7, IL-9, and IL-21. IL-15 signals through a receptor complex composed of the IL-2/IL-15 receptor β (IL-15R β) (CD122) subunit, which is shared with IL-2 and the common gamma chain (γ C) (CD132) receptor subunit, which is also utilized by all of the additional family members. Notably, IL-15 is a growth factor for T cells and NK cells, and plays an important role in the development, proliferation, and activation of these immune cells. Although IL-15 has a potential for therapeutic use, natural IL-15 has therapeutic development issues, namely a low biological potency and a short half-life.

[0065] The term “interleukin-15” or “IL-15” refers to any native IL-15 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless

otherwise indicated. IL-15 includes unprocessed IL-15 as well as any form of IL-15 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-15, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-15 is shown in SEQ ID NO: 4. IL-15 also includes “wild-type IL-15” or a naturally occurring IL-15. The sequence of a native human IL-15 molecule is shown in SEQ ID NO: 4. For the purpose of the present disclosure, the term wild-type IL-15 also encompasses forms of IL-15 comprising one or more amino acid mutations. For example, the fusion proteins described herein can comprise an IL-15 polypeptide having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to the amino acid sequence of SEQ ID NO: 4.

[0066] In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-15 sequences or variant sequences (e.g., a IL-15 polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

[0067] The IL-15 polypeptide can comprise a full-length native sequence or truncations thereof. In some embodiments, the IL-15 polypeptide is a truncated IL-15 polypeptide. The truncated IL-15 polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino

acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about 10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 polypeptide having an amino acid sequence of SEQ ID NO: 5.

[0068] In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %.

In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

TGFβ inhibiting polypeptides

[0069] As described herein, the fusion proteins provided herein generally comprise an interleukin polypeptide (e.g., IL-2 or IL-15) fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity (e.g., two or more polypeptides that bind a TGFβ protein). The transforming growth factor β (TGFβ) superfamily is a large group of soluble factors (e.g., proteins) that initiate and control activation, proliferation, and differentiation of many cell types, thus playing important roles in embryonal development and homeostasis. The TGFβ superfamily includes several subfamilies: the activin/inhibin family, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), the TGFβ subfamily, and glial cell line-derived neurotrophic factor (GDNF) family. TGFβ superfamily has three types: TGFβ 1, TGFβ 2, and TGFβ 3. TGFβ superfamily proteins have been discovered in a variety of species, including invertebrates as well as vertebrates. TGFβ malfunction can lead to developmental disorders, severe defects in organ function, and is associated with several diseases, including various types of cancer.

[0070] The inhibition and suppression of TGFβ superfamily activity can be achieved through the use of fusion proteins comprising, in addition to an interleukin polypeptide, two or more TGFβ superfamily receptor polypeptides. In the context of the fusion proteins described herein, the two or more TGFβ superfamily receptor polypeptides yield a multi-valent single chain polypeptide capable of binding two or more TGFβ proteins. Generally, ligands of the TGFβ superfamily bind to receptor complexes consisting of type I and type II receptor subunits. As disclosed herein, polypeptides derived from TGFβ superfamily receptors are useful for inhibiting or suppressing TGFβ superfamily ligands. For example, in some embodiments, the TGFβ superfamily receptor is selected from the group consisting of: an activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, and a TGFβ receptor polypeptide or fragment thereof.

[0071] Within the TGFβ superfamily of proteins, the TGFβ sub-family of proteins comprises TGFβ1, TGFβ2, and TGFβ3. One of the biological effects of TGFβ proteins is the inhibition of proliferation of most normal epithelial cells using an autocrine mechanism of action, and this suggests a tumor suppressor role for TGFβ. Loss of autocrine TGFβ activity and/or responsiveness

to exogenous TGF β appears to provide some epithelial cells with a growth advantage leading to malignant progression. This suggests a pro-oncogenic role for TGF β in addition to its tumor suppressor role. Among TGF β s, β 1, β 2, β 3, TGF β 1 is the most potent immunosuppressive cytokine described to date, exerts deleterious effects (e.g., immunosuppression) on several components of the immune system response against cancer cells, and is most frequently overexpressed by carcinomas. Notably, TGF β proteins (e.g., TGF β 1) diminishes or reduces the effector functions of macrophages, B cells, cytotoxic T cells, dendritic cells, and NK cells, where TGF β acts as negative regulator of IFN γ production via its mediators SMAD2, SMAD3, and SMAD4. In addition to immunosuppression, TGF β proteins can act as a prometastatic and proangiogenic factor in late-stage cancer by constitutively inducing epithelial to mesenchymal transition (EMT) and tumor associated angiogenesis.

[0072] Most cell types express three sizes of receptors for TGF β , designated Type I (53 kDa), Type II (70 - 85 kDa), and Type III (250 - 350 kDa). The Type I receptor is a membrane-bound serine/threonine kinase that apparently requires the presence of the Type II receptor to bind TGF β . The Type II receptor is also a membrane-bound serine/threonine kinase that binds TGF β 1 and TGF β 3 with high affinity and TGF β 2 with much lower affinity. The Type I and Type II receptors together form a heterodimeric signaling complex that is essential for the transduction of the anti-proliferative signals of TGF β . The Type III receptor is a transmembrane proteoglycan with a large extracellular domain and a 43 amino acid residue cytoplasmic domain. The cytoplasmic domain of the Type III receptor lacks an obvious signaling motif and the receptor may not be involved directly in signal transduction. Notably, the soluble extracellular domain of the T β RII, consisting of the extracellular domain of the receptor binds TGF- β 1 and TGF- β 3 with high affinity.

[0073] Accordingly, the TGF β receptor T β RII polypeptides are useful in TGF β binding polypeptides of the multi-functional fusion proteins described herein. Particularly, TGF β receptor T β RII polypeptides are useful for inhibiting or reducing TGF β 1 activity or signaling associated therewith. The term “soluble transforming growth factor (TGF) β receptor type II B or sT β RII” as used herein refers to a soluble, or non-membrane form of the alternatively spliced transforming growth factor β type II receptor, preferably the ectodomain of the TGF β type II receptor from any species or source and includes the full-length ectodomain as well as fragments or portions of the ectodomain. In some embodiments, the sT β RIIB is human or mouse. The human TGF β receptor II has the amino acid sequence of SEQ ID NOs: 5 or 6 (short and long isoforms, respectively). The term “sT β RII fragment” as used herein means at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % or 100 % of the full-length ectodomain.

80 %, 90 %, 95 %, or more of the entire length of the reference polypeptide. In one embodiment, the IL-2 is truncated at the N-terminal or C-terminal end to permit cloning.

[0074] In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

[0075] In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having

sequence identity to SEQ ID NO: 7 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

[0076] As described herein, the two or more sTBRII receptor polypeptides utilize the ectodomain of a native sTBRII receptor. Accordingly, the sTBRII receptor polypeptide used herein comprises SEQ ID NO: 8 (e.g., the short isoform of sTBRII), SEQ ID NO: 9 (e.g., the long isoform of sTBRII), or a combination thereof.

[0077] In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

[0078] In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some

embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an TBRII sequence having sequence identity to SEQ ID NO: 9 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

[0079] The sTBRII polypeptide of SEQ ID NO: 8 or SEQ ID NO: 9 can comprise additional truncations thereof. In some embodiments, the sTBRII polypeptide is a truncated sTBRII polypeptide. The truncated sTBRII polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide). In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about 10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide). In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids. In some embodiments, the fusion protein comprises an sTBRII that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids. In some embodiments, the fusion protein comprises an sTBRII that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide).

[0080] Suitable sTBRII sequences or variant sequences (e.g., sTBRII polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any

of the methods for sequestering or binding TGF β described herein (e.g., as described in any one of Examples 1-3).

Multi-Functional and Multi-Valent Fusion Protein Compositions

[0081] Provided herein are fusion polypeptides comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide. Also provided herein are fusion polypeptides comprising: a first IL polypeptide that binds to and agonizes the IL receptor; a second polypeptide that binds to and sequesters soluble TGF β ; and a third polypeptide that binds to and sequesters soluble TGF β .

[0082] In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 2. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 3. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In certain embodiments the IL-15 polypeptide comprises SEQ ID NO: 5. In certain embodiments the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5. In certain embodiments, the IL polypeptide is a truncated polypeptide. In some embodiments, the IL-2 sequence comprises an alanine at position 62 (e.g., as in SEQ ID NO: 3).

[0083] In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II polypeptide. In some embodiments, the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGF β superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In certain embodiments the soluble TGF β receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

[0084] In another embodiment, the second polypeptide that binds to and sequesters soluble TGF β and/or the third polypeptide that binds to and sequesters soluble TGF β comprises a soluble TGF β receptor II polypeptide. In some embodiments, the second polypeptide that binds to and sequesters soluble TGF β and/or the third polypeptide that binds to and sequesters soluble TGF β comprises a soluble TGF β receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In certain embodiments the soluble TGF β receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

[0085] In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide target a TGF- β 1 polypeptide, a TGF- β 2 polypeptide, a TGF- β 3 polypeptide, a activin β A polypeptide, a activin β B polypeptide, a activin β C polypeptide, a activin β E polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an artaman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide.

[0086] In another embodiment, the second polypeptide and/or the third polypeptide binds to and sequesters a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof. In certain embodiments, the second polypeptide and/or the third polypeptide binds to and sequesters a TGF β 1 polypeptide.

[0087] A polypeptide of the fusion polypeptide can be “fused” or “linked” via a linker amino acid sequence. The linker may be a polypeptide linker or other linker of suitable flexibility so as not to inhibit binding of either targeting polypeptide (e.g., the IL2 polypeptide or TBR11 receptor polypeptide). The linker polypeptide can be unstructured (e.g., lacking secondary structure), structured, or a combination thereof. In some embodiments, the linker sequence is a natural amino

acid sequence of the IL polypeptide or soluble TBR II polypeptide. In some embodiments, the linker is not native to the IL polypeptide or TGF receptor polypeptide. For example, a non-native linker can comprise poly-glycine, poly-alanine, poly-serine amino acid or a combination thereof (e.g., GSSG (SEQ ID NO: 26), GGSS (SEQ ID NO: 27), GSAGG (SEQ ID NO: 28), etc.).

[0088] In some embodiments, the linker is a non-nature or synthetic linker. The term "synthetic linker" as used herein includes a chemical moiety comprising or derived from a group of atoms that is covalently attached to a targeting agent, and that is also covalently attached to a cytotoxic moiety. Linkers include compounds comprising or derived from divalent radicals such as an alkylene, an arylene, a heteroarylene, moieties such as: $-(CR_2)_nO(CR_2)_n-$ wherein R_2 is independently repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, polyetheramines such as Jeffamine™) and n is independently >1 , in particular n may be 1 to 15; compounds including the linkers described in Example 1, N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC) and N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide as well as peptides, such as but not limited to repeating units of G, A and C (for example up to 10) with one or more lys residues or other suitable chemical groups for linking to a targeting agent and a cytotoxic moiety. The linker is optionally C1-30 alkylene, unsubstituted or substituted with one or more substituents, and/or optionally interrupted with one or more heteromoieties independently selected from O, S, NR₁, and/or optionally interrupted with one or more of C(O) and C(S), wherein R₁ is independently selected from H, and C1-6 alkyl. The linker can comprise a non-cleavable (stable linker) or cleavable unit (labile linker) such as a peptide bond or a disulfide bond. The linker can be conjugated to the targeting agent and/or the cytotoxic moiety via reactive functional groups.

[0089] Both cleavable and non-cleavable linkers can be used in the synthesis of fusion proteins (ADCs). Cleavable linkers include motifs that are either sensitive to lysosomal proteases or sensitive to an acidic pH (such as hydrazone, which is hydrolysed to cleave the linker in gemtuzumab ozogamicin and inotuzumab ozogamicin), or they can contain disulfide bridges that can be reduced by glutathione. The steric hindrance of disulfide bridges can be optimized to limit premature cleavage inside the cell. Generally, the disulfide linker is initially cleaved to release the thiol compound. Acid-cleavable linkers, such as hydrazone, are designed to remain stable at the neutral pH in the blood circulation, but in acidic cellular compartments they undergo hydrolysis and release the cytotoxic drug.

[0090] In some embodiments, the linker (e.g., peptide linker) comprises about 10 amino acids to about 100 amino acids. In some embodiments, the linker comprises about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 25 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 50 amino acids, about 10 amino acids to about 75 amino acids, about 10 amino acids to about 100 amino acids, about 15 amino acids to about 20 amino acids, about 15 amino acids to about 25 amino acids, about 15 amino acids to about 30 amino acids, about 15 amino acids to about 40 amino acids, about 15 amino acids to about 50 amino acids, about 15 amino acids to about 75 amino acids, about 15 amino acids to about 100 amino acids, about 20 amino acids to about 25 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 75 amino acids, about 20 amino acids to about 100 amino acids, about 25 amino acids to about 30 amino acids, about 25 amino acids to about 40 amino acids, about 25 amino acids to about 50 amino acids, about 25 amino acids to about 75 amino acids, about 25 amino acids to about 100 amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 75 amino acids, about 30 amino acids to about 100 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 75 amino acids, about 40 amino acids to about 100 amino acids, about 50 amino acids to about 75 amino acids, about 50 amino acids to about 100 amino acids, or about 75 amino acids to about 100 amino acids. In some embodiments, the linker comprises about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 75 amino acids, or about 100 amino acids. In some embodiments, the linker comprises at least about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, or about 75 amino acids. In some embodiments, the linker comprises at most about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 75 amino acids, or about 100 amino acids.

[0091] Generally, different polypeptide elements of the fusion polypeptides can be arranged or ordered, from N-terminus to C-terminus, in any number of different combinations. For example, described herein are fusion polypeptides that comprise, from N-terminus to C-terminus, an IL polypeptide, a first TGF β superfamily receptor polypeptide, and a second TGF β superfamily receptor polypeptide. By way of further example, the fusion polypeptides as described herein, can

also comprise, from N-terminus to C-terminus, a first TGF β superfamily receptor polypeptide, an IL polypeptide, and a second TGF β superfamily receptor polypeptide. In some embodiments, the fusion polypeptide further comprises a pharmacokinetic extender polypeptide (e.g., an Fc polypeptide or HSA polypeptide), wherein the fusion polypeptides further comprising the pharmacokinetic extender can be arranged or ordered, from N-terminus to C-terminus, in any number of different combinations. **FIG. 3** shows a non-limiting schematic representation of exemplary embodiments of fusion polypeptides that comprise an IL polypeptide, a first TGF β superfamily receptor polypeptide, and a second TGF β superfamily receptor polypeptide (e.g., SEQ ID NOs: 10-25)

[0092] In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10 to 20.

[0093] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises

an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 10.

[0094] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 11.

[0095] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 12.

[0096] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion comprises SEQ ID NO: 13.

[0097] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion comprises SEQ ID NO: 14.

[0098] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98%

sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion comprises SEQ ID NO: 15.

[0099] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion comprises SEQ ID NO: 16.

[0100] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion comprises SEQ ID NO: 17.

[0101] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90%

sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion comprises SEQ ID NO: 18.

[0102] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion comprises SEQ ID NO: 19.

[0103] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion comprises SEQ ID NO: 20.

[0104] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at

least 80% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion comprises SEQ ID NO: 21.

[0105] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion comprises SEQ ID NO: 22.

[0106] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion comprises SEQ ID NO: 23.

[0107] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion comprises SEQ ID NO: 24.

[0108] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion comprises SEQ ID NO: 25.

[0109] In some embodiments, the bivalent FIST fusion polypeptide comprises one or more amino acid modifications (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 15 amino acid modifications mutations (e.g., substitutions,

deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), or about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), or about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, the modification increases an affinity for an IL-2 receptor. In certain embodiments, the IL-2 receptor comprises IL-2R α (termed CD25). In certain embodiments, the IL-2 receptor comprises IL-2R β . In certain embodiments, the IL-2 receptor comprises IL-2R γ . In certain embodiments, the modification increases an affinity for IL-2R β and decreases an affinity for IL-2R α . Such mutations for affecting IL-2 affinity for an IL-2 receptor include those known in the field. Suitable bivalent FIST sequences or variant sequences (e.g., fusion polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods for activating an immune cell (e.g., increased IFN-gamma secretion, increased proliferation, etc.) and sequestering or binding TGF β (e.g., TGF β binding, etc.) described herein (e.g., as described in any one of Examples 1-5).

[0110] In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 25.

[0111] The fusion polypeptides disclosed herein can further comprise a pharmacokinetic modulating polypeptide (e.g., a polypeptide that improves the pharmacokinetic profile of a therapeutic agent). For example, the fusion protein can further comprise an immunoglobulin Fc polypeptide or human serum albumin (HAS) polypeptide fused to the N-terminus of the IL polypeptide. In some embodiments, the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide. In some embodiments, the immunoglobulin Fc region is an IgG Fc region. In some embodiments, the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments, the PK modulator comprises an albumin polypeptide. In some embodiments, the albumin polypeptide is a human albumin polypeptide.

[0112] In certain embodiments the fusion polypeptides of the current disclosure are included in a pharmaceutical composition comprising one or more pharmaceutically acceptable excipients, carriers, and diluents. In certain embodiments, the fusion proteins of the current disclosure are administered suspended in a sterile solution. In certain embodiments, the solution comprises about 0.9% NaCl. In certain embodiments, the solution comprises about 5.0% dextrose. In certain embodiments, the solution further comprises one or more of: buffers, for example, acetate, citrate,

histidine, succinate, phosphate, bicarbonate and hydroxymethylaminomethane (Tris); surfactants, for example, polysorbate 80, polysorbate 20, and poloxamer 188; polyol/disaccharide/polysaccharides, for example, glucose, dextrose, mannose, mannitol, sorbitol, sucrose, trehalose, and dextran 40; amino acids, for example, glycine or arginine; antioxidants, for example, ascorbic acid, methionine; or chelating agents, for example, Ethylenediaminetetraacetic acid or ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid.

[0113] In certain embodiments, the fusion proteins of the current disclosure are shipped/stored lyophilized and reconstituted before administration. In certain embodiments, lyophilized fusion polypeptide formulations comprise a bulking agent such as, mannitol, sorbitol, sucrose, trehalose, dextran 40, or combinations thereof. The lyophilized formulation can be contained in a vial comprised of glass or other suitable non-reactive material. The fusion proteins when formulated, whether reconstituted or not, can be buffered at a certain pH, generally less than 7.0. In certain embodiments, the pH can be between 4.5 and 6.5, 4.5 and 6.0, 4.5 and 5.5, 4.5 and 5.0, or 5.0 and 6.0.

[0114] In certain embodiments, described herein is a method of preparing a cancer treatment comprising admixing one or more pharmaceutically acceptable excipients, carriers, or diluents and a fusion polypeptide of the current disclosure. In certain embodiments, described herein is a method of preparing a cancer treatment for storage or shipping comprising lyophilizing one or more fusion polypeptides of the current disclosure.

[0115] The fusion polypeptides described herein (e.g., SEQ ID NOs: 10 to 25) can be encoded by a nucleic acid. A nucleic acid is a type of polynucleotide comprising two or more nucleotide bases. In certain embodiments, the nucleic acid is a component of a vector that can be used to transfer the polypeptide encoding polynucleotide into a cell. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a genomic integrated vector, or “integrated vector,” which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an “episomal” vector, e.g., a nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as “expression vectors.” Suitable vectors comprise plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, viral vectors and the like. In the expression vectors regulatory elements such as promoters, enhancers, polyadenylation signals for use in controlling transcription can be derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants

may additionally be incorporated. Vectors derived from viruses, such as lentiviruses, retroviruses, adenoviruses, adeno-associated viruses, and the like, may be employed. Plasmid vectors can be linearized for integration into a chromosomal location. Vectors can comprise sequences that direct site-specific integration into a defined location or restricted set of sites in the genome (e.g., AttP-AttB recombination). Additionally, vectors can comprise sequences derived from transposable elements.

Methods of Use

[0116] The fusion polypeptides disclosed herein are useful as an effective angiostatic and anticancer compound for cancer therapy. For example, the disclosed fusion polypeptides are an effective immunotherapy that induces a unique gene expression profile downstream of IL-2 receptor not achieved by its single components or the combination, which leads to new pharmacological properties. These novel multi-functional proteins can simultaneously activate several immune system mechanisms and signaling pathways that act in synergy to effectively eliminate cancer cells. At the cellular level, the immunotherapy platform activates a panoply of lymphoid cells (e.g., T, B, NK and NK-T cells) and indirectly prime antigen-presenting cells (e.g., dendritic cells and macrophages) due to, in part, GM-CSF induction triggering the cascade of specific immunological reactions against malignant cells. At the molecular level, it activates key transcription factors and signaling molecules crucial to potentiate innate and adaptive immune responses. The fusion polypeptides described herein target and synchronize various arms of the immune system against cancer.

[0117] Accordingly, disclosed herein are methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; thereby inhibiting or reducing tumor growth and/or progression in the individual. Furthermore, provided are the fusion polypeptides for use in methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; thereby inhibiting or reducing tumor growth and/or progression in the individual.

[0118] Also disclosed herein are methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the

individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; wherein the fusion polypeptide activates an immune cell. Furthermore, provided are the fusion polypeptides for use in methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; wherein the fusion polypeptide activates an immune cell. In some embodiments the immune cell is selected from the group consisting of: a T cell, an NK cell, a B cell, or a combination thereof. In certain embodiments, activation comprises increasing cell proliferation, increasing cytokine signaling (e.g., IFN γ), and/or decreasing TGF β 1-mediated suppression.

[0119] Additionally, disclosed are methods of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide, thereby neutralizing or killing tumor cells in the individual. Furthermore, provided are the fusion polypeptides for use in methods of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide, thereby neutralizing or killing tumor cells in the individual.

[0120] Disclosed are also methods of treating or ameliorating cancer in an individual having a cancer, comprising administering to the subject a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the individual. Furthermore, provided are the fusion polypeptides for use methods of treating or ameliorating cancer in an individual having a cancer, comprising administering to the subject a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the individual.

[0121] Also provided are methods of activating a cell expressing an IL receptor comprising contacting a cell with a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide. In some embodiments, the cell is an immune cell. The immune cell can be contacted,

and subsequently activated, in vivo or ex vivo. In vivo, the immune cell can reside within a tumor microenvironment. Furthermore, provided are the fusion polypeptides for use in methods of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide.

[0122] Methods utilizing the ex-vivo activation of immune cells using the fusion polypeptides disclosed herein are also embodied. For example, disclosed are methods of activating immune cells ex vivo, comprising contacting an immune cell with a composition comprising: (a) an interleukin (IL) polypeptide; (b) a first TGF β superfamily receptor polypeptide; and (c) a second TGF β superfamily receptor polypeptide; thereby producing an activated immune cell. Such methods can be applied to the treatment of cancer or a tumor by administering the activated immune cells to an individual having cancer or a tumor. For example, the fusion polypeptides described herein are useful in Methods for the ex-vivo activation of immune cells. The ex vivo treatment of immune cells is also useful in the generation and expansion of immune cells for the generation of chimeric antigen receptor cell therapies (e.g., CAR-T or CAR-NK cells and B cells). For example, a leukopack (e.g., a leukapheresis product) comprising immune cells can be contacted with the fusion polypeptides disclosed herein to expand T cells or NK cells to be used in a chimeric antigen receptor cell therapy. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer.

[0123] In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 2. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 3. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In certain embodiments the IL-15 polypeptide comprises SEQ ID NO: 5. In certain embodiments the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99%

sequence identity to SEQ ID NO: 5. In certain embodiments, the IL polypeptide is a truncated polypeptide.

[0124] In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprise a soluble TGF β receptor II polypeptide. In some embodiments, the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide comprise a soluble TGF β receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGF β superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In certain embodiments the soluble TGF β receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

[0125] In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide target a TGF- β 1 polypeptide, a TGF- β 2 polypeptide, a TGF- β 3 polypeptide, a activin β A polypeptide, a activin β B polypeptide, a activin β C polypeptide, a activin β E polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an artaman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof. In some embodiments, the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide.

[0126] In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an

amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25.

[0127] In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10 to 20.

[0128] The term “cancer” includes primary malignant tumors (e.g., those whose cells have not migrated to sites in the subject's body other than the site of the original tumor) and secondary malignant tumors (e.g., those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor).

[0129] “Tumor” refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0130] The methods disclosed herein are useful for treating cancers comprising a solid tumor or treating solid tumors. The term “solid tumor” refers to, for example, breast cancer, ovarian cancer, cancer of the colon and generally the GI (gastro-intestinal) tract, cervix cancer, lung cancer, in particular small-cell lung cancer, and non-small-cell lung cancer, head and neck cancer, bladder cancer, cancer of the prostate or Kaposi's sarcoma. The fusion polypeptides neutralize and/or inhibit the growth of solid tumors. Furthermore, depending on the tumor type and the particular combination used a decrease of the tumor volume can be obtained. The combinations disclosed herein are also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases. The combinations disclosed herein are in particular suitable for the treatment of poor prognosis patients, especially such poor prognosis patients having metastatic melanoma or pancreatic cancer.

[0131] Furthermore, the methods disclosed herein are useful in the treatment of hematological cancer. The term “hematological cancer” refers to a cancer of the blood, and includes leukemia and malignant lymphoproliferative disorders, among others. “Leukemia” refers to a cancer of the blood, in which too many white or red blood cells are made, thus crowding out the other parts that make up the blood, such as platelets and normal red blood cells. It is understood that cases of leukemia are classified as acute or chronic. Cancer cells in acute leukemias are blocked at an immature stage, yet they continue to multiply. Chronic leukemias progress more slowly, with cancer cells developing to full maturity. Furthermore, the white blood cells may be myelogenous or lymphoid. Thus, certain forms of leukemia may be, by way of example, acute lymphocytic (or lymphoblastic) leukemia (ALL); acute myelogenous leukemia (AML); chronic lymphocytic leukemia (CLL); or chronic myelogenous leukemia (CML); and myelodysplastic syndrome. “Malignant hematological disorders” may refer to a lymphoma, such as Hodgkin's lymphoma, and non-Hodgkin's lymphoma, or multiple myeloma among others.

[0132] In certain embodiments, disclosed herein, are fusion proteins useful for the treatment of a cancer or tumor. Treatment refers to a method that seeks to improve or ameliorate the condition being treated. With respect to cancer, treatment includes, but is not limited to, reduction of tumor volume, reduction in growth of tumor volume, increase in progression-free survival, or overall life expectancy. In certain embodiments, treatment may affect remission of a cancer being treated. In certain embodiments, treatment encompasses use as a prophylactic or maintenance dose intended to prevent reoccurrence or progression of a previously treated cancer or tumor. It is understood by those of skill in the art that not all individuals will respond equally or at all to a treatment that is administered, nevertheless these individuals are considered to be treated.

[0133] The fusion polypeptides provided herein are useful for the activation of an immune response (e.g., anti-tumor immune response). In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. Accordingly, treating or a treatment, in some embodiments, comprises activating immune effector cells that target a tumor cell and/or reducing the activation of immunosuppressive cells (e.g., Treg cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), tumor-associated neutrophils (TANs), and cancer-associated fibroblasts (CAFs))

[0134] The term “T cell” refers to a subset of lymphocytic cells that are present in PBMC and express a surface marker of “CD3” (T-cell receptor). Unless otherwise indicated T cells are intended to include CD4+ (e.g., T-helper cells) and CD8+ (e.g., cytotoxic killer cells).

[0135] The methods provided herein are also useful for the suppression or reduction of immunosuppressive immune cells such as T regulatory cells. The terms “Treg” or “regulatory T cell” refer to CD4+CD25+FOXP3+ T cells that suppresses CD4+CD25– and CD8+ T cell proliferation and/or effector function, or that otherwise down-modulate an immune response. Notably, Treg may down-regulate immune responses mediated by CD8 T cells, Natural Killer cells, Natural Killer T cells as well as other immune cells. Further encompassed, in some embodiments, is the blockade or prevention of immunosuppressive cell differentiation or activation.

[0136] In certain embodiments, the cancer or tumor is a solid cancer or tumor. In certain embodiments, the cancer or tumor is a blood cancer or tumor. In certain embodiments, the cancer or tumor comprises breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head, neck, ovarian, prostate, brain, pancreatic, skin, bone, bone marrow, blood, thymus, uterine, testicular, and liver tumors. In certain embodiments, tumors which can be treated with the fusion proteins described herein comprise adenoma, adenocarcinoma, angiosarcoma, astrocytoma, epithelial carcinoma, germinoma, glioblastoma, glioma, hemangioendothelioma, hemangiosarcoma, hematoma, hepatoblastoma, leukemia, lymphoma, medulloblastoma, melanoma, neuroblastoma, osteosarcoma, retinoblastoma, rhabdomyosarcoma, sarcoma and/or teratoma. In certain embodiments, the tumor/cancer is selected from the group of acral lentiginous melanoma, actinic keratosis, adenocarcinoma, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, astrocytic tumors, Bartholin gland carcinoma, basal cell carcinoma, bronchial gland carcinoma, capillary carcinoid, carcinoma, carcinosarcoma, cholangiocarcinoma, chondrosarcoma, cystadenoma, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, ependymal sarcoma, Swing's sarcoma, focal nodular hyperplasia, gastrinoma, germ line tumors, glioblastoma, glucagonoma, hemangioblastoma, hemangioendothelioma, hemangioma, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, insulinitis, intraepithelial neoplasia, intraepithelial squamous cell neoplasia, invasive squamous cell carcinoma, large cell carcinoma, liposarcoma, lung carcinoma, lymphoblastic leukemia, lymphocytic leukemia, leiomyosarcoma, melanoma, malignant melanoma, malignant mesothelial tumor, nerve sheath tumor, medulloblastoma, medulloepithelioma, mesothelioma, mucoepidermoid carcinoma, myeloid leukemia,

neuroblastoma, neuroepithelial adenocarcinoma, nodular melanoma, osteosarcoma, ovarian carcinoma, papillary serous adenocarcinoma, pituitary tumors, plasmacytoma, pseudosarcoma, prostate carcinoma, pulmonary blastoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, squamous cell carcinoma, small cell carcinoma, soft tissue carcinoma, somatostatin secreting tumor, squamous carcinoma, squamous cell carcinoma, undifferentiated carcinoma, uveal melanoma, verrucous carcinoma, vagina/vulva carcinoma, VIPoma, and Wilm's tumor. In certain embodiments, the tumor/cancer to be treated with one or more fusion proteins described herein comprise brain cancer, head and neck cancer, colorectal carcinoma, acute myeloid leukemia, pre-B-cell acute lymphoblastic leukemia, bladder cancer, astrocytoma, preferably grade II, III or IV astrocytoma, glioblastoma, glioblastoma multiforme, small cell cancer, and non-small cell cancer, preferably non-small cell lung cancer, lung adenocarcinoma, metastatic melanoma, androgen-independent metastatic prostate cancer, androgen-dependent metastatic prostate cancer, prostate adenocarcinoma, and breast cancer, preferably breast ductal cancer, and/or breast carcinoma. In certain embodiments, the cancer treated with the fusion proteins of this disclosure comprises glioblastoma. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises pancreatic cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises ovarian cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises lung cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises prostate cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises colon cancer. In certain embodiments, the cancer treated comprises glioblastoma, pancreatic cancer, ovarian cancer, colon cancer, prostate cancer, or lung cancer. In a certain embodiment, the cancer is refractory to other treatment. In a certain embodiment, the cancer treated is relapsed. In a certain embodiment, the cancer is a relapsed/refractory glioblastoma, pancreatic cancer, ovarian cancer, colon cancer, prostate cancer, or lung cancer.

[0137] The term "tumor microenvironment" refers to a microenvironment (e.g., a region/area wherein malignant cells or clusters of malignant cells are located) in and surrounding a solid tumor to support the growth and metastasis of the tumor cells. The tumor microenvironment includes surrounding blood vessels, immune cells, fibroblasts, other cells, soluble factors, signaling molecules, an extracellular matrix, and mechanical cues that can promote neoplastic transformation, support tumor growth and invasion, protect the tumor from host immunity, foster therapeutic resistance, and provide niches for dormant metastases to thrive. The tumor and its

surrounding microenvironment are closely related and interact constantly. Tumors can influence their microenvironment by releasing extracellular signals, promoting tumor angiogenesis and inducing peripheral immune tolerance, while the immune cells in the microenvironment can affect the growth and evolution of cancerous cells.

[0138] In certain embodiments, the fusion proteins can be administered to a subject in need thereof by any route suitable for the administration of fusion protein-containing pharmaceutical compositions, such as, for example, subcutaneous, intraperitoneal, intravenous, intramuscular, intratumoral, or intracerebral, etc. In certain embodiments, the fusion proteins are administered intravenously. In certain embodiments, the fusion proteins are administered subcutaneously. In certain embodiments, the fusion proteins are administered intratumoral. In certain embodiments, the fusion proteins are administered on a suitable dosage schedule, for example, weekly, twice weekly, monthly, twice monthly, once every two weeks, once every three weeks, or once a month etc. In certain embodiments, the fusion proteins are administered once every three weeks. The fusion proteins can be administered in any therapeutically effective amount. In certain embodiments, the therapeutically acceptable amount is between about 0.1 mg/kg and about 50 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 1 mg/kg and about 40 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 5 mg/kg and about 30 mg/kg. Therapeutically effective amounts include amounts are those sufficient to ameliorate one or more symptoms associated with the disease or affliction to be treated.

[0139] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0140] As used herein, the terms “treatment” or “treating” are used in reference to a pharmaceutical or other intervention regimen for obtaining beneficial or desired results in the recipient. Beneficial or desired results include but are not limited to a therapeutic benefit and/or a prophylactic benefit. A therapeutic benefit may refer to eradication or amelioration of symptoms or of an underlying disorder being treated. Also, a therapeutic benefit can be achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the

subject may still be afflicted with the underlying disorder. A prophylactic effect includes delaying, preventing, or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof. For prophylactic benefit, a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease may undergo treatment, even though a diagnosis of this disease may not have been made.

[0141] The term “a therapeutically effective amount” of a compound of the present application refers to an amount of the compound of the present application that will elicit the biological or medical response of a subject, for example, reduction or inhibition of tumor cell proliferation, or ameliorate symptoms, alleviate conditions, slow or delay disease progression, or prevent a disease, etc. In one non-limiting embodiment, the term “a therapeutically effective amount” refers to the amount of a compound of the present application that, when administered to a subject, is effective to at least partially alleviate, inhibit, prevent and/or ameliorate a condition, or a disorder or a disease, or at least partially inhibit activity of a targeted enzyme or receptor.

[0142] The term “target cells” refers to a type of cell, cell population, or composition of cells which are the desired cells to be collected, isolated, or separated by the present disclosure. Target cells represent cells that various procedures described herein require or are designed to purify, collect, engineer etc. What the specific cells are will depend on the context in which the term is used. For example, if the objective of a procedure is to isolate a particular kind of stem cell, that cell would be the target cell of the procedure. The terms “target cells” and “desired cells” are interchangeable and have the same meaning regarding the present disclosure. Target cells can exist in a genus-species relationship. For example, if target cells comprised leukocytes, the target cells would include T cells.

[0143] The methods disclosed herein are particularly useful or comprise the activation of immune cells. The term “immune cell” refers generally to cells of the immune system. Immune cells are derived from myeloid or lymphoid cell lineages. Generally, the methods disclosed herein are directed towards, but not limited to, the activation of immune effector cells.

[0144] The term “immune effector cell” refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes. In certain embodiments, T cells,

NK cells, B cells, NKT cells and gamma/delta T cells or a combination thereof are activated by the fusion polypeptide.

[0145] The term “immune effector function” or “immune effector response,” as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack to a target cell. E.g., an immune effector function or response refers to a property of a T cells or NK cells that promotes killing or the inhibition of growth or proliferation, of a target cell. Immune effector function includes direct cytotoxicity, cytokine release, chemokine release, phagocytosis, or other immune function that primes or perpetuates an immune response.

[0146] The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

[0147] The term “myeloid cell” refers to terminally differentiated cells of the myeloid lineage. These cells include neutrophils, eosinophils and monocytes/macrophages, myeloid dendritic cells. In one embodiment of any aspect of the present disclosure, the myeloid cell is a neutrophil, eosinophil or monocyte/macrophage/dendritic cells.

[0148] The term “macrophage” and/or “macrophage-like cells” generally refers to macrophages, monocytes, and cells of macrophage/monocyte lineage, and any other similar cells which perform the functions generally associated with macrophages, such as phagocytosis or antigen presentation to other classes of immune cells such as T-cells and B-cells in order to sensitize these cells to a particular target, including but not limited to viruses, bacterial cells, other foreign cells, cancer cells, and other undesired proliferating cells.

[0149] The term “lymphocyte” as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells kill tumors and cells infected by viruses.

[0150] The term “natural killer (NK) cells” refers to cells of the immune system that kill target cells in the absence of a specific antigenic stimulus, and without restriction according to MHC class. Target cells may be tumor cells or cells harboring viruses. NK cells are characterized by the presence of CD56 and the absence of CD3 surface markers.

[0151] The term “T cell” refers to a subset of lymphocytic cells that express a surface marker of “CD3” (T-cell receptor). Unless otherwise indicated T cells are intended to include CD4⁺ (e.g., T-helper cells) and CD8⁺ (e.g., cytotoxic killer cells).

[0152] The term “endogenous cells” is used to refer to cells derived from a donor (or the patient), as distinguished from cells from a cell line. Endogenous cells are generally heterogeneous

populations of cells from which a specific cell type can be isolated or enriched. Endogenous cells may be intended for autologous or allogeneic treatment of a patient.

[0153] Throughout this application, various embodiments may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0154] As used in the specification and claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a sample” includes a plurality of samples, including mixtures thereof.

[0155] The terms “determining,” “measuring,” “evaluating,” “assessing,” “assaying,” and “analyzing” are often used interchangeably herein to refer to forms of measurement. The terms include determining if an element is present or not (for example, detection). These terms can include quantitative, qualitative or quantitative and qualitative determinations. Assessing can be relative or absolute. “Detecting the presence of” can include determining the amount of something present in addition to determining whether it is present or absent depending on the context.

[0156] The terms “subject,” “individual,” or “patient” are often used interchangeably herein. A “subject” can be a biological entity containing expressed genetic materials. The biological entity can be a plant, animal, or microorganism, including, for example, bacteria, viruses, fungi, and protozoa. The subject can be tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro. The subject can be a mammal. The mammal can be a human. In some embodiments, the subject may be diagnosed or suspected of being at high risk for cancer. In some embodiments, the subject may be diagnosed or suspected of being at high risk for having a tumor. In some embodiments, the subject is not necessarily diagnosed or suspected of being at high risk for cancer.

[0157] The term “*in vivo*” is used to describe an event that takes place in a subject’s body.

[0158] The term “*ex vivo*” is used to describe an event that takes place outside of a subject’s body. An *ex vivo* assay is not performed on a subject. Rather, it is performed upon a sample separate from a subject. An example of an *ex vivo* assay performed on a sample is an “*in vitro*” assay.

[0159] The term “*in vitro*” is used to describe an event that takes place contained in a container for holding laboratory reagent such that it is separated from the biological source from which the material is obtained. *In vitro* assays can encompass cell-based assays in which living or dead cells are employed. *In vitro* assays can also encompass a cell-free assay in which no intact cells are employed.

[0160] As used herein, the term “about” a number refers to that number plus or minus 10% of that number. The term “about” a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

[0161] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

EXAMPLES

[0162] The following examples are included for illustrative purposes only and are not intended to limit the scope of the disclosure.

Example 1 – Improved IL-2 activation of immune cells

[0163] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide (bFIST) activate higher levels of IFN-gamma and CXCL10 in human NK cells as compared to fusion proteins comprising an IL-2 polypeptide and single sTBRII receptor polypeptide (monovalent FIST).

[0164] Human T cells and NK cells were isolated from PBMC by negative selection and were stimulated with FIST (IL2-sTBRII, monovalent) or a FIST variant with bivalent sTBRII traps (IL2-sTBRII-sTBRII) for 48 hours. After 48h of incubation, the conditioned media were collected to quantify the levels of IFN-gamma from T cells and CXCL10 from NK cells by ELISA. As shown in the data of **FIG. 1**, bFIST demonstrates significantly higher IL-2 receptor activation than FIST (monovalent FIST).

Example 2 – Improved TGFβ1 binding

[0165] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide

(bFIST) more effectively bind active TGF β 1 as compared to fusion proteins comprising an IL-2 polypeptide and single sTBR2 receptor polypeptide (monovalent FIST).

[0166] Human active TGF β 1 (2 ng/mL) previously activated with 4 mM HCl containing 0.1% human or bovine serum albumin (BSA) was incubated with sTBR2 isoform 2 (long), FIST (monovalent FIST) or bFIST (5-20 nM) for 30 mins at RT and free active TGF β in solution was quantified by ELISA (ELISA for human TGF β 1 immunoassay).

[0167] bFIST effectively blocks active TGF β 1 in solution at significant lower concentrations than sTBR2 as illustrated in that **FIG. 2**. These results indicate that IL-2 as part of the fusion protein also modifies the sTBR2 portion and its binding properties to active TGF β . Furthermore, the bivalent sTBR2 trap variant has more effective TGF β blocking activity.

Example 3 – Blockage of TGF β Isoforms in Solution

[0168] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBR2 receptor polypeptide, and a second sTBR2 receptor polypeptide more effectively binds TGF β isoforms.

[0169] bFISTv3 and FIST (monovalent FIST) at equimolar concentrations were compared in their ability to inhibit TGF β 1-mediated suppression of CTLL-2 proliferation (IL-2 dependent T Lymphocyte cell line). The blocking activity of TGF β isoforms in solution by bFISTv3, FIST (monovalent FIST), or control (TBR2 isoform 2) was determined by incubating bFISTv3, FIST (monovalent FIST), or control with 1 ng/mL of active TGF β isoforms. Equimolar concentrations (range of 5-5x10⁻⁶ nM) of bFISTv3, FIST (monovalent FIST) or control (TBR2 isoform 2) were compared in their ability to block active TGF β isoforms in solution. bFISTv3, FIST (monovalent FIST) or control were incubated with 1 ng/mL of active TGF β isoforms diluted in phosphate buffered saline (PBS) for 30 minutes and the free TGF β (unblocked) was quantified by ELISAs specific for each TGF β isoforms.

[0170] bFISTv3 demonstrated a significantly increased affinity with picomolar potency for TGF β 1 (9 picomoles) and TGF β 3 (70 picomoles) compared to FIST and the control TBR2 isoform 2 (**FIG. 4A-C**). **Figure 4A** shows blocking of the TGF β 1 binding. **Figure 4B** shows blocking of the TGF β 2 binding. **Figure 4C** shows blocking of the TGF β 3 binding. **Table 1** shows binding values for TGF β blocking activity in solution. The TGF β 1 and TGF β 3 isoforms are the most oncogenic isoforms and potent inducers of tumor-immune resistance, angiogenesis, metastases,

and immunosuppression. TGF β 1 and TGF β 3 isoforms are also known inducers of fibrotic diseases. In contrast, TGF β 2 was not blocked by the fusion protein or control. TGF β 2 is an important regulator of hematopoiesis, cardiovascular function and glucose and fatty acid metabolism.

[0171] Table 1: IC₅₀ values for active TGF β blocking activity in solution

Molecule	TGF β 1	TGF β 2	TGF β 3
TbRII (isoform II)	0.02 nM	NA	0.26 nM
FIST	1.29 nM	NA	3.6 nM
bFISTv3	0.009 nM	NA	0.07 nM

Example 4 – Inhibition of TGF β 1-mediated Suppression of CTLL-2 Proliferation

[0172] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTbRII receptor polypeptide, and a second sTbRII receptor polypeptide more effectively induces cytotoxic T cell proliferation and prevents the TGF β 1-mediated suppression.

[0173] Equimolar concentrations (range of 50-5x10⁻⁵ nM) of bFISTv3, FIST (monovalent FIST) or controls (IL-2 and TbRII isoform 2) were compared in their ability to inhibit active TGF β 1-mediated suppression of CTLL-2 proliferation. CTLL-2 cells (5 x 10³ cells/well) were stimulated with equimolar concentration of bFISTv3, FIST (monovalent FIST) or controls for 72 hours at 37°C with/without active TGF β 1 (1 ng/mL). After the incubation period, cell proliferation was determined by a luminescent cell viability assay. The absolute IC₅₀ was determined with a dose-response curve using nonlinear regression model with a sigmoidal dose response. The surviving rate (%) = (Lum_{Test article} - Lum_{Medium control}) / (Lum_{Vehicle control} - Lum_{Medium control}) × 100%. The cell number was quantified by a luminescence signal versus cell dose curve. bFISTv3 induced CTLL-2 cell proliferation but prevents the TGF β 1-mediated suppression of CTLL-2 proliferation more effectively than FIST (monovalent FIST). **Figure 5A:** Proliferation of CTLL-2 stimulated with bFISTv3. **Figure 5B:** Proliferation of CTLL-2 stimulated with bFISTv3 in the presence of active TGF β 1 (1 ng/mL). **Figure 5C:** Proliferation of CTLL-2 stimulated with FIST (monovalent FIST). **Figure 5D:** Proliferation of CTLL-2 stimulated with FIST (monovalent FIST) in the presence of active TGF β 1 (1 ng/mL). **Figure 5E:** Proliferation of CTLL-2 stimulated with IL-2. **Figure 5F:** Proliferation of CTLL-2 stimulated IL-2 in the presence of active TGF β 1 (1 ng/mL). Data are shown as mean $\pm$ SD, **p<0.005. TGF β 1 is known to inhibit IL-2 signaling and the proliferation of T cells through multiple pathways. TGF β 1 inhibits the phosphorylation and

activation of components of the JAK/STAT cascade downstream of IL-2R, as well as exerts inhibitory activity at the nuclear level on a subset of IL-2 target genes including c-Myc, cyclin D2, and cyclin.

[0174] Table 2: IC50 values for CTLL-2 cell proliferation in response to the stimuli of fusion proteins or controls

Molecule	IC50	top	bottom	slope
IL-2	0.057	106	1.04	~12
IL2+TβRII	0.131	116	1.26	1.6
FIST	0.063	121	3.8	1.56
bFISTv3	0.459	137	0.07	0.69

[0175] Table 3: IC50 values for the inhibition of TGFβ1-dependent suppression of CTLL-2 cell proliferation

Molecule	IC50	top	bottom	slope
IL-2	~0.051	37	1.22	~6.85
IL2+TβRII	0.322	94	2.21	1.01
FIST	0.120	54	-3.4	0.39
bFISTv3	0.650	106	0	0.90

Example 5 – bFIST Proliferation of Effector Memory T cells and Terminal Differentiated Effector Memory Cells

[0176] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively induces cytokine production and proliferation of both effector memory (T_{EM}) and terminal differentiated effector memory (T_{EMRA}) CD8 T cells.

[0177] Purified human T cells derived from PBMC-stimulated with CEF peptide pool were labeled with CellTrace™ Violet to track T cell proliferation (CellTrace™ mean fluorescence (MFI) decreases as the cell proliferation increases). Proliferation of CD8⁺ T cells, effector memory T cells (CCR7⁻CD45RA⁺CD62L⁻CD8⁺ T cells), and terminal differentiated effector memory CD8 T cells (CCR7⁻CD45RA⁺CD62L⁻CD8⁺ T cells) stimulated with equimolar concentration of bFISTv3, FIST (monovalent FIST), or TβRII isoform 2.

Human PBMC from healthy donors were stimulated with CEF peptide pool overnight to induce the generation of antigen-specific T cells. T cells (CD3⁺) were purified from PBMC derived from

healthy donors by immunomagnetic negative selection using a Human T cell isolation kit. Purified T cells were labeled with CellTrace™ Violet before being stimulated with 5 nmoL of bFISTv3, FIST (monovalent FIST) and IL-2 for 48 hours. Stimulated T cells were labeled with fluorescence-conjugated antibodies specific for the memory T cell populations. After the incubation period, cell supernatants were collected to quantify the concentration of IFN γ by ELISA and the cells were collected to determine the cell proliferation of each T cell subpopulation by flow cytometry. bFISTv3 induces IFN γ production from T cells and significantly higher proliferation of both effector memory (T_{EM}) and terminal differentiated effector memory (T_{EMRA}) CD8 T cells (e.g., when compared to FIST). **FIG. 6A** shows bFISTv3 induced proliferation of effector memory T cells (T_{EM}) and terminal differentiated effector memory cells (T_{EMRA}). **FIG. 6B** shows FIST induced proliferation of effector memory T cells (T_{EM}) and terminal differentiated effector memory cells (T_{EMRA}). **FIG. 6C** shows IL-2 induced proliferation of effector memory T cells (T_{EM}) and terminal differentiated effector memory cells (T_{EMRA}). **FIG. 6D** shows the average of CellTrace™ MFI values of stimulated T_{EM} derived from two donors (a decrease in MFI denotes increased proliferation). **FIG. 6E** shows average CellTrace™ MFI values of stimulated T_{EMRA} derived from two donors (a decrease in MFI denotes increased proliferation). **FIG. 6F** shows quantification of IFN γ production by T cells stimulated with bFISTv3, FIST (monovalent FIST), or IL-2 for 48 hours. Data are shown as mean $\pm$ SD, * p<0.05. The effector memory T lymphocyte are antigen-primed lymphocytes that can confer rapid immune protection upon a second challenge with tumor-associated antigens. The memory T cell subpopulations CCR7⁺ can migrate to inflame tissues (e.g., tumor site) and exert immediate effector functions like cytotoxicity against cancer cells. CCR7⁺ memory CD8⁺ T cells are characterized by the production of IFN γ and perforin-containing granules essential for their anti-tumor cytolytic activity. In particular, the perforin expression is prominent in the terminal differentiated effector memory T cells (CD45RA⁺CCR7⁺CD62L⁺).

Example 6 – bFIST inhibits TGFb1-mediated suppression of primary NK cell proliferation and increases IFN γ production

[0178] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sT β RII receptor polypeptide, and a second sT β RII receptor polypeptide more effectively inhibits and/or reduces TGFb1-mediated of primary NK cell proliferation and IFN γ production.

[0179] Human NK cells were purified from PBMC derived from healthy donors by immunomagnetic negative selection using a human NK cell isolation kit. Purified NK cells were labeled stimulated with equimolar concentrations (25 nM, 12.5 nM and 6.25 nM) of bFISTv3, FIST (monovalent FIST) and IL-2 for 5 days. After the incubation period, the cell culture supernatant was collected to quantify the concentration of IFN γ by ELISA. The number of viable cells was quantified with the fluorescence-based assay using a cell number/luminescence curve and the percentage of cell proliferation was calculated versus the positive control (IL-2+TbRII) as the maximum stimulus of NK cell proliferation for these experimental conditions.

[0180] Purified human NK cells derived from peripheral mononuclear cells (PBMC) were stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or control (IL-2 + TbRII) for 5 days with or without active TGFb1 (1 ng/mL). The culture supernatant was collected to quantify the amount of IFN γ by ELISA and the number of viable cells in culture was determined by quantitating the amount of ATP present, which indicates the metabolically active cells. **FIG. 7A** shows the percentage of NK cell proliferation in the absence of active TGFb1. **FIG. 7B** shows the percentage of NK cell proliferation in the presence of active TGFb1. **FIG. 7C** shows the quantification of IFN γ produced by stimulated NK cells cultured in the absence of active TGFb1. **FIG. 7D**, quantification of IFN γ produced by stimulated NK cells cultured with active TGFb1 (1 ng/mL). **FIG. 7E**, quantification of CXCL10 produced by stimulated NK cells cultured with of active TGFb1 (1 ng/mL). The data are representative of two independent experiments performed in duplicates. Statistic comparisons between bFISTv3 and FIST are indicated (data are shown as mean $\pm$ SD, *p<0.05, **p<0.005, ***p<0.0005).

Example 7 – bFIST Increases NK Cell-mediated Cytotoxicity and Activation

[0181] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTbRII receptor polypeptide, and a second sTbRII receptor polypeptide more effectively increases and/or stimulates natural killer (NK) cell-mediated cytotoxicity and activation.

[0182] The NK92 cells were serum-starved for 9 hours before being stimulated with equimolar concentrations of the bFISTv3, FIST (monovalent FIST) and TbRII isoform 2 (control) in the presence or absence of active TGF β 1 (1 ng/mL) for 48 hours. After the incubation period, the amount of IFN γ was quantified by ELISA. The NK92 cells were collected and co-cultured with

target cells (K-562 cells, Chronic Myelogenous Leukemia) at different effector: target ratios (1:1, 3:1, 6:1). The NK92 cytolytic activity against cancer cells was determined by measuring % of specific tumor target death through fixable viability dye staining after NK: K-562 co-culture for three hours at 37°C, 5% CO₂ incubator. The % of dead cells was calculated based on the equation:

$$\% \text{ specific lysis} = \frac{100 \times (\% \text{ lysis in sample} - \% \text{ basal death})}{100 - \% \text{ basal death}}$$

[0183] TGF- β is a potent immunosuppressive cytokine that severely affects immune cell functions, including NK cell activation, IFN- γ production, and the expression of activating receptors NKG2D and NKp30, thereby reducing the cytotoxic ability of NK cells and impairing their antitumor function. bFIST induces both robust immune cell activation and effective blockage (with picomolar potency) of active TGF β 1. These two effects in tandem promote higher NK cell proliferation, activation, and cytotoxicity against cancer cells. **FIG. 8A** shows quantification of IFN γ production by NK92 cells as indicator of NK92 cell activation (as described above, human NK92 cells were serum-starved for 9 hours before being stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, control (IL-2 + T β R2) for 48 hours with or without active TGF β 1 (1 ng/mL) and the cell supernatant was collected). **FIG. 8B**, shows induced cytotoxicity of bFISTv3- or FIST-stimulated NK92 cells against K-562 cells at 6:1 ratio (effector:target). Statistic comparisons between bFISTv3 and FIST are indicated. Data are shown as mean $\pm$ SD, *p<0.05.

Example 8 – bFIST stimulates and increases B cell maturation, proliferation and IFN γ production

[0184] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sT β R2 receptor polypeptide, and a second sT β R2 receptor polypeptide more effectively (1) stimulates and/or increases B cell maturation, proliferation and IFN γ production and (2) reduces and/or inhibits TGF β 1 suppression of B cell maturation, proliferation and IFN γ production.

[0185] Human B cells were purified from PBMC derived from healthy donors by immunomagnetic negative selection using a Human B cell isolation kit. Purified B cells were pre-labeled with CFSE to trace cell proliferation (the marker intensity decreases as the cell proliferation increases) before being stimulated with equimolar concentrations (20 nM, 10 nM and 5 nM) of bFISTv3, FIST and IL-2 for 5 days. After the incubation period, B cells were labeled with CD86 and HLA-DR and the percentages of double positive (CD86⁺ HLA-DR⁺) B cells and proliferation

were quantified by flow cytometry. The cell culture supernatants were collected to quantify the concentration of IFN γ by ELISA. The statistic comparisons between bFISTv3 and FIST are indicated. Data are shown as mean $\pm$ SD, *p<0.05, **p<0.005, ***p<0.0005.

[0186] Bivalent FIST effectively increases and/or stimulates B cell activation, proliferation, and IFN γ production (e.g., compared to FIST or IL-2). Bivalent FIST also induces the upregulation of costimulatory molecule (CD86) and HLA-DR (MHC class II) molecule expression that indicates B-cell responsiveness to bivalent FIST stimulatory effects. Further, bivalent FIST also activates B cells into effector cells with high expression of antigen-presenting cell (APC) maturation markers and IFN γ production

[0187] **FIG. 9A-D** shows data from purified human B cells (CD19 $^{+}$ CD3 $^{-}$) derived from peripheral mononuclear cells (PBMC) were labeled with Carboxyfluorescein succinimidyl ester (CFSE) to trace cell proliferation before being stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, control (IL-2 + T β RII) for 5 days with and without active TGF β 1 (1 ng/mL). Stimulated B cells were labeled with conjugated antibodies specific for HLA-DR and CD86 to quantify the percentage of mature B cells by flow cytometry. **FIG. 9A** shows a comparison between bFISTv3 (20 nmoL) and FIST (20 nmoL) in the induction of double positive CD86 $^{+}$ HLA-DR $^{+}$ B cells cultured with/without active TGF β 1. **FIG. 9B** shows the percentage of CD86 $^{+}$ HLA-DR $^{+}$ B cells stimulated with 20, 10, 5 nmoL of bFISTv3, FIST (monovalent FIST) or, control (IL-2+T β RII) cultured with/without active TGF β 1. **FIG. 9C** shows a comparison between bFISTv3 (20 nmoL) and FIST (20 nmoL) in the induction of CD86 $^{+}$ HLA-DR $^{+}$ B cell proliferation with/without active TGF β 1. The mean fluorescence intensity (MFI) values of CFSE (cell tracer) are indicated. **FIG. 9D** shows quantification of IFN γ production in the supernatant of stimulated B cells cultured with and without active TGF β 1.

Example 9 – bFIST Inhibits the Upregulation of Epithelial to Mesenchymal Transition (EMT) Markers on Lung Cancer Cells

[0188] Exemplifying the fusion polypeptide compositions and methods disclosed herein, the following example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sT β RII receptor polypeptide, and a second sT β RII receptor polypeptide more effectively inhibits and/or reduces the upregulation of Epithelial to Mesenchymal Transition (EMT) markers on lung cancer cells.

[0189] A-549 lung cancer cells were treated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, controls (IL-2 and IL-2 + T β RII) with/without active TGF β 1 for 72 hours.

After the incubation period, the cells were collected and labeled with conjugated antibodies specific for E-Cadherin, N-Cadherin, and PD-L1. The percentage of cells expressing these EMT markers was quantified by Flow cytometry.

Epithelial-mesenchymal transition (EMT) enables carcinoma cells to suppress their epithelial features changing to mesenchymal ones. As result of the EMT process, carcinoma cells to acquire mobility and the capacity to migrate from the primary tumor site to distant organs and form metastases. The loss of E-Cadherin expression is a crucial step in EMT and a hallmark of mesenchymal phenotype that characterize the transformation from non-metastatic to metastatic cancer cells. The treatment with bFIST reduces the TGF β 1-mediated upregulation of EMT markers (decrease of E-cadherin, increase of N-cadherin and increase of PD-L1 expression). **FIG. 10A** shows the percentage of A-549 cells (lung cancer cells) expressing E-Cadherin after being treated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, controls (IL-2 and IL-2 + TbRII) with/without active TGF β 1 for 72 hours. **FIG. 10B** shows the percentage of A-549 cells expressing N-Cadherin and **FIG. 10C** shows the percentage of A-549 cells expressing PD-L1. The data are representative of two independent experiments performed in duplicates. As shown, The treatment of lung cancer cells with bFIST increases of E-cadherin expression, decreases of N-cadherin, and decreases of PD-L1 expression.

[0190] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

SEQUENCES

SEQ ID NO	SEQUENCE	DESCRIPTION
1	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT	Human IL2
2	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT	FIST_IL2
3	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT	FIST_IL2M IL-2 mutated F62A
4	MVLGTIDLCSCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTE SDVHPSCCKVTAMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNG NVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINITS	Human_IL15
5	CFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCCKVT AMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFINITS	FIST_IL15
6	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFP QLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVAVWRKNDENIT LETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETTFMCSCSSDECND NIIFSEYNTSNPDLLLVIQVGTGISLLPPLGVAISVIIIFYCYRVNRQQKL SSTWETGKTRKLMEFSEHCAHLEDDRSDISSTCANNINHNTPELLFIELD TLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYEEYASWKTEKDIFS DINLKHENILQFLTAERKTELKQYWLITAFHAKGNLQEYLTRHVIS WEDLRKLGSSLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNILVKNDL TCCLCDFGLSLRLDPTLSVDDLANSQVGTARYMAPEVLESRMNLEN VESFKQTDVYSMALVLWEMTSRCNAVGEVKDYEPFPGSKVREHPCVE SMKDNVLRDRGRPEIPSWLNHQGIQMVCELTTECWDHDPPEARLTAQ CVAERFSELEHLDRLSGRSCSEEKIPEDGSLNTTK	Human TGFB

7	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSDVEMEAQKDEIICPSCNR TAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNC SITSICEKPQEVAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP CIMKEKKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIQVTGI SLLPPLGV AISVIIIFYCYRVNRQQKLSSTWETGKTRKLMEFSEHCAIILE DDRSDISSTCANNINHNTELLPIELDTLVGKGRFAEVYKAKLKQNTSEQ FETVAVKIFPYEEYASWKTEKDIFSDINLKHENILQFLTAEERKTELGK QYWLITAFHAKGNLQEYLTRHVISWEDLRKLGSSLARGIAHLHSDHTP CGRPKMPIVHRDLKSSNILVKNDLTCCCLDFGLSLRLDPTLSVDDLANS GQVGTARYMAPEVLESRMNLENVESFKQTDVYSMALVLWEMTSRCN AVGEVKDYEPFPGSKVREHPCVESMKDNVLRDRGRPEIPSWLNHQGI QMV CETLTECWDHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKIP EDGSLNTTK	Human TGFB long
8	IPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLEDAASP KCVMEKKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD	bFIST_TGFB R_short
9	IPPHVQKSDVEMEAQKGEMICVTCNRTHPLKHVNNDMIVTDNNGAV KLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKNDEN ITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKKPGETFFMCSCSSD ECNDNIIFSEDYNTSNPD	bFIST_TGFB R_long
10	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLEDAASP KCVMEKKKKPGETFFMCSCSADECNDNIIFIPPHVQKSVNNDMIVTDN NGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWR KNDENITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKKPGETFFMC CSADECNDNIIFSEDYNTSNPD	bFISTv.2

11	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLE DAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPDIPPH VQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSIT SICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLEDAASPKCV MKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD	bFISTv.2.1
12	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRTAHPLKHAVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKND ENITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETFFMCSCSS DECNDNIIFIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHG FTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD	bFISTv.3
13	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQIPPHVKSVDVEMEAQKGEMICVTCNRTAHPLKHAVNNDMIVTD NNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWR RKNDENITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETFFM CSCSSDECNDNIIFSEDYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKLP QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENIT LETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADEC NDNIIFSEDYNTSNPD	bFISTv.3.1

14	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLEDAASP KCVMKEKKKPGETTFMCSCSADECNDNIIFIPPHVQKSDVEMEAQKGE MICVTCNRTAHLKHNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVAVWRKNDENITLETVCHDPKLTYPHGFT LEDAASPKCVMKEKKKPGETTFMCSCSSDECNDNIIFSEDYNTSNPD	bFISTv.4
15	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLE DAASPKCVMKEKKKPGETTFMCSCSADECNDNIIFSEDYNTSNPDIPPH VQKSDVEMEAQKGEMICVTCNRTAHLKHNNDMIVTDNNGAVKLP QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKNDENIT LETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETTFMCSCSSDECND NIIFSEDYNTSNPD	bFISTv.4.1
16	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRTAHLKHNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKND ENITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETTFMCSCSS DECNDNIIFIPPHVQKSDVEMEAQKGEMICVTCNRTAHLKHNNDMIV TDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAV VWRKNDENITLETVCHDPKLTYPHGFTLEDAASPKCVMKEKKKPGETF FMCSCSSDECNDNIIFSEDYNTSNPD	bFISTv.5

17	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQIPPHVKSDEVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTD NNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW RKNNDENITLETVCHDPKLTYPHGFLEDAASPKCVMKEKKKPGETFFM CSCSSDECNDNIIFSEDYNTSNPDIPPHVKSDEVEMEAQKGEMICVTCNR TAHPLKHVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNC SITSICEKPQEVCAVWRKNNDENITLETVCHDPKLTYPHGFLEDAASPK CVMKEKKKPGETFFMCSSDECNDNIIFSEDYNTSNPD	bFISTv.5.1
18	MYRMQLLSICIALSLALVTNSISAMVRSVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHY TQKSLSLSPGKAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTR MLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKSDV EMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAVKLPQLCKFC DVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNNDENITLETVCH DPKLTYPHGFLEDAASPKCVMKEKKKPGETFFMCSSDECNDNIIFIP PHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCS ITSICEKPHEVCAVWRKNNDENITLETVCHDPKLTYPHGFLEDAASPKC VMKEKKKPGETFFMCSSADECNDNIIFSEDYNTSNPD	bFISTv.6

19	MYRMQLLSICIALSLALVTNSISAMVRSVECPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHY TQKSLSLSPGKAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTR MLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKSVN NDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPH EVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKK PGETFFMCSCSADECNDNIIFIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDFVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPD	bFISTv.7
20	MYRMQLLSICIALSLALVTNSIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDFVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPDAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLT RMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKS VNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEK PHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEK KKPGETFFMCSCSADECNDNIIFSEDYNTSNPD	bFISTv.8
21	MYRMQLLSICIALSLALVTNSIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDFVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPDAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLT RMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKS DVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAVKLPQLCK FCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSSDECNDNI FSEDYNTSNPD	bFISTv.9

22	MYRMQLLSICIALSLALVTNSIPPHVKSDVEMEAQKGEMICVTCNR PLKLVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCV MKEKKKPGETTFMCSCSSDECNDNIIFSEDYNTSNPDAPTSSSTKKTQL QLEHLLLDLQMLNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFCQSIISTLQKSDVEMEAQKGEMICVTCNR LKLVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITS CEKPQEVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVM KEKKKPGETTFMCSCSSDECNDNIIFSEDYNTSNPD	bFISTv.10
23	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRPLKLVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKND ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETTFMCSCSS DECNDNIIFSEDYNTSNPD	FISTv.11
24	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRPLKLVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVAVWRKND ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETTFMCSCSS DECNDNIIFSEDYNTSNPD	FISTv.12

25	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYPHGFTLEDAASP KCVMKKEKKKPGETTFMCSCSADECNDNIIFSEDYNTSNPD	FISTv. 13
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CLAIMS

Listing of Claims:

1. A fusion polypeptide, comprising:
an interleukin (IL) polypeptide;
a first TGF β superfamily receptor polypeptide; and
a second TGF β superfamily receptor polypeptide.
2. A fusion polypeptide comprising:
an IL polypeptide that binds to and agonizes an IL receptor; a first TGF β superfamily receptor polypeptide that binds to and sequesters soluble TGF β ; and a second TGF β superfamily receptor polypeptide that binds to and sequesters soluble TGF β .
3. The fusion polypeptide of any one of claims 1 to 2, wherein the IL polypeptide comprises an IL-2 polypeptide or IL-15.
4. The fusion polypeptide of any one of claims 1 to 3, wherein the IL polypeptide comprises an IL-2 polypeptide.
5. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide comprises SEQ ID NO: 2 or SEQ ID NO: 3.
6. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, or an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3.
7. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide consists of SEQ ID NO: 2 or SEQ ID NO: 3.
8. The fusion polypeptide of any one of claims 1 to 3, wherein the IL polypeptide comprises an IL-15 polypeptide.
9. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide comprises SEQ ID NO: 5.
10. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5.
11. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide consists of SEQ ID NO: 5.
12. The fusion polypeptide of any one of claims 1 to 11, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises an

activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, or a TGF β receptor II polypeptide or fragment thereof.

13. The fusion polypeptide of any one of claims 1 to 12, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a TGF β receptor II (TBRII) polypeptide or fragment thereof.

14. The fusion polypeptide of any one of claims 1 to 13, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBRII) polypeptide or fragment thereof.

15. The fusion polypeptide of any one of claims 1 to 14, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBRII) polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof.

16. The fusion polypeptide of any one of claims 1 to 15, wherein the first TGF β superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGF β superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9.

17. The fusion polypeptide of any one of claims 14 to 16, wherein the soluble TGF β receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

18. The fusion polypeptide of any one of claims 1 to 17, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF- β 1 polypeptide, a TGF- β 2 polypeptide, a TGF- β 3 polypeptide, a activin β A polypeptide, a activin β B polypeptide, a activin β C polypeptide, a activin β E polypeptide, a bone morphogenetic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an artaman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide.

19. The fusion polypeptide of any one of claims 1 to 18, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof.
20. The fusion polypeptide of any one of claims 1 to 19, wherein the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide.
21. The fusion polypeptide of any one of claims 1-20, wherein the truncated TGF β superfamily receptor polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof.
22. The fusion protein of claim 21, wherein the truncated TGF β superfamily receptor polypeptide is a truncated soluble TGF β receptor II (sT β RII) polypeptide.
23. The fusion polypeptide of claim 22, wherein the truncation comprises greater than 2, 5, 7, 10, 15, 20, 25, or 30 amino acids.
24. The fusion polypeptide of any one of claims 1 to 23, wherein the IL polypeptide comprises a truncated IL polypeptide.
25. The fusion polypeptide of claim 24, wherein the truncated IL polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof.
26. The fusion polypeptide of claim 24, wherein the truncated IL polypeptide comprises a truncated IL-2 polypeptide or IL-15 polypeptide.
27. The fusion polypeptide of any one of claims 1 to 26, further comprising a linker polypeptide or a linker molecule attaching the first TGF β superfamily receptor polypeptide and the second TGF β superfamily receptor polypeptide.
28. The fusion polypeptide of any one of claims 1 to 27, further comprising a linker polypeptide fusing the IL polypeptide and the first TGF β superfamily receptor polypeptide.
29. The fusion polypeptide of any one of claims 1 to 28, further comprising a pharmacokinetic (PK) modulator.
30. The fusion polypeptide of claim 29, wherein the pharmacokinetic modulator comprises an immunoglobulin constant (Fc) region polypeptide.
31. The fusion polypeptide of claim 30, wherein the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide.
32. The fusion polypeptide of claim 30, wherein the immunoglobulin Fc region is an IgG Fc region.

33. The fusion polypeptide of claim 30, wherein the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region.
34. The fusion polypeptide of claim 29, wherein the PK modulator comprises an albumin polypeptide.
35. The fusion polypeptide of claim 34, wherein the albumin polypeptide is a human albumin polypeptide.
36. The fusion polypeptide of any one of claims 1 to 35, wherein the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10-25.
37. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10.
38. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11.
39. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12.
40. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13.
41. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14.
42. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15.
43. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16.
44. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17.
45. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18.
46. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19.
47. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20.
48. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21.

49. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22.
50. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23.
51. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24.
52. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25.
53. The fusion polypeptide of any one of claims 1 to 52, wherein the fusion polypeptide consists of any one of SEQ ID NOs: 10-25.
54. The fusion polypeptide of any one of claims 1 to 53, wherein the fusion polypeptide activates target cells expressing an IL-2 or IL-15 receptor.
55. The fusion polypeptide of claim 54, wherein the target cell is an immune cell.
56. The fusion polypeptide of claim 55, wherein the immune cell is a T cells, natural killer cells NKT cells, B cells, or gamma delta T cells.
57. A pharmaceutical composition comprising the fusion polypeptide of any one of claims 1 to 56, comprising a pharmaceutically acceptable carrier or excipient.
58. A method of inhibiting or reducing tumor growth and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising:
- (a) an interleukin (IL) polypeptide;
 - (b) a first TGF β superfamily receptor polypeptide; and
 - (c) a second TGF β superfamily receptor polypeptide;
- thereby inhibiting or reducing tumor growth and/or progression in the individual.
59. The method of claim 58, wherein the tumor is a primary tumor.
60. The method of any one of claims 58 to 59, wherein the tumor is a metastatic tumor.
61. The method of any one of claims 58 to 60, wherein the cancer comprises a solid tumor.
62. The method of any one of claims 58 to 61, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.
63. The method of claim 62, wherein the immune cells express IL-2.
64. The method of claim 63, wherein the immune cells comprise T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells.

65. The method of any one of claims 58 to 64, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cells within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.
66. The method of any one of claims 58 to 65, wherein the cancer is a hematological cancer.
67. The method of any one of claims 58 to 66, wherein the cancer is a cancer comprising a solid tumor.
68. A method of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising:
- (a) an interleukin (IL) polypeptide;
 - (b) a first TGF β superfamily receptor polypeptide; and
 - (c) a second TGF β superfamily receptor polypeptide;
- thereby neutralizing or killing tumor cells in the individual.
69. The method of claim 68, wherein the tumor is a primary tumor.
70. The method of any one of claims 68 to 69, wherein the tumor is a metastatic tumor.
71. The method of any one of claims 68 to 70, wherein the cancer comprises a solid tumor.
72. The method of any one of claims 68 to 71, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.
73. The method of claim 72, wherein the immune cells express IL-2 or IL-15 receptors.
74. The method of claim 72, wherein the immune cells comprise T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells.
75. The method of any one of claims 68 to 74, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.
76. The method of any one of claims 68 to 75, wherein the cancer is a hematological cancer.
77. The method of any one of claims 68 to 76, wherein the cancer comprises a solid tumor.
78. A method of treating or ameliorating cancer in a subject having a cancer, comprising administering to the subject a fusion polypeptide comprising a
- (a) an interleukin (IL) polypeptide;
 - (b) a first TGF β superfamily receptor polypeptide; and
 - (c) a second TGF β superfamily receptor polypeptide;
- thereby treating or ameliorating the cancer in the subject.
79. The method of claim 78, wherein the tumor is a primary tumor.
80. The method of any one of claims 78 to 79, wherein the tumor is a metastatic tumor.

81. The method of any one of claims 78 to 80, wherein the cancer comprises a solid tumor.
82. The method of any one of claims 78 to 81, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.
83. The method of claim 82, wherein the immune cells express IL-2 or IL-15 receptors.
84. The method of claim 82, wherein the immune cells comprise T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells.
85. The method of any one of claims 78 to 84, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.
86. The method of any one of claims 78 to 85, wherein the cancer is a hematological cancer.
87. The method of any one of claims 78 to 86, wherein the cancer comprises a solid tumor.
88. The method of any one of claims 78 to 87, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide binds a TGF β 1 polypeptide, a TGF β 2 polypeptide, a TGF β 3 polypeptide, or any combination thereof.
89. A method of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising
- (a) an interleukin (IL) polypeptide;
 - (b) a first TGF β superfamily receptor polypeptide; and
 - (c) a second TGF β superfamily receptor polypeptide.
90. The method of claim 89, wherein the cell is an immune cell.
91. The method of claim 90, wherein the immune cell comprises T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells.
92. The method of any one of claims 89 to 91, wherein the immune cells are contacted in vivo.
93. The method of any one of claims 89 to 91, wherein the immune cells are contacted ex vivo.
94. The method of claim 93, wherein a leukopak or leukapheresis product comprises the immune cells.
95. The method of claim 93, wherein the immune cells are derived from an apheresis sample.
96. The method of claim 93, wherein the immune cells are subsequently used for a cell-based therapy.
97. The method of any one of claims 89 to 96, wherein the IL polypeptide is IL-2 or IL-15.

98. The method of any one of claims 89 to 97, wherein the first TGF β superfamily receptor polypeptide and/or the second TGF β superfamily receptor polypeptide comprises a soluble TGF β receptor II (TBRII) polypeptide or fragment.
99. A host cell, comprising a nucleic acid molecule comprising a nucleic acid sequence encoding the recombinant fusion polypeptide as in any of claims 1 to 56.
100. Use of the fusion polypeptide of any one of claims 1 to 56, for inhibiting or reducing tumor growth and/or progression in an individual having cancer.
101. Use of the fusion polypeptide of any one of claims 1 to 56, for neutralizing or killing tumor cells in an individual having cancer.
102. Use of the fusion polypeptide of any one of claims 1 to 56, for treating or ameliorating cancer in a subject having a cancer.
103. Use of the fusion polypeptide of any one of claims 1 to 56, for activating a cell expressing an IL receptor.
104. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for inhibiting or reducing tumor growth and/or progression in an individual having cancer.
105. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for neutralizing or killing tumor cells in an individual having cancer.
106. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for treating or ameliorating cancer in a subject having a cancer.
107. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for activating a cell expressing an IL receptor.
108. The fusion polypeptide of any one of claims 1 to 56, for use in inhibiting or reducing tumor growth and/or progression in an individual having cancer.
109. The fusion polypeptide of any one of claims 1 to 56, for use in neutralizing or killing tumor cells in an individual having cancer.
110. The fusion polypeptide of any one of claims 1 to 56, for use in treating or ameliorating cancer in a subject having a cancer.
111. The fusion polypeptide of any one of claims 1 to 56, for use in activating a cell expressing an IL receptor.