ENGINEERED TGFβ RECEPTOR REPURPOSED FOR IL-9 SIGNALING AND USES THEREOF IN IMMUNE CELLS

BACKGROUND

One of the hurdles for adoptive cell therapies (ACT) in treating solid tumors is the suppressive tumor microenvironment (TME), and TGFβ is established as one of the major contributing factors. As a soluble immunosuppressive cytokine, TGFβ inhibits anti-tumor immune responses via different mechanisms including, for example, inhibiting T effector cell activation, while promoting T regulatory cell differentiation. High level of TGFβ expression had been reported in multiple solid cancers such as liver cancer, breast cancer, prostate cancer, glioma, bladder cancer, thymic epithelial tumor, etc., casting barriers for effective tumor immunotherapy.

Another hurdle for ACT is poor persistence of infused cells in patients. Certain type of ACT requires concurrent infusion of high doses of interleukin 2 or IL-2 (i.e. Aldesleukin) to sustain cell persistence in order for robust and prolonged anti-tumor responses which, however, is often associated with systemic toxicities in the patients.

Therefore, there is a need to address these above two hurdles in order to develop an immunotherapy with an increased efficacy yet a reduced toxicity.

SUMMARY OF THE INVENTION

In order to address the above two hurdles, the present disclosure provides an engineered chimeric TGFβ receptor to be expressed in immune cells (e.g. T lymphocytes or T cells) that is repurposed, upon TGFβ binding, to transduce immunosupportive interleukin 9 (IL-9) signaling rather than the immunosuppressive TGFβ signaling mediated by endogenous TGFβ receptors. Immune cells engineered to express such engineered chimeric TGFβ receptor have much better survival and proliferation capacity upon repeated or persistent antigen stimulation, especially in a solid tumor having a TGFβ-enrich immunosuppressive environment, when compared to unmodified immune cells which typically undergo T cell exhaustion or activation-induced cell death under repeated antigen stimulation. Such engineered immune cells are expected to have an enhanced persistence and better control in solid tumors, thereby carrying a promise for improving current adoptive cell therapies.

In a first aspect, an engineered transforming growth factor beta (TGFβ) receptor chain is provided.

The engineered TGFβ receptor chain encodes a transmembrane polypeptide when expressed in an immune cell, and the transmembrane polypeptide comprises an extracellular portion and an intracellular portion, which are operably connected by a transmembrane portion. The extracellular portion comprises an extracellular domain (ECD) of any one of TGFβR1 or TGFβR2, or a functional portion or a functional variant thereof; and the intracellular portion comprises an intracellular domain (ICD) of any one of IL9R or IL2Rγ, or a functional portion or a functional variant thereof.

In the engineered TGFβ receptor chain, the transmembrane portion can comprise a transmembrane domain (TM) of any transmembrane protein, such as TGFβR1, TGFβR2, IL2Rγ, IL4R, IL9R, IL7R, IL15R, IL10R, IL21R, CD28, CTLA4, PD1, TIM3, TIGIT, CD4, CD8, or 4-1BB, etc., or a functional portion or a functional variant thereof. In some embodiments, the transmembrane portion comprises a transmembrane domain (TM) of one of TGFβR1, TGFβR2, IL2Rγ or IL9R, or a functional portion or a functional variant thereof.

According to some embodiments of the engineered TGFβ receptor chain, the extracellular portion comprises an ECD of TGFβR1, which comprises a sequence having at least 70% identity to SEQ ID NO: 1; and the intracellular portion comprises an ICD of IL9R, which comprises a sequence having at least 70% identity to SEQ ID NO: 3.

Further in some embodiments, the transmembrane portion comprises a transmembrane domain (TM) of TGFβR1, which comprises a sequence having at least 70% identity to SEQ ID NO: 5. In certain embodiments, the engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 9.

Further in some other embodiments, the transmembrane portion comprises a transmembrane domain (TM) of IL9R, which comprises a sequence having at least 70% identity to SEQ ID NO: 7. In certain embodiments, the engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 10.

According to yet some other embodiments of the engineered TGFβ receptor chain, the extracellular portion comprises an ECD of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and the intracellular portion comprises an ICD of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

Further in some embodiments, the transmembrane portion comprises a transmembrane domain (TM) of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 5. In certain embodiments, the engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 11.

Further in some other embodiments, the transmembrane portion comprises a transmembrane domain (TM) of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 8. In certain embodiments, the engineered TGFβ receptor chain compries a sequence having at least 70% identity to SEQ ID NO: 12.

According to yet some other embodiments of the engineered TGFβ receptor chain, the extracellular portion comprises an ECD of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and the intracellular portion comprises an ICD of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 3.

Further in some embodiments, the transmembrane portion comprises a transmembrane domain (TM) of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 6. In certain embodiments, the engineered TGFβ receptor chain compries a sequence having at least 70% identity to SEQ ID NO: 13.

Further in some other embodiments, the transmembrane portion comprises a transmembrane domain (TM) of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 7. In certain embodiments, the engineered TGFβ receptor chain compries a sequence having at least 70% identity to SEQ ID NO: 14.

According to yet some other embodiments of the engineered TGFβ receptor chain, the extracellular portion comprises an ECD of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and the intracellular portion comprises an ICD of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

Further in some embodiments, the transmembrane portion comprises a transmembrane domain (TM) of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 6. In certain embodiments, the engineered TGFβ receptor chain compries a sequence having at least 70% identity to SEQ ID NO: 15.

Further in some other embodiments, the transmembrane portion comprises a transmembrane domain (TM) of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 8. In certain embodiments, the engineered TGFβ receptor chain compries a sequence having at least 70% identity to SEQ ID NO: 16.

In a second aspect, an engineered TGFβ receptor system is further provided, which substantially comprises at least one engineered TGFβ receptor chain, each based on the engineered TGFβ receptor chain according to any embodiments as provided in the first aspect as described above.

Herein the engineered TGFβ receptor system may comprise a first engineered TGFβ receptor chain and a second engineered TGFβ receptor chain. It is configured such that an

extracellular portion of the first engineered TGFβ receptor chain and an extracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, and an ECD of TGFβR2 or a functional portion or a functional variant thereof, or vice versa, and it is further configured such that an intracellular portion of the first engineered TGFβ receptor chain and an intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ICD of IL9R or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, or vice versa.

According to some embodiments of the engineered TGFβ receptor system, the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and an ICD of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 3. The extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and an ICD of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

Herein further in some embodiments of the engineered TGFβ receptor system, the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 5; and the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 6. In certain embodiments, the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 9; and the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 15.

Herein further in some other embodiments of the engineered TGFβ receptor system, the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 7; and the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 8. In certain embodiments, the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to

SEQ ID NO: 10; and the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 16.

According to some other embodiments of the engineered TGFβ receptor system, the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and an ICD of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 4. The extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and an ICD of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 3.

Herein further in some embodiments of the engineered TGFβ receptor system, the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 5; and the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 6. In certain embodiments, the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 11; and the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 13.

Herein further in some other embodiments of the engineered TGFβ receptor system, the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 8; and the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 7. In certain embodiments, the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 12; and the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 14.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell, the immune cell stimulated with TGFβ exhibits an activation of the JAK-STAT signaling pathway compared to when TGFβ stimulation is absent.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell, the immune cell stimulated with TGFβ exhibits increased phosphorylation of at least one of STAT1, STAT3 or STAT5 compared to when TGFβ stimulation is absent.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 and/or STAT5 in the immune cell stimulated with TGFβ is at least 50% more than that when TGFβ stimulation is absent.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, and when the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 and/or STAT5 in the immune cell stimulated with TGFβ is at least 2.5 fold that when TGFβ stimulation is absent.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof. When the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 in the immune cell stimulated with TGFβ is at least 4.0 fold that when TGFβ stimulation is absent.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell cultured in the presence of IL-2, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a slower reduction in proliferation in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a better viability in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, and when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2, the population of immune cells stimulated with TGFβ have a viability of at least 50% 12 days after IL-2 is withdrawn.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof. When the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2, the population of immune cells stimulated with TGFβ have a viability of at least 65% 12 days after IL-2 is withdrawn.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, and when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 300 fold.

In some embodiments of the engineered TGFβ receptor system, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof. When the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 500 fold.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2 and under repeated antigen stimulation, the population of immune cells stimulated with TGFβ exhibit an enhanced viability compared to when the engineered TGFβ receptor system is absent in the immune cell.

In some embodiments of the engineered TGFβ receptor system, when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2 and under repeated antigen stimulation, the population of immune cells stimulated with TGFβ have a viability of at least 70% 9 days after IL-2 is withdrawn.

In a third aspect, a vector system is further provided, which comprises at least one recombinant vector (short as "vector" hereinafter) that encodes the engineered TGFβ receptor system according to any embodiments as provided above in the second aspect. It is configured such that when the vector system is transduced in an immune cell, the immune cell is capable of activating JAK-STAT signaling upon TGFβ stimulation.

According to some embodiments of the vector system, the engineered TGFβ receptor system comprises a first engineered TGFβ receptor chain and a second engineered TGFβ receptor chain. An extracellular portion of the first engineered TGFβ receptor chain and an extracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, and an ECD of TGFβR2 or a functional portion or a functional variant thereof, or vice versa. An intracellular portion of the first engineered TGFβ receptor chain and an intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ICD of IL9R or a functional portion or a

functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, or vice versa.

Herein in some embodiments of the vector system, the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain in the engineered TGFβ receptor system are encoded in one vector. Optionally, the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are encoded within one open reading frame (ORF) in the one vector. Further optionally, the ORF comprises a polynucleotide encoding a self-cleaving peptide, operably connecting a polynucleotide encoding the first engineered TGFβ receptor chain and a polynucleotide encoding the second engineered TGFβ receptor chain, wherein the self-cleaving peptide is selected from T2A, P2A, F2A, or E2A.

Herein in some other embodiments, the vector system comprises two vectors, which encode the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain, respectively.

In some embodiments of the vector system, a first polynucleotide encoding a first signal peptide is operably connected to a polynucleotide encoding the first engineered TGFβ receptor chain, and a second polynucleotide encoding a second signal peptide is operably connected to a polynucleotide encoding the second engineered TGFβ receptor chain. Herein the first signal peptide and the extracellular portion of the first engineered TGFβ receptor chain are optionally derived from a same protein, and the second signal peptide and the extracellular portion of the second engineered TGFβ receptor chain are optionally derived from a same protein.

Herein, the vector system may be based on a viral vector, such as that derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV), lentivirus, or retrovirus.

In a fourth aspect, an immune cell expressing the engineered TGFβ receptor system according to any embodiment as provided in the second aspect is provided.

According to some embodiments of the immune cell, when stimulated with TGFβ, the immune cell exhibits an activation of the JAK-STAT signaling pathway compared to when TGFβ stimulation is absent.

Herein further in some embodiments, when stimulated with TGFβ, the immune cell exhibits increased phosphorylation of at least one of STAT1, STAT3 or STAT5 compared to when TGFβ stimulation is absent.

Herein further in some embodiments, when the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 and/or STAT5 in the immune cell is at least 50% more than that when TGFβ stimulation is absent.

Herein according to some embodiments of the immune cell, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, and when the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 and/or STAT5 in the immune cell is at least 2.5 fold that when TGFβ stimulation is absent.

Herein further in some embodiments, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof. When the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 in the immune cell is at least 4.0 fold that when TGFβ stimulation is absent.

According to some embodiments of the immune cell, when cultured in the presence of IL-2, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

According to some embodiments of the immune cell, when cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a slower reduction in proliferation in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

According to some embodiments of the immune cell, when cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a better viability in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

Herein further in some embodiments of the immune cell, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when cultured in the absence of IL-2, a population of the immune cell stimulated with TGFβ has a viability of at least 50% 12 days after IL-2 is withdrawn.

Herein further in some embodiments, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a

functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof. When cultured in the absence of IL-2, a population of the immune cell stimulated with TGFβ has a viability of at least 65% 12 days after IL-2 is withdrawn.

According to some embodiments of the immune cell, when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

Herein further in some embodiments, the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 300 fold.

Herein further in some embodiments, the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 500 fold.

According to some embodiments of the immune cell, when cultured in the absence of IL-2 and under repeated antigen stimulation, a population of the immune cell stimulated with TGFβ exhibit an enhanced viability compared to when the engineered TGFβ receptor system is absent in the immune cell.

Herein further in some embodiments, when cultured in the absence of IL-2 and under repeated antigen stimulation, a population of the immune cells stimulated with TGFβ have a viability of at least 70% 9 days after IL-2 is withdrawn.

In any embodiment of the immune cell as described above, the immune cell can be a T cell, a natural killer (NK) cell, or a tumor infiltrating lymphocyte (TIL), and the immune cell can further express a target-recognizing receptor capable of binding a target molecule on a target cell. Herein optionally, the target-recognizing receptor is selected from a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-TCR. The Aspire-TCR has been disclosed in international application number PCT/US2023/085790, whose disclosure is incorporated by reference in its entirety.

In a fifth aspect, a method for treating a tumor in a patient in need thereof is further provided, which comprises: administering a population of the immune cell according to any embodiment as provided above in the fourth aspect.

Herein according to some embodiments of the method, the immune cell further expresses a target-recognizing receptor capable of binding a target molecule on a target cancer cell within the tumor, wherein the target-recognizing receptor is selected from a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-TCR.

According to some embodiments of the method, the tumor is a solid tumor characterized to have a high level of TGFβ expression therewithin, and may be a tumor from a liver cancer, breast cancer, prostate cancer, glioma, bladder cancer, or thymic epithelial tumor, etc.

As used herein, the term "high level of TGFβ expression" is defined such that expression of TGFB1 at mRNA level is at least 10% higher compared to adjacent non-tumor tissue.

As used herein and throughout the disclosure, “a” or “an” means “at least one” or “one or more.”

As used herein, the term “immune cell” or "immune cells" can mean any of T lymphocytes (including αβ T cells or γδ T cells), tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, or NK T cells, or any of these above cells that have been engineered (e.g. cells expressing TCR or Chimeric antigen receptor (CAR)). In the Examples provided in the disclosure, the T lymphocytes, or T cells, are used as illustrating yet non-limiting example for the immune cells.

As used herein, the term “linker” (L), or “linker domain” or “linker region” as used herein refer to an oligo- or polypeptide region from about 1 to 100 amino acids in length, which links together any of the domains/regions. Linkers can be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers can be used when it is desirable to ensure that two adjacent domains do not

sterically interfere with one another. Linkers can be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example P2A, T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), Thosea asigna virus (T2A) or combinations, variants and functional equivalents thereof. Other linkers will be apparent to those of skill in the art and can be used in the methods described herein.

As used herein, the term "functional variant" is referred to as a sequence variant of a particular functional domain of interest with a pre-defined sequence (e.g., TGFβR1 ECD, TGFβR2 ECD, IL9R ICD, IL2Rγ ICD, TGFβR1 TM, or TGFβR2 TM, etc.) that contains one or more sequence alterations, such as that substitution(s), deletion(s), insertion(s), transposition(s), etc., yet still wholly or partially retains the functionalities of the particular functional domain with the pre-defined sequence. Similarly, the term "functional portion" is referred to as a portion, and not all, of a particular functional domain of interest with a pre-defined sequence (e.g., TGFβR1 ECD, TGFβR2 ECD, IL9R ICD, IL2Rγ ICD, TGFβR1 TM, or TGFβR2 TM, etc.) that substantially retains, wholly or partially, the functionalities of the particular functional domain with the whole sequence.

As used herein, the term "increase", "increased", "enhance", "enhanced", "elevated", "reduce", or "reduced", "alter", "altered", "change", "changed", "higher", "lower", or alike, refers to the level (e.g. the pSTAT3 level) change of no less than 10% compared to a reference level.

As used herein, the phrase “substantially unchanged” means that a level of a variable under examination (e.g. the pSTAT3 level) changes by less than 5% if comparing a later timepoint with an earlier reference timepoint. If a change is greater than or equal to 5%, such a change can be deemed as an “increase” or “reduce” as mentioned herein.

As used herein, the phrase “target cells” refers to cells that can be specifically targeted or killed by immune cells.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is

a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. For example, the comparison of sequences and determination of percent identity between two sequences can be accomplished using a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

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

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1D illustrate the block diagrams (FIG. 1A and FIG. 1C) of two classes of the engineered transforming growth factor beta (TGFβ) receptor system, and the corresponding TGFβ-responsive signaling complexes formed thereby (FIG. 1B and FIG. 1D, corresponding to FIG. 1A and FIG. 1C respectively) provided by certain embodiments of the disclosure.

FIGS. 2A-2D illustrate the block diagrams of constructs encoding the engineered TGFβ receptor systems according to four embodiments of the disclosure.

FIGS. 3A-3C show the transduction efficiency of two TGFβR1R2-IL9R receptors and its impact on T cell expansion.

FIGS. 4A-4C show the comparison results in TGFβ-stimulated pSTAT1/3/5 signaling.

FIGS. 5A-5D show the comparison results in cell proliferation and survival in T cells cultured with or without IL-2 and with or without TGFβ1.

FIGS. 6A-6D show the comparison results in cell proliferation and survival after repetitive antigen stimulation, with or without IL-2 and with or without TGFβ1.

FIGS. 7A-7C show the transduction efficiency of TGFβR1R2-IL9R receptor in tumor-infiltrating lymphocytes (TILs) and the impacts on cell proliferation or survivial.

FIG. 8 shows the TGFβ-stimulated activation of pSTAT signaling in TILs expressing the TGFβR1R2-IL9R chimeric receptor.

FIGS. 9A-9B show the effects of the TGFβR1R2-IL9R receptor modification in TILs in terms of cell expansion with or without IL-2 and with or without TGFβ1.

FIGS. 10A-10B show the effects of the TGFβR1R2-IL9R receptor modification in TILs in terms of T cell proliferation upon repetitive antigen stimulation, with or without IL-2 and with or without TGFβ1.

FIG. 11 provides the sequence listing for the disclosure.

DETAILED DESCRITPION

The present disclosure provides an engineered chimeric transforming growth factor beta (TGFβ) receptor system, a recombinant vector system, and an engineered immune cell expressing the engineered chimeric TGFβ receptor system, and a method for treating a patient in need using the engineered immune cells. The engineered chimeric TGFβ receptor system is configured such that, when expressed in an immune cell, the immune cell is re-purposed to stimulate the IL-9 signaling pathway upon TGFβ binding. More details are provided below.

Engineered TGFβ receptor system

FIGS. 1A-1D illustrate two classes of the engineered TGFβ receptor system, and the corresponding TGFβ-responsive signaling complexes formed thereby, provided by certain embodiments of the disclosure. Each of the two classes of the engineered TGFβ receptor system (i.e. class I class II, corresponding to FIG. 1A and FIG. 1C respectively) comprises a first engineered TGFβ receptor chain and a second engineered TGFβ receptor chain. Each of the first and second engineered TGFβ receptor chains comprises an extracellular portion, a transmembrane portion, and an intracellular portion, which are operably connected to one another.

Specifically, in class I of the engineered TGFβ receptor system as illustrated in FIG. 1A, the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprises an extracellular domain (ECD) of a mammalian TGFβR1 or a functional portion or a functional variant thereof, and an ICD of a mammalian IL9R or a functional portion or a functional variant thereof; and the extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprises an extracellular domain (ECD) of a mammalian TGFβR2 or a functional portion or a functional variant thereof, and an ICD of a mammalian IL2Rγ or a functional portion or a functional variant thereof.

In class II of the engineered TGFβ receptor system as illustrated in FIG. 1C, the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprises an extracellular domain (ECD) of a mammalian TGFβR1 or a functional portion or a functional variant thereof, and an ICD of a mammalian IL2Rγ or a functional portion or a functional variant thereof; and the extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprises an extracellular domain (ECD) of a mammalian TGFβR2 or a functional portion or a functional variant thereof, and an ICD of a mammalian IL9R or a functional portion or a functional variant thereof.

Herein, the mammalian TGFβR1, mammalian TGFβR2, mammalian IL9, or mammalian IL2Rγ can be derived from any mammals such as humans, apes, rats, mouses, dogs, cats, etc. According to some embodiments, each ECD and each ICD as described above in the first and second chain in each class of the engineered TGFβ receptor system are all derived from human, and as such, the ECD of the human TGFβR1, the ECD of the human TGFβR2, the ICD of the human IL9, or the ICD of the human IL2Rγ included in the first and second engineered TGFβ receptor chain(s) respectively comprise a sequence as set forth in SEQ ID NOS: 1, 2, 3 and 4.

In some embodiments, the extracellular portion in the first engineered TGFβ receptor chain in both class I and class II of the engineered TGFβ receptor system (FIGS. 1A and 1C) may comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 1; the extracellular portion in the second engineered TGFβ receptor chain in both class I and class II of the engineered TGFβ receptor system (FIGS. 1A and 1C) may comprise an ECD of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 2. Each of the intracellular portion in the first engineered TGFβ receptor chain in class I of the engineered TGFβ receptor system (FIG. 1A) or the intracellular portion in the second engineered TGFβ receptor chain in class II of the engineered TGFβ receptor system (FIG. 1C) may comprise an ICD of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 3; and the intracellular portion in the second engineered TGFβ receptor chain in class I of the engineered TGFβ receptor system (FIG. 1A) or the intracellular portion in the first engineered TGFβ receptor chain in class II of the engineered TGFβ receptor system (FIG. 1C) may comprise an ICD of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

In each engineered TGFβ receptor chain in each class of the engineered TGFβ receptor system as illustrated in FIGS. 1A and 1C, the transmembrane portion (i.e. TM1, TM2, TM3, or TM4) that operably connects the extracellular portion and the intracellular portion may optionally be derived from a transmembrane domain of any transmembrane proteins (e.g. TGFβR1, TGFβR2, IL2Rγ, IL4T, IL9R, IL7R, IL15R, IL10R, IL21R, CD28, CTLA4, PD1, TIM3, TIGIT, CD4, CD8, or 4-1BB, etc.). In some embodiment, the transmembrane portion may be derived from a same protein as the extracellular portion, which may comprise a transmembrane domain (TM) of TGFβR1 (SEQ ID NO: 5) or of TGFβR2 (SEQ ID NO: 6), or a functional portion or a functional variant thereof. Yet in some other embodiments, the transmembrane portion may be derived from a same protein as the intracellular portion, which may comprise a transmembrane domain (TM) of IL9R (SEQ ID NO: 7) or of IL2Rγ (SEQ ID NO: 8), or a functional portion or a functional variant thereof. Table 1 summarizes the sequences for each structural domain of several embodiments of the engineered TGFβ receptor chains.

Table 1: Domain structures and amino acid sequences for several embodiments of the engineered TGFβ receptor chain used in the engineered TGFβ receptor system provided in the disclosure.

Extracellular Domain (ECD)	Transmembrane Domain (TM)	Intracellular Domain (ICD)	Engineered TGFβ Receptor Chain
TGFβR1(ECD): SEQ ID NO: 1	TGFβR1(TM): SEQ ID NO: 5	IL9R(ICD): SEQ ID NO: 3	TGFβR1(ECD)-TGFβR1(TM)-IL9R(ICD): SEQ ID NO: 9
TGFβR1(ECD): SEQ ID NO: 1	IL9R(TM): SEQ ID NO: 7	IL9R(ICD): SEQ ID NO: 3	TGFβR1(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 10
TGFβR1(ECD): SEQ ID NO: 1	TGFβR1(TM): SEQ ID NO: 5	IL2Rγ(ICD): SEQ ID NO: 4	TGFβR1(ECD)-TGFβR1(TM)-IL2Rγ(ICD): SEQ ID NO: 11
TGFβR1(ECD): SEQ ID NO: 1	IL2Rγ(TM): SEQ ID NO: 8	IL2Rγ(ICD): SEQ ID NO: 4	TGFβR1(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 12
TGFβR2(ECD): SEQ ID NO: 2	TGFβR2(TM): SEQ ID NO: 6	IL9R(ICD): SEQ ID NO: 3	TGFβR2(ECD)-TGFβR2(TM)-IL9R(ICD): SEQ ID NO: 13
TGFβR2(ECD): SEQ ID NO: 2	IL9R(TM): SEQ ID NO: 7	IL9R(ICD): SEQ ID NO: 3	TGFβR2(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 14
TGFβR2(ECD): SEQ ID NO: 2	TGFβR2(TM): SEQ ID NO: 6	IL2Rγ(ICD): SEQ ID NO: 4	TGFβR2(ECD)-TGFβR2(TM)-IL2Rγ(ICD): SEQ ID NO: 15

TGFβR1(ECD): SEQ ID NO: 2	IL2Rγ(TM): SEQ ID NO: 8	IL2Rγ(ICD): SEQ ID NO: 4	TGFβR2(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 16

Recombinant vectors

In another aspect, the present disclosure further provides a recombinant vector system that is designed to express the engineered chimeric TGFβ receptor system as described above in immune cells. Such recobminant vector system may comprise at least one recmbinant vector (e.g., expression vector).

Herein, each recombinant vector may include an isolated polynucleotide disclosed herein (e.g., a polynucleotide that encodes a polypeptide disclosed herein), host cells into which are introduced the recombinant vectors (i.e., such that the host cells contain the polynucleotide and/or a vector comprising the polynucleotide), and the production of recombinant polypeptides or fragments thereof by recombinant techniques.

As used herein, a "vector" is any construct capable of delivering one or more polynucleotide(s) of interest to a host cell when the vector is introduced to the host cell. An "expression vector" is capable of delivering and expressing the one or more polynucleotide(s) of interest as an encoded polypeptide in a host cell into which the expression vector has been introduced. Thus, in an expression vector, the polynucleotide of interest is positioned for expression in the vector by being operably linked with regulatory elements such as a promoter, enhancer, and/or a poly-A tail, either within the vector or in the genome of the host cell at or near or flanking the integration site of the polynucleotide of interest such that the polynucleotide of interest will be translated in the host cell introduced with the expression vector.

A vector can be introduced into the host cell by methods known in the art, e.g., electroporation, chemical transfection (e.g., DEAE-dextran), transformation, transfection, and infection and/or transduction (e.g., with recombinant virus). Thus, non-limiting examples of vectors include viral vectors (which can be used to generate recombinant virus), naked DNA or RNA, plasmids, cosmids, phage vectors, and DNA or RNA expression vectors associated with cationic condensing agents.

The present disclosure provides a recombinant vector comprising a nucleic acid construct suitable for genetically modifying a cell, which can be used for treatment of pathological disease or condition.

Any vector or vector type can be used to deliver genetic material to the cell. These vectors include but are not limited to plasmid vectors, viral vectors, bacterial artificial

chromosomes (BACs), yeast artificial chromosomes (YACs), and human artificial chromosomes (HACs). Viral vectors can include but are not limited to recombinant retroviral vectors, recombinant lentiviral vectors, recombinant adenoviral vectors, foamy virus vectors, recombinant adeno-associated viral (AAV) vectors, hybrid vectors, and plasmid transposons (e.g., sleeping beauty transposon system, and PiggyBac transposon system) or integrase based vector systems. Other vectors that are known in the art can also be used in connection with the methods described herein.

In some embodiments, the vector is a viral vector. The viral vector can be grown in a culture medium specific for viral vector manufacturing. Any suitable growth media and/or supplements for growing viral vectors can be used in accordance with the embodiments described herein.

In some embodiments, the vector used is a recombinant retroviral vector. A retroviral vector is capable of directing the expression of a nucleic acid molecule of interest. A retrovirus is present in the RNA form in its viral capsule and forms a double-stranded DNA intermediate when it replicates in the host cell. Similarly, retroviral vectors are present in both RNA and double-stranded DNA forms. The retroviral vector also includes the DNA form which contains a recombinant DNA fragment and the RNA form containing a recombinant RNA fragment. The vectors can include at least one transcriptional promoter/enhancer, or other elements which control gene expression. Such vectors can also include a packaging signal, long terminal repeats (LTRs) or portion thereof, and positive and negative strand primer binding sites appropriate to the retrovirus used. Long terminal repeats (LTRs) are identical sequences of DNA that repeat many times (e.g., hundreds or thousands of times) found at either end of retrotransposons or proviral DNA formed by reverse transcription of retroviral RNA. They are used by viruses to insert their genetic material into the host genomes. Optionally, the vectors can also include a signal which directs polyadenylation, selectable markers such as Ampicillin resistance, Neomycin resistance, TK, hygromycin resistance, phleomycin resistance histidinol resistance, or DHFR, as well as one or more restriction sites and a translation termination sequence. For example, such vectors can include a 5' LTR, a leading sequence, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3' LTR or a portion thereof. Additionally, retroviral vector used herein can also refers to the recombinant vectors created by removal of the retroviral gag, pol, and env genes and replaced with the gene of interest.

In some embodiments, the vector can include an additional nucleic acid encoding an inhibitory protein (e.g., a checkpoint inhibitor). In various embodiments, the cell expresses the

genetically engineered antigen receptor and the inhibitory protein. In various embodiments, the inhibitory protein is constitutively expressed.

In some embodiments, the vector or construct can contain a single promoter that drives the expression of one or more nucleic acid molecules. In some embodiments, such promoters can be multicistronic (bicistronic or tricistronic). For example, in some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes separated from one another by sequences encoding a self-cleavage peptide (e.g., P2A or T2A) or a protease recognition site (e.g., furin). The ORF thus encodes a single polyprotein, which, either during (in the case of 2A e.g., T2A) or after translation, is cleaved into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream.

Various cell lines can be used in connection with the vectors as described herein. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells; and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the binding molecule. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

According to some embodiments, the vector system may comprise two vectors, which are designed to respectively express the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain in the engineered TGFβ receptor system.

According to some other embodiments, the vector system may comprise one vector, which expresses both the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain in the engineered TGFβ receptor system. As such, the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are substantially in one single vector. Further in some embodiments, these two engineered TGFβ receptor chains can be encoded within one open reading frame (ORF) in the one vector. Furthermore, the ORF may comprise a polynucleotide encoding a self-cleaving peptide (e.g. T2A, P2A, F2A, or E2A, etc.), which operably connects a polynucleotide encoding the first engineered TGFβ receptor

chain and a polynucleotide encoding the second engineered TGFβ receptor chain. In some other embodiments, each of the two engineered TGFβ receptor chains may be in a different ORF in the same vector.

In any of the embodiments of the vector system as described above, each of the polynucleotide that encodes the first engineered TGFβ receptor chain and the polynucleotide that encodes the second engineered TGFβ receptor chain is further operably connected to a polynucleotide encoding a signal peptide (SP). As such, the polypeptide encoded by the polynucleotide in the vector(s) of the vector system substantially comprises a first signal peptide over the N-terminus of the first engineered TGFβ receptor chain, and comprises a second signal peptide over the N-terminus of the second engineered TGFβ receptor chain, of the engineered TGFβ receptor system.

In some embodiments, the first signal peptide and the extracellular portion of the first engineered TGFβ receptor chain are derived from a same protein, and the second signal peptide and the extracellular portion of the second engineered TGFβ receptor chain are derived from a same protein. As such, in either class I (FIG. 1A) or class II (FIG. 1C) of the engineered TGFβ receptor system as described above, according to some specific embodiments, the first signal peptide for the first engineered TGFβ receptor chain can comprise the signal peptide of TGFβR1 (SEQ ID NO: 17) or a functional portion or functional variant thereof, and the second signal peptide for the second engineered TGFβ receptor chain can comprise the signal peptide of TGFβR2 (SEQ ID NO: 18) a functional portion or functional variant thereof. Table 2 summarizes the amino acid sequences for several embodiments of the engineered TGFβ receptor chains that are encoded by the vector system provided in the disclosure.

Table 2: Amino acid sequences for several embodiments of the engineered TGFβ receptor chain encoded by the vector system provided in the disclosure.

Signal Peptide (SP)	Engineered TGFβ Receptor Chain (without SP)	Engineered TGFβ Receptor Chain (with SP)
TGFβR1(SP): SEQ ID NO: 17	TGFβR1(ECD)-TGFβR1(TM)-IL9R(ICD): SEQ ID NO: 9	TGFβR1(SP)-TGFβR1(ECD)-TGFβR1(TM)-IL9R(ICD): SEQ ID NO: 19
TGFβR1(SP): SEQ ID NO: 17	TGFβR1(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 10	TGFβR1(SP)-TGFβR1(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 20
TGFβR1(SP): SEQ ID NO: 17	TGFβR1(ECD)-TGFβR1(TM)-IL2Rγ(ICD): SEQ ID NO: 11	TGFβR1(SP)-TGFβR1(ECD)-TGFβR1(TM)-IL2Rγ(ICD): SEQ ID NO: 21

TGFβR1(SP): SEQ ID NO: 17	TGFβR1(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 12	TGFβR1(SP)-TGFβR1(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 22
TGFβR2(SP): SEQ ID NO: 18	TGFβR2(ECD)-TGFβR2(TM)-IL9R(ICD): SEQ ID NO: 13	TGFβR2(SP)-TGFβR2(ECD)-TGFβR2(TM)-IL9R(ICD): SEQ ID NO: 23
TGFβR2(SP): SEQ ID NO: 18	TGFβR2(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 14	TGFβR2(SP)-TGFβR2(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 24
TGFβR2(SP): SEQ ID NO: 18	TGFβR2(ECD)-TGFβR2(TM)-IL2Rγ(ICD): SEQ ID NO: 15	TGFβR2(SP)-TGFβR2(ECD)-TGFβR2(TM)-IL2Rγ(ICD): SEQ ID NO: 25
TGFβR2(SP): SEQ ID NO: 18	TGFβR2(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 16	TGFβR2(SP)-TGFβR2(ECD)-IL2Rγ(TM)-IL2Rγ(ICD): SEQ ID NO: 26

Herein, the term "linker" (L) or "linker domain" or "linker region" as used herein refer to an oligo- or polypeptide region from about 1 to 100 amino acids in length, which links together any of the domains/regions. Linkers can be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers can be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers can be cleavable or non-cleavable.

"Nucleic acid" as used herein can include "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained from natural sources, which can contain natural, non-natural or altered nucleotides. Furthermore, the nucleic acid comprises complementary DNA (cDNA). It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it can be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

The nucleic acids as described herein can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides. In some of any such embodiments, the nucleotide sequence is codon-optimized.

The present disclosure also provides the nucleic acids comprising a nucleotide sequence complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

In some embodiments, the nucleotide sequence encoding two functional polypeptides (e.g. the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain) are separated by a peptide sequence that causes ribosome skipping. In some embodiments, the peptide that causes ribosome skipping is a P2A, F2A, or T2A peptide. In some embodiments, the nucleic acid is synthetic. In some embodiments, the nucleic acid is cDNA.

In some embodiments, each of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain described herein is encoded by a nucleotide sequence that has been codon-optimized. In certain embodiments, each of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain further comprises a signal peptide (SP). In particular embodiments, each of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain described herein is isolated or purified or is recombinant.

The disclosure also provides a nucleic acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any nucleotide sequence as described herein, and an amino acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any amino acid sequence as described herein. In some embodiments, the disclosure relates to nucleotide sequences encoding any peptides that are described herein, or any amino acid sequences that are encoded by any nucleotide sequences as described herein.

In some embodiments, the nucleic acid sequence is at least or about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300, 350, 400, 500, or 600 nucleotides. In some embodiments, the amino acid sequence is at least or about 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acid residues. In some embodiments, the nucleic acid sequence is less than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300, 350, 400, 500, or 600 nucleotides. In some embodiments, the amino acid sequence is less than 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acid residues.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same

amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

FIGS. 2A-2D illustrate the block diagrams of four constructs respectively encoding the engineered TGFβ receptor systems according to four specific embodiments (i.e. Embodiment 1-4, sequences summarized in Table 3) of the disclosure. As illustrated, each embodiment substantially takes a configuration of one single ORF encoding both the first and second engineered TGFβ receptor chains separated by an "F2A" self-cleaving peptide (SEQ ID NO: 27).

Table 3: Amino acid sequences for the four embodiments of the construct encoding the engineered TGFβ receptor system provided in the disclosure.

Embodiment	Construct	Alias or other names
1	TGFβR1(SP)-TGFβR1(ECD)-TGFβR1(TM)-IL9R(ICD)-F2A-TGFβR2(SP)-TGFβR2(ECD)-TGFβR2(TM)-IL2Rγ(ICD): SEQ ID NO: 28 (nucleotide sequence: SEQ ID NO: 32)	"TGFβR1-IL9R-TGFβR2-IL2Rγ (TGFβR1/R2 TM)"; or "TR1/2-IL9R (TR TM)"
2	TGFβR1(SP)-TGFβR1(ECD)-IL9R(TM)-IL9R(ICD)-F2A-TGFβR2(SP)-TGFβR2(ECD)-IL2Rγ(TM)-IL2Rγ (ICD): SEQ ID NO: 29	"TGFβR1-IL9R-TGFβR2-IL2Rγ (IL9R/IL2Rγ TM)"; or "TR1/2-IL9R (9R TM)"
3	TGFβR1(SP)-TGFβR1(ECD)-TGFβR1(TM)-IL2Rγ(ICD)-F2A-TGFβR2(SP)-TGFβR2(ECD)-TGFβR2(TM) -IL9R (ICD): SEQ ID NO: 30	"TGFβR1-IL2Rγ-TGFβR2-IL9R (TGFβR1/R2 TM)"
4	TGFβR1(SP)-TGFβR1(ECD)-IL2Rγ(TM)-IL2Rγ(ICD)-F2A-TGFβR2(SP)-TGFβR2(ECD)-IL9R(TM)-IL9R(ICD): SEQ ID NO: 31	"TGFβR1-IL2Rγ-TGFβR2-IL9R (IL9R/IL2Rγ TM)"

Engineered cells

The present disclosure further provides engineered cells, and in particular immune cells (e.g., T cells) that comprise the engineered TGFβ receptor system deescribed herein. These engineered cells can be used to treat various disorders or disease as described herein (e.g., virus infection, cancers, virus-induced disorders).

In various embodiments, the cell that is engineered can be obtained from e.g., humans and non-human animals. In various embodiments, the cell that is engineered can be obtained from bacteria, fungi, humans, rats, mice, rabbits, monkeys, pig or any other species. Preferably, the cell is from humans, rats or mice. More preferably, the cell is obtained from humans. In various embodiments, the cell that is engineered is a blood cell. Preferably, the cell is a leukocyte (e.g., a T cell), lymphocyte or any other suitable blood cell type. In some embodiments, the cell is a peripheral blood cell. In some embodiments, the cell is a T cell, tumor-infiltrating lymphocyte (TIL), B cell or NK cell.

In some embodiments, the cell is a T cell. In some embodiments, the T cells can express a cell surface receptor that recognizes a specific antigenic moiety on the surface of a target cell. The cell surface receptor can be a wild type or recombinant T cell receptor (TCR), a chimeric antigen receptor (CAR), or any other surface receptor capable of recognizing an antigenic moiety that is associated with the target cell. T cells can be obtained by various methods known in the art, e.g., in vitro culture of T cells (e.g., tumor infiltrating lymphocytes) isolated from patients. TCR gene-modified T cells can be obtained by transducing T cells (e.g., isolated from the peripheral blood of patients), with a viral vector. In some embodiments, the T cell is a TCR gene-modified T cell. In some embodiments, the T cells are CD4+ T cells, CD8+ T cells, or regulatory T cells. In some embodiments, the T cells are T helper type 1 T cells and T helper type 2 T cells. In some embodiments, the T cell expressing this receptor is an αβ-T cell. In alternate embodiments, the T cell expressing this receptor is a γδ-T cell.

In some embodiments, the cell is an NK cell. In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the binding molecule, e.g., TCR, can be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

Different cell types can be obtained from appropriate isolation methods. The isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, e.g., surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers can be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes

separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

Also provided are methods, nucleic acids, compositions, and kits, for expressing the binding molecules, and for producing the genetically engineered cells expressing such binding molecules. The genetic engineering generally involves introduction of a nucleic acid encoding the therapeutic molecule, e.g. TCR, CAR, e.g. TCR-like CAR, polypeptides, fusion proteins, into the cell, such as by retroviral transduction, transfection, or transformation. In some embodiments, gene transfer is accomplished by first stimulating the cell, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, e.g., as measured by expression of a cytokine or activation marker, followed by transduction of the activated cells, and expansion in culture to numbers sufficient for clinical application.

In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors. In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), or spleen focus forming virus (SFFV). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In some embodiments, the vector is a lentivirus vector. In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation. In

some embodiments, recombinant nucleic acids are transferred into T cells via transposition. Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection, protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment and strontium phosphate DNA co-precipitation. Many of these methods are descried e.g., in WO2019195486, which is incorporated herein by reference in its entirety.

Also provided are populations of engineered cells, compositions containing such cells and/or enriched for such cells, such as in which cells expressing the binding molecule make up at least 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more percent of the total cells in the composition or cells of a certain type such as T cells, $CD8^+$ or $CD4^+$ cells.

Method for preparation of engineered cells

The present disclosure further provides a method or process for manufacturing and using the engineered cells for treatment of pathological diseases or conditions.

The cells for introduction of the engineered TGFβ receptor system can be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

Accordingly, the cells in some embodiments are primary cells, e.g., primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g. transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues,

liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, or non-human primate.

In some embodiments, the blood cells collected from the subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated "flow-through" centrifuge. In some aspects, a washing step is accomplished by tangential flow filtration (TFF). In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, Ca^{2+}/Mg^{2+} free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media. In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

In some embodiments, the method comprises one or more steps of: e.g., isolating the T cells from a patient's blood; transducing the population T cells with a viral vector including the nucleic acid construct encoding a genetically engineered antigen receptor; expanding the transduced cells in vitro; and/or infusing the expanded cells into the patient, where the engineered cells (e.g., engineered T cells) will seek and destroy antigen positive tumor cells. In some embodiments, the nucleic acid construct further includes a sequence encoding an inhibitory protein. In some embodiments, these engineered cells (e.g., engineered T cells) can block PD-1/PD-L1 immunosuppression and strengthen the antitumor immune response. In some embodiments, the method further comprises: transfection of T cells with the viral vector containing the nucleic acid construct.

In some embodiments, the methods involve introducing any vectors described herein into a cell in vitro or ex vivo. In some embodiments, the vector is a viral vector and the introducing is carried out by transduction. In some embodiments, the one or more agent is an inhibitory nucleic acid (e.g., siRNA). In some embodiments, the one or more agent is a fusion protein comprising a DNA-targeting protein and a nuclease or an RNA-guided nuclease (e.g.,

a clustered regularly interspaced short palindromic nucleic acid (CRISPR)-associated nuclease).

The transfection of T cells may be achieved by using any standard method such as calcium phosphate, electroporation, liposomal mediated transfer, microinjection, biolistic particle delivery system, or any other known methods by skilled artisan. In some embodiments, transfection of T cells is performed using the calcium phosphate method.

According to various embodiments described herein, the present disclosure provides an immunotherapy against tumors. In some embodiments, the engineered cells (e.g., engineered T cells) recognize a tumor associated antigen. In some embodiments, these engineered cells (e.g., engineered T cells) demonstrate a stronger antitumor response and reduced T cell exhaustion.

The present disclosure provides a method to create a personalized anti-tumor immunotherapy. In some embodiments, the engineered cells can be produced from a patient's blood cells. These engineered cells are then reinfused into the patient as a cellular therapy product. This product can be applied to any patient who has a tumor.

Methods of treatment

In yet another aspect, methods of treatment are also provided, which can be used for various therapeutic purposes. In one aspect, the disclosure provides methods for treating a tumor in a subject, methods of reducing the rate of the increase of volume of a tumor in a subject over time, methods of reducing the risk of developing a metastasis, or methods of reducing the risk of developing an additional metastasis in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of a cancer. In some embodiments, the treatment can result in the reduction of in the number, severity, and/or duration of one or more symptoms of the cancer in a subject. Herein the subject can be a patient in need thereof (i.e. someone having, or identified or diagnosed as having, the tumor).

According to some embodiments, a method for treating a tumor in a patient in need thereof is provided, which comprises: administering a population of the immune cell according to any embodiment as provided above in the fourth aspect. Herein, the population of the immune cell can be in a therapeutically effective amount.

Herein according to some embodiments of the method, the immune cell further expresses a target-recognizing receptor capable of binding a target molecule on a target cancer cell within the tumor, wherein the target-recognizing receptor is selected from a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-TCR.

According to some embodiments of the method, the tumor is a solid tumor, such as breast cancer (e.g., triple-negative breast cancer), carcinoid cancer, cervical cancer, endometrial cancer, glioma, head and neck cancer, liver cancer, lung cancer, small cell lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, colorectal cancer, gastric cancer, testicular cancer, thyroid cancer, bladder cancer, urethral cancer, or hematologic malignancy. In some embodiments, the cancer is unresectable melanoma or metastatic melanoma, non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, or metastatic hormone-refractory prostate cancer.

According to some embodiments of the method, the solid tumor is characterized to have a high level of TGFβ expression therewithin, and may be a tumor from a liver cancer, breast cancer, prostate cancer, glioma, bladder cancer, or thymic epithelial tumor, etc.

In some embodiments, the compositions and methods disclosed herein can be used for treatment of patients at risk for a cancer. Patients with cancer can be identified with various methods known in the art.

Furthermore, the disclosure provides methods for treating infection or infection associated conditions in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of the disease. These methods generally involve administering a therapeutically effective amount of genetic engineered cells disclosed herein to a subject in need thereof. In some embodiments, the disease or condition treated is an infectious disease or condition, such as, but not limited to, viral, retroviral, bacterial, and protozoal infections, immunodeficiency, Human Papilloma Virus (HPV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, BK polyomavirus.

As used herein, by an "effective amount" or "therapeutically effective amount" is meant an amount or dosage sufficient to effect beneficial or desired results including halting, slowing, retarding, or inhibiting progression of a disease, e.g., a cancer. An effective amount will vary depending upon, e.g., an age and a body weight of a subject to which the therapeutic agent and/or therapeutic compositions is to be administered, a severity of symptoms and a route of administration, and thus administration can be determined on an individual basis.

An effective amount can be administered in one or more administrations. By way of example, an effective amount of a composition is an amount sufficient to ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of a cancer in a patient or is an amount sufficient to ameliorate, stop, stabilize, reverse, slow and/or delay proliferation of a cell (e.g., a biopsied cell, any of the cancer cells described herein, or cell line (e.g., a cancer cell line)) in

vitro. As is understood in the art, an effective may vary, depending on, inter alia, patient history as well as other factors such as the type (and/or dosage) of compositions used.

Effective amounts and schedules for administrations may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage that must be administered will vary depending on, for example, the mammal that will receive the treatment, the route of administration, the particular type of therapeutic agents and other drugs being administered to the mammal. Guidance in selecting appropriate doses can be found in the literature. In addition, a treatment does not necessarily result in the 100% or complete treatment or prevention of a disease or a condition. There are multiple treatment/prevention methods available with a varying degree of therapeutic effect which one of ordinary skill in the art recognizes as a potentially advantageous therapeutic mean.

In any of the methods described herein, the engineered cells and, and/or at least one additional therapeutic agent can be administered to the subject at least once a week (e.g., once a week, twice a week, three times a week, four times a week, once a day, twice a day, or three times a day). In some embodiments, at least two different engineered cells (e.g., cells express different binding molecules) are administered in the same composition (e.g., a liquid composition). In some embodiments, engineered cells and at least one additional therapeutic agent are administered in the same composition (e.g., a liquid composition). In some embodiments, engineered cells and the at least one additional therapeutic agent are administered in two different compositions. In some embodiments, the at least one additional therapeutic agent is administered as a pill, tablet, or capsule. In some embodiments, the at least one additional therapeutic agent is administered in a sustained-release oral formulation.

In some embodiments, the one or more additional therapeutic agents can be administered to the subject prior to, concurrently with, or after administering the engineered cells to the subject.

In some embodiments, one or more additional therapeutic agents can be administered to the subject. The additional therapeutic agent can be a checkpoint inhibitor (CPI). In some embodiments, the checkpoint inhibitor is an inhibitory protein, e.g., an antibody or antigen binding fragment thereof. The checkpoint inhibitor can inhibit or block one or more immune checkpoints, including e.g., PD-1, PD-L1, PD-L2, 2B4 (CD244), 4-1BB, A2aR, B7.1, B7.2, B7-H2, B7-H3, B7-H4, B7-H6, BTLA, butyrophilins, CD160, CD48, CTLA4, GITR, gp49B, HHLA2, HVEM, ICOS, ILT-2, ILT-4, KIR family receptors, LAG-3, OX-40, PIR-B, SIRPalpha (CD47), TFM-4, TIGIT, TIM-1, TIM-3, TIM-4, VISTA and combinations thereof. In some embodiments, the inhibitory protein blocks PD-1 or PD-Ll. In various embodiments,

the inhibitory protein comprises an anti-PD-1 scFv. The inhibitory protein is capable of leading to reduced expression of PD-1 or PD-L1 and/or inhibiting upregulation of PD- 1 or PD-L1 in T cells in the population and/or physically obstructing the formation of the PD- 1/PD-L1 complex and subsequent signal transduction. In some embodiments, the inhibitory protein blocks PD-1. In some embodiments, the additional therapeutic agent is an anti-OX40 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-LAG-3 antibody, an anti-TIGIT antibody, an anti-BTLA antibody, an anti-CTLA-4 antibody, or an anti-GITR antibody. In some embodiments, the additional therapeutic agent is an anti-CTLA4 antibody (e.g., ipilimumab), an anti-CD20 antibody (e.g., rituximab), an anti- EGFR antibody (e.g., cetuximab), an anti-CD319 antibody (e.g., elotuzumab), or an anti-PD1 antibody (e.g., nivolumab).

In one some embodiments, the additional therapeutic agent can comprise one or more inhibitors selected from the group consisting of an inhibitor of B-Raf, an EGFR inhibitor, an inhibitor of a MEK, an inhibitor of ERK, an inhibitor of K-Ras, an inhibitor of c-Met, an inhibitor of anaplastic lymphoma kinase (ALK), an inhibitor of a phosphatidylinositol 3-kinase (PI3K), an inhibitor of an Akt, an inhibitor of mTOR, a dual PI3K/mTOR inhibitor, an inhibitor of Bruton's tyrosine kinase (BTK), and an inhibitor of Isocitrate dehydrogenase 1 (IDH1) and/or Isocitrate dehydrogenase 2 (IDH2). In some embodiments, the additional therapeutic agent is an inhibitor of indoleamine 2,3-dioxygenase-1) (IDO1) (e.g., epacadostat). In some embodiments, the additional therapeutic agent can comprise one or more inhibitors selected from the group consisting of an inhibitor of HER3, an inhibitor of LSD1, an inhibitor of MDM2, an inhibitor of BCL2, an inhibitor of CHK1, an inhibitor of activated hedgehog signaling pathway, and an agent that selectively degrades the estrogen receptor.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of Trabectedin, nab-paclitaxel, Trebananib, Pazopanib, Cediranib, Palbociclib, everolimus, fluoropyrimidine, IFL, regorafenib, Reolysin, Alimta, Zykadia, Sutent, temsirolimus, axitinib, everolimus, sorafenib, Votrient, Pazopanib, IMA-901, AGS-003, cabozantinib, Vinflunine, an Hsp90 inhibitor, Ad-GM-CSF, Temazolomide, IL-2, IFNa, vinblastine, Thalomid, dacarbazine, cyclophosphamide, lenalidomide, azacytidine, lenalidomide, bortezomid, amrubicine, carfilzomib, pralatrexate, and enzastaurin.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of an adjuvant, a TLR agonist, tumor necrosis factor (TNF) alpha, IL-1, HMGB1, an IL-10 antagonist, an IL-4 antagonist, an IL-13 antagonist, an IL-17 antagonist, an HVEM antagonist, an ICOS agonist, a treatment targeting

CX3CL1, a treatment targeting CXCL9, a treatment targeting CXCL10, a treatment targeting CCL5, an LFA-1 agonist, an ICAM1 agonist, and a Selectin agonist.

In some embodiments, carboplatin, nab-paclitaxel, paclitaxel, cisplatin, pemetrexed, gemcitabine, FOLFOX, or FOLFIRI are administered to the subject. In some embodiments, the additional therapeutic agent is selected from asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine and/or combinations thereof.

Compositions and formulations

The present disclosure provides compositions (including pharmaceutical and therapeutic compositions) containing the engineered cells, produced by the methods disclosed herein. Also provided are methods, e.g., therapeutic methods for administrating the engineered cells and compositions thereof to subjects, e.g., patients.

Compositions including the engineered cells for administration, including pharmaceutical compositions and formulations, such as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof are provided. The pharmaceutical compositions and formulations can include one or more optional pharmaceutically acceptable carrier or excipient. In some embodiments, the composition includes at least one additional therapeutic agent.

A pharmaceutically acceptable carrier refers to an ingredient in a pharmaceutical composition, other than an active ingredient. The pharmaceutically acceptable carrier does not interfere with the active ingredient and is nontoxic to a subject. A pharmaceutically acceptable carrier can include, but is not limited to, a buffer, excipient, stabilizer, or preservative. The pharmaceutical formulation refers to process in which different substances and/or agents are combined to produce a final medicinal product. The formulation studies involve developing a preparation of drug acceptable for patient. Additionally, a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

In some embodiments, the choice of carrier is determined in part by the particular cell (e.g., T cell or NK cell) and/or by the method of administration. A variety of suitable formulations are available. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives can include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some embodiments, a mixture of two or more

preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some embodiments, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

The formulations can include aqueous solutions. The formulation or composition can also contain more than one active ingredient useful for a particular indication, disease, or condition being treated with the engineered cells, preferably those with activities complementary to the engineered cells, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition can further include other pharmaceutically active agents or drugs, such as checkpoint inhibitors, fusion proteins, chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, and/or vincristine.

The pharmaceutical composition in some embodiments contains the engineered cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. The desired dosage can be delivered by a single bolus administration of the engineered cells, by multiple bolus administrations of the engineered cells, or by continuous infusion administration of the engineered cells.

The engineered cells and compositions can be administered using standard administration techniques, formulations, and/or devices. Administration of the engineered cells can be autologous or heterologous. For example, immunoresponsive T cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject after genetically modifying them in accordance with various embodiments described herein. Peripheral blood derived immunoresponsive T cells or their progeny (e.g., in vivo, ex vivo or in vitro derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. Usually, when administering a therapeutic composition (e.g., a pharmaceutical composition containing a genetically modified immunoresponsive cell), it is generally formulated in a unit dosage injectable form (solution, suspension, emulsion).

Formulations disclosed herein include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the engineered cells are administered to the subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

The compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which can in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water,

saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the engineered cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, and/or colors, depending upon the route of administration and the preparation desired. Standard texts can in some aspects be consulted to prepare suitable preparations.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, and sorbic acid. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

The formulations to be used for in vivo administration are generally sterile. Sterility can be readily accomplished, e.g., by filtration through sterile filtration membranes.

The compositions or pharmaceutical compositions as described herein can be included in a container, pack, or dispenser together with instructions for administration.

Methods of administration

Provided are also methods of administering the engineered cells, populations, and compositions, and uses of such cells, populations, and compositions to treat or prevent diseases, conditions, and disorders, including cancers. In some embodiments, the methods described herein can reduce the risk of the developing diseases, conditions, and disorders as described herein.

In some embodiments, the engineered cells, populations, and compositions, described herein are administered to a subject or patient having a particular disease or condition to be treated, e.g., via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, cells and compositions prepared by the provided methods, such as engineered compositions and end-of-production compositions following incubation and/or other processing steps, are administered to a subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, e.g., ameliorate one or more symptom of, the disease

or condition, such as by lessening tumor burden in cancer expressing an antigen recognized by the engineered cells (e.g., engineered T cells).

Methods for administration of cells for adoptive cell therapy are known and can be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in U.S. 2003/0170238; U.S. Pat. No. 4,690,915; Rosenberg, "Cell transfer immunotherapy for metastatic solid cancer—what clinicians need to know." Nature reviews Clinical oncology 8.10 (2011): 577; Themeli et al. "Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy." Nature biotechnology 31.10 (2013): 928; Tsukahara et al. "CD19 target-engineered T-cells accumulate at tumor lesions in human B-cell lymphoma xenograft mouse models." Biochemical and biophysical research communications 438.1 (2013): 84-89; Davila et al. "CD19 CAR-targeted T cells induce long-term remission and B Cell Aplasia in an immunocompetent mouse model of B cell acute lymphoblastic leukemia." PloS one 8.4 (2013); each of which is incorporated herein by reference in its entirety.

In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by autologous transfer, in which the T cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the engineered cells are derived from a subject, e.g., patient, in need of a treatment and the engineered cells, following isolation and processing are administered to the same subject.

In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by allogeneic transfer, in which the T cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, e.g., a first subject. In such embodiments, the engineered cells then are administered to a different subject, e.g., a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

In some embodiments, the HLA class or HLA supertype of the subject is identified. In some embodiments, the subject is treated with a cell therapy that can recognize the antigen in the context of the HLA class or HLA supertype.

In some embodiments, the subject has been treated with a therapeutic agent targeting the disease or condition, e.g. the tumor, prior to administration of the engineered cells or composition containing the engineered cells. In some aspects, the subject is refractory or non-responsive to the other therapeutic agent. In some embodiments, the subject has persistent or

relapsed disease, e.g., following treatment with another therapeutic intervention, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), e.g., allogenic HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy.

In some embodiments, the subject is responsive to the other therapeutic agent, and treatment with the therapeutic agent reduces disease burden. In some aspects, the subject is initially responsive to the therapeutic agent, but exhibits a relapse of the disease or condition over time. In some embodiments, the subject has not relapsed. In some such embodiments, the subject is determined to be at risk for relapse, such as at high risk of relapse, and thus the engineered cells are administered prophylactically, e.g., to reduce the likelihood of or prevent relapse. In some embodiments, the subject has not received prior treatment with another therapeutic agent.

In some embodiments, the engineered cells are administered at a desired dosage, which in some aspects includes a desired dose or number of cells or cell type(s) and/or a desired ratio of cell types. Thus, the dosage of cells in some embodiments is based on a total number of cells (or number per kg body weight) and a desired ratio of the individual populations or sub-types, such as the CD4+ to CD8+ ratio. In some embodiments, the dosage of cells is based on a desired total number (or number per kg of body weight) of cells in the individual populations or of individual cell types. In some embodiments, the dosage is based on a combination of such features, such as a desired number of total cells, desired ratio, and desired total number of cells in the individual populations.

In some embodiments, the populations or sub-types of cells, such as CD8+ and CD4+ T cells, are administered at or within a tolerated difference of a desired dose of total cells, such as a desired dose of T cells. In some embodiments, the desired dose is a desired number of cells or a desired number of cells per unit of body weight of the subject to whom the engineered cells are administered, e.g., cells/kg. In some embodiments, the desired dose is at or above a minimum number of cells or minimum number of cells per unit of body weight. In some embodiments, among the total cells, administered at the desired dose, the individual populations or sub- types are present at or near a desired output ratio (such as CD4+ to CD8+ ratio), e.g., within a certain tolerated difference or error of such a ratio.

In some embodiments, the engineered cells are administered at or within a tolerated difference of a desired dose of one or more of the individual populations or sub-types of cells, such as a desired dose of CD4+ cells and/or a desired dose of CD8+ cells. In some embodiments, the desired dose is a desired number of cells of the sub-type or population, or a desired number

of such cells per unit of body weight of the subject to whom the engineered cells are administered, e.g., cells/kg. In some embodiments, the desired dose is at or above a minimum number of cells of the population or sub-type, or minimum number of cells of the population or sub-type per unit of body weight.

Thus, in some embodiments, the dosage is based on a desired fixed dose of total cells and a desired ratio, and/or based on a desired fixed dose of one or more, e.g., each, of the individual sub-types or sub-populations. Thus, in some embodiments, the dosage is based on a desired fixed or minimum dose of T cells and a desired ratio of CD4+ to CD8+ cells, and/or is based on a desired fixed or minimum dose of CD4+ and/or CD8+ cells.

In certain embodiments, the engineered cells are administered to the subject at a range of about one million to about 100 billion cells, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges.

In some embodiments, the dose of total cells and/or dose of individual sub- populations of cells is within a range of between at or about 104 and at or about 109 cells/kilograms (kg) body weight, such as between 105 and 106 cells/kg body weight, for example, at least or at least about or at or about 1×105 cells/kg, 1.5×105 cells/kg, 2×105 cells/kg, or 1×106 cells/kg body weight. For example, in some embodiments, the engineered cells are administered at, or within a certain range of error of, between at or about 104 and at or about 109 T cells/kilograms (kg) body weight, such as between 105 and 106 T cells/kg body weight, for example, at least or at least about or at or about 1×105 T cells/kg, 1.5×105 T cells/kg, 2×105 T cells/kg, or 1×106 T cells/kg body weight.

In some embodiments, the engineered cells are administered at or within a certain range of error of between at or about 104 and at or about 109 CD4+ and/or CD8+ cells/kilograms (kg) body weight, such as between 105 and 106 CD4+ and/or CD8+ cells/kg body weight, for

example, at least or at least about or at or about 1×105 CD4+ and/or CD8+ cells/kg, 1.5×105 CD4+ and/or CD8+ cells/kg, 2×105 CD4+ and/or CD8+ cells/kg, or 1×106 CD4+ and/or CD8+ cells/kg body weight.

In some embodiments, the engineered cells are administered at or within a certain range of error of, greater than, and/or at least about 1×106, about 2.5×106, about 5×106, about 7.5×106, or about 9×106 CD4+ cells, and/or at least about 1×106, about 2.5×106, about 5×106, about 7.5×106, or about 9×106 CD8+ cells, and/or at least about 1×106, about 2.5×106, about 5×106, about 7.5×106, or about 9×106 T cells. In some embodiments, the engineered cells are administered at or within a certain range of error of between about 108 and 1012 or between about 1010 and 1011 T cells, between about 108 and 1012 or between about 1010 and 1011 CD4+ cells, and/or between about 108 and 1012 or between about 1010 and 1011 CD8+ cells.

In some embodiments, the engineered cells are administered at or within a tolerated range of a desired output ratio of multiple cell populations or sub-types, such as CD4+ and CD8+ cells or sub-types. In some aspects, the desired ratio can be a specific ratio or can be a range of ratios. for example, in some embodiments, the desired ratio (e.g., ratio of CD4+ to CD8+ cells) is between at or about 1:5 and at or about 5:1 (or greater than about 1:5 and less than about 5:1), or between at or about 1:3 and at or about 3:1 (or greater than about 1:3 and less than about 3:1), such as between at or about 2:1 and at or about 1:5 (or greater than about 1:5 and less than about 2:1, such as at or about 5:1, 4.5:1, 4:1, 3.5:1, 3:1, 2.5:1, 2:1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1:1, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9: 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, or 1:5. In some aspects, the tolerated difference is within about 1%, about 2%, about 3%, about 4% about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50% of the desired ratio, including any value in between these ranges. In some aspects, the TCR described here provides improved expression and activity, thereby providing therapeutic effects even at a low effector to target (E:T) ratio.

Optimal response to therapy can depend on the ability of the engineered recombinant receptors such as TCRs, to be consistently and reliably expressed on the surface of the engineered cells and/or bind the target antigen. For example, in some cases, properties of certain recombinant receptors, e.g., TCRs, can affect the expression and/or activity of the recombinant receptor, in some cases when expressed in a cell, such as a human T cell, used in cell therapy. In some contexts, the level of expression of particular recombinant receptors, e.g., TCRs, can be low, and activity of the engineered cells, such as human T cells, expressing such recombinant receptors, may be limited due to poor expression or poor signaling activity. In

some cases, consistency and/or efficiency of expression of the recombinant receptor, and activity of the receptor is limited in certain cells or certain cell populations of available therapeutic approaches. In some cases, a large number of engineered cells (e.g., engineered T cells) (a high effector to target (E:T) ratio) is required to exhibit functional activity. In some embodiments, the desired ratio (E:T ratio) is between at or about 1:10 and at or about 10:1 (or greater than about 1:10 and less than about 10:1), or between at or about 1:1 and at or about 10:1 (or greater than about 1:1 and less than about 5:1), such as between at or about 2:1 and at or about 10:1. In some embodiments, the E:T ratio is greater than or about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1.

For the prevention or treatment of disease, the appropriate dosage may depend on the type of disease to be treated, the type of cells or recombinant receptors, the severity and course of the disease, whether the engineered cells are administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the engineered cells, and the discretion of the attending physician. The compositions and cells are in some embodiments suitably administered to the subject at one time or over a series of treatments.

The engineered cells described herein can be administered by any suitable means, for example, by bolus infusion, by injection, e.g., intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjectval injection, subconjuntival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In some embodiments, a given dose is administered by a single bolus administration of the engineered cells. In some embodiments, it is administered by multiple bolus administrations of the engineered cells, for example, over a period of no more than 3 days, or by continuous infusion administration of the engineered cells.

In some embodiments, the engineered cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The engineered cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the engineered cells are

co- administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the engineered cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the engineered cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents includes a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

Following administration of the engineered cells, the biological activity of the engineered cell populations in some embodiments is measured, e.g., by any of a number of known methods. Parameters to assess include specific binding of engineered cells (e.g., engineered T cells) to the antigen, in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al. "Construction and pre-clinical evaluation of an anti-CD19 chimeric antigen receptor." Journal of immunotherapy (Hagerstown, Md.: 1997) 32.7 (2009): 689 and Hermans et al. "The VITAL assay: a versatile fluorometric technique for assessing CTL-and NKT-mediated cytotoxicity against multiple targets in vitro and in vivo." Journal of immunological methods 285.1 (2004): 25-40. In certain embodiments, the biological activity of the engineered cells is measured by assaying expression and/or secretion of one or more cytokines, such as CD107a, IFNγ, IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

EXAMPLES

In the following, in order to describe the inventions provided in the disclosure in greater detail, several examples are provided. Please note that these examples are for illustrating purpose only and shall not be interpreted to impose any limitation to the scope of the disclosure.

EXAMPLE 1

In this example, functional studies over two embodiments of the engineered TGFβ receptor system as provided above, i.e., "TGFβR1-IL9R-TGFβR2-IL2Rγ (TGFβR1/R2 TM)" (Embodiment 1; FIG. 2A) and "TGFβR1-IL9R-TGFβR2-IL2Rγ (IL9R/IL2Rγ TM)" (Embodiment 2; FIG. 2B), were performed and compared side by side. These two embodiments have exactly same configurations in aspects such as the signal peptides (SPs), extracellular domains (ECDs), intracellular domains (ICDs) for the two engineered TGFβ receptor chains, except that different transmembrane domains (TMs) are employed. To be more specific, in

Embodiment 1, the TMs from TGFβR1 and TGFβR2 are respectively employed for the two engineered TGFβ receptor chains, whereas in Embodiment 2, the TMs from IL9R and IL2Rγ are respectively employed for the engineered TGFβ receptor chains.

1.1 Evaluation of TGFβR1R2-IL9R(TR TM) and TGFβR1R2-IL9R(9R TM) transgenes expression in T cells and impacts on T cell proliferation and survival.

Briefly, the two constructs TGFβR1R2-IL9R(TR TM) and TGFβR1R2-IL9R(9R TM) for the two embodiments of the engineered TGFβ receptor system were retrovirally transduced to peripheral blood mononuclear cells (PBMCs), and the cell number and viability were counted every 2-3 days (FIGS. 3B and 3C). Day 3 after transduction, cell number was counted and 0.2 million cells were taken and stained with TGFβR2 antibody to assess the expression of the chimeric receptors (FIG. 3A). As shown by the figures, the two chimeric receptors, i.e., TGFβR1R2-IL9R(TR TM) and TGFβR1R2-IL9R(9R TM), were well expressed in PBMCs (FIG. 3A) and they apparently did not affect cell proliferation (FIG. 3B) or cell survival (FIG. 3C), and there was no apparent difference in terms of the cell expansion or survival for the PBMCs modified with the two engineered TGFβ receptors (i.e. "TR1/2-IL9R (TR TM) modified" and "TR1/2-IL9R (9R TM) modified" in the figures) or for the untransduced control cells (i.e. "Unmodified", or "UTD" in the figures).

1.2 Comparison of TGFβ-stimulated JAK-STAT activation in T cells modified with the two TGFβR1R2-IL9R receptors.

JAK-STAT is a major signal transduction pathway mediated by cytokine receptors, and IL9R has been established, upon binding of the cognate IL-9 ligand molecules, to activate several signal transducer and activator (STAT) proteins, i.e. STAT1, STAT3 and STAT5, causing their phosphorylation. In order to examine whether the engineered TGFβR1/2-IL9R chimerical receptor systems can activate phosphorylation of STATs (i.e. "pSTAT") upon binding of the cognate ligand TGFβ, and if so, to further examine whether there is any difference in the JAK-STAT activation in response to TGFβ between PBMCs modified with the two engineered TGFβ receptors, the following experiments were carried out.

Briefly, after stimulated with TGFβ1(10 ng/mL) for 30 minutes, the untransduced or unmodified ("UT") T cells and the two TGFβR1R2-IL9R modified T cells were fixed with 4% paraformaldehyde and permeabilized with 100% methanol, followed by pSTAT flow staining. pSTAT activation were analyzed by flow cytometry, and the mean fluorescent intensity (MFI) of antibody staining were used to quantify STAT activation.

As shown in FIGS. 4A-4C, the unmodified T cells showed no STAT1/3/5 signaling in response to TGFβ1 stimulation. However, there was a surprisingly big difference in the TGFβ-stimulated JAK-STAT activation between the two modified T cells. In the TR1/2-IL9R (9R TM) modified T cells stimulated with TGFβ1 for 30 min, the level of phosphorylated STAT1(pSTAT1), phosphorylated STAT3 (pSTAT3) and phosphorylated STAT5 (pSTAT5) are respectively ~1.44 fold (FIG. 4A), ~2.48 fold (FIG. 4B), and ~1.67 fold (FIG. 4C) the level of pSTAT1, pSTAT3 and pSTAT5 in the same cells when TGFβ stimulation was absent; and in surprising contrast, the TR1/2-IL9R (TR TM) modified T cells stimulated with TGFβ1 showed a much stronger pSTAT activation, with the level of pSTAT1, pSTAT3 and pSTAT5 in the TR1/2-IL9R (TR TM) modified T cells upon TGFβ stimulation for 30 min respectively ~1.85 fold (FIG. 4A), ~4.62 fold (FIG. 4B), and ~3.33 fold (FIG. 4C) the level of pSTAT1, pSTAT3 and pSTAT5 in the same cells when TGFβ stimulation was absent.

Thus this data indicate that TGFβ1 induces much stronger STAT1/3/5 signaling in the TR1/R2-IL9R(TR TM) modified T cells than in the TR1/R2-IL9R(9R TM) modified T cells.

1.3 Comparison of the proliferation capacity of T cells modified with the two TGFβR1R2-IL9R receptors in response to TGFβ.

Briefly, the unmodified or untransduced ("UT") and the TR1/R2-IL9R modified PBMCs were cultured with IL-2 (300 IU/mL) (FIGS. 5B and 5D) or without IL-2 (FIGS. 5A and 5C), and the cell numbers (TOP) and cell viability (Bottom) were counted at indicated time points for evaluation of the cell proliferation (FIGS. 5A and 5B) and survival (FIGS. 5C and 5D).

FIG. 5A and FIG. 5C show the comparison results where all three groups of the T cells were cultured in the absence of IL-2. As shown, when TGFβ1 was absent, both engineered TR1/R2-IL9R receptors could surprisingly inhibit the cell exhaustion (left panels of FIGS. 5A and 5C), with the cell number reducing to the original level (i.e. at Day 0) at Day 9 in the TR1/R2-IL9R (TR TM)-modified T cells, and at ~Day 11 in the TR1/R2-IL9R (9R TM)-modified T cells. In contrast, the cell number reduced to the original level at ~Day 5 in the unmodified control cells. When TGFβ1 was present, both the TR1/2-IL9R (TR TM)-modified T cells and the TR1/2-IL9R (9R TM)-modified T cells showed an enhanced proliferation capacity compared with the unmodified control cells, with the former showing a more pronounced enhancement than the latter (right panel of FIG. 5A). In addition, while the cell number reduced to the original level at ~Day 5 in the unmodified control cells, the cell number reduced to the original level at ~Day 9 in the TR1/R2-IL9R (9R TM)-modified T cells, and the

cell number did not even reduce to the original level at ~Day 12 in the TR1/R2-IL9R (TR TM)-modified T cells. Further in terms of the survivability, when IL-2 was absent, whereas there was no difference between the unmodified T cells and the TR1/2-IL9R (9R TM)-modified T cells, the TR1/2-IL9R (TR TM)-modified T cells surprisingly showed a significant better viability compared with the other two groups of T cells (right panel of FIG. 5C). Specifically, at Day 12 after IL-2 was withdrawn from the culture, the viability of both the unmodified T cells and the TR1/2-IL9R (9R TM)-modified T cells dropped to < ~45%, whereas the viability of the TR1/2-IL9R (TR TM)-modified T cells remained as high as ~70%. This data suggests that in the absence of IL-2, TGFβ stimulation allows the TR1/2-IL9R (TR TM)-modified T cells to have a surprisingly better persistence (better survivability and enhanced proliferation) compared with the TR1/2-IL9R (9R TM)-modified T cells.

FIG. 5B and FIG. 5D show the comparison results where all three groups of the T cells were cultured in the presence of IL-2. As shown in FIG. 5D, none of the two TR1/2-IL9R receptors seemed to show effects on the cell viability when IL-2 is present, regardless whether TGFβ1 was absent or present. As further shown in FIG. 5B, both TR1/2-IL9R receptors seemed to confer an enhanced proliferation capacity to the T cells modified thereby when TGFβ1 is present (right panel of FIG. 5B).

1.4 Comparison of the proliferation capacity of T cells modified with the two TGFβR1R2-IL9R receptors upon repeated antigen activation in response to TGFβ.

Briefly, the untransduced and TR1/R2-IL9R modified PBMC were co-cultured with fresh HeLa (anti-CD3) cells at ET ratio of 1:1 every 3-4 days (as shown by the repeated stimulation, "STI" at each time point) in the presence of IL-2 supply (100 IU/mL) (FIGS. 6B and 6D) or without IL-2 (FIGS. 6A and 6C). Cell numbers (FIGS. 6A and 6B) and cell viability (FIGS. 6C and 6D) were counted at indicated time points. Herein, the HeLa (anti-CD3) cells are engineered to express an anti-CD3 scFv-B7.1 fusion protein on surface thereof which can reportedly provoke potent T cell activation and cytotoxicity (Judith Leitner, et al., J Immunol Methods. 2010 Oct 31; 362(1-2): 131–141).

FIG. 6A and FIG. 6C show the comparison results where all three groups of the T cells were cultured in the absence of IL-2. As shown, when cultured in the presence of TGFβ1 and under repeated antigen stimulation, both the TR1/2-IL9R (TR TM)-modified T cells and the TR1/2-IL9R (9R TM)-modified T cells showed significantly better survival compared with unmodified T cells, with the former showing a slightly more pronounced effect (see right panel of FIG. 6C); as further shown in the right panel of FIG. 6A, whereas TGFβ1 substantially

suppressed the proliferation of the unmodified T cells, both the TR1/2-IL9R (TR TM)-modified T cells and the TR1/2-IL9R (9R TM)-modified T cells showed a significantly enhanced proliferation capacity, and it is noteworthy that the TR1/2-IL9R (TR TM) receptor conferred a significantly higher enhancement compared with the TR1/2-IL9R (9R TM) receptor (e.g. at Day 12, the cell expansion ratio of the TR1/2-IL9R (TR TM)-modified T cells was ~3 fold that of the TR1/2-IL9R (9R TM)-modified T cells).

As shown in FIG. 6B and FIG. 6D where all three groups of the T cells were cultured in the absence of IL-2, there as substantially no difference between the TR1/2-IL9R (TR TM)-modified T cells and the TR1/2-IL9R (9R TM)-modified T cells in terms of proliferation or survivability regardless whether TGFβ1 was absent or present.

The above data together indicate that the transmembrane domain (TM) has an important effect on the engineered TR1/R2-IL9R receptor. Compared with the TGFβR1R2-IL9R receptor with IL9R/IL2Rγ TMs, the TGFβR1R2-IL9R receptor with the TGFβR1 and TGFβR2 TMs demonstrates unexpectedly better capability in TGFβ-stimulated JAK-STAT activation (esp. STAT3 and STAT5), in conferring the T cells modified thereby to survive the cell exhaustion when TGFβ is present while IL-2 is absent, esp. when under repeated or persistent antigen stimulation.

EXAMPLE 2

In this example, futher functional studies are performed in tumor-infiltrating lymphocytes (TILs) over the Embodiment 1 of the engineered TGFβ receptor system, i.e. "TGFβR1-IL9R-TGFβR2-IL2Rγ (TGFβR1/R2 TM)" (illustrated in FIG. 2A).

2.1 Evaluation of TGFβR-IL9R transgene expression in TILs and impacts on proliferation and survival.

Briefly, the construct expressing the TGFβR1/2-IL9R chimeric receptor system was retrovirally transduced to TILs. After transduction, cell numbers at indicated time points (e.g. Day 1, Day 6, Day 8, Day 10, and Day 12, etc.) during the expansion stage were counted and 0.2 million cells were taken and stained with TGFβR2 antibody every few days, to assess the expression of the eneigneered TGFβ receptor. As shown in FIGS. 7A-7C, the engineered TGFβ receptor system (i.e. TGFβR1R2-IL9R) can be well expressed in TILs (see FIG. 7A), and do not seem to affect cell proliferation (see FIG. 7B) or survival (see FIG. 7C) when comparing the TGFβR1/2-IL9R modified cells with the unmodified control cells.

2.2 Evaluation of JAK-STAT activation in TGFβR-IL9R-modified TILs in response to TGFβ.

TILs were transduced with the TGFβR1/2-IL9R construct, and after stimulated with TGFβ1 (10 ng/mL) for 30 minutes, both unmodified and TGFβR1R2-IL9R modified cells were fixed with 4% paraformaldehyde and permeabilized with 100% methanol, followed by pSTAT flow staining. pSTAT activation was analyzed by flow cytometry, and the mean fluorescent intensity (MFI) of antibody staining were used for quantify STAT activation. As illustrated in FIG. 8, TGFβ1 is capable of inducing strong pSTAT3 (~1.38 fold) and pSTAT5 (~1.39 fold) signaling, yet relatively weak pSTAT1 (~1.11 fold) signaling in TGFβR1R2-IL9R modified TILs compared to unmodified TILs.

2.3 Evaluation of the proliferation capacity of TGFβR-IL9R-modified T cells in response to TGFβ.

To evaluate whether the engineered TGFβR1R2-IL9R system confers a proliferation capability to TILs modified thereby, the following experiments are performed. Briefly, the untransduced (i.e. unmodified) and TGFβR1R2-IL9R modified TILs (FIGS. 9A and 9B) were cultured in the presence of IL-2 (3000 IU/mL) (FIG. 9B) or in the absence of IL-2 (FIG. 9A), and then cell numbers were counted at indicated time points (Day 0, Day 3, Day 5, etc.).

As shown by the results in FIG. 9A where the TILs were cultured in the absence of IL-2, the cell numbers began droping/decreasing continuously in TILs starting from Day 3 post-transduction in general. In the absence of TGFβ1, there was substantially no difference in terms of the cell number reduction between the unmodified and TGFβR1R2-IL9R modified TILs; in the presence of TGFβ1, however, an apparently slower reduction was seen in the TGFβR1R2-IL9R modified TILs (FIG. 9B, right panel, from Day 3 to Day 12, only with the exception on Day 9) compared to the unmodified control cells, suggesting that in the absence of IL-2, TGFβ1 causes the TGFβR1R2-IL9R modified TILs to have a better persistence than the unmodified TILs.

As further shown by the results in FIG. 9B where the TILs were cultured in the presence of IL-2, the cell numbers continuously increased in TILs in general. As shown in FIG. 9B, although the TGFβR1R2-IL9R modified TILs showed a slightly slower expansion compared with the unmodified control cells in the absence of TGFβ1, a significantly enhanced expansion was observed in the TGFβR1R2-IL9R modified TILs compared to unmodified control cells (see right panel; e.g. at Day 12, the cell expansion rate is > ~2.5 fold higher in the modified cells compared to that in the unmodified cells) when TGFβ1 was present. More specifically,

the presence of TGFβ1 substantially inhibited the proliferation of the unmodified TILs starting from Day 7 (if compared to when the TGFβ1 is absent), while in sharp contrast, the presence of TGFβ1 substantially still stimulated the proliferation of the TGFβR1R2-IL9R modified TILs. As such, in the presence of IL-2, TGFβ1 causes the TGFβR1R2-IL9R modified TILs to have a significantly enhanced proliferation capacity than the unmodified T cells.

2.4 Evaluation of the proliferation capacity of TGFβR-IL9R-modified T cells upon repeated tumor challenge in response to TGFβ.

To further evaluate whether the engineered TGFβR1R2-IL9R system confers resistance to exhaustion of T cells under repeated or persisting antigen stimulation, the following experiments are performed. Briefly, the untransduced (i.e. unmodified) and TGFβR1R2-IL9R modified TILs were co-cultured with fresh HeLa (anti-CD3) cells at ET ratio of 1:1 every 3-4 days in the presence of IL-2 supply (1000 IU/mL) (FIG. 10B) or without IL-2 (FIG. 10A). Cell number were counted at indicated time points.

FIG. 10A shows the results of experiments performed on TILs unmodified or modified with the engineered TGFβR1R2-IL9R system cultured under repeated antigen stimulation (i.e. "STI") and in the absence of IL-2. When TGFβ1 was absent (see the left panel), both modified and unmodified TILs could hardly expand (although modified TILs showed a slightly better expansion). When TGFβ1 was present (see right panel), proliferation of the unmodified TILs cultured under repeated antigen stimulation and without IL-2 was both strongly inhibited, while in contrast, TGFβ1 could strongly promote the proliferation of the TGFβR1R2-IL9R modified TILs.

FIG. 10B shows the results of experiments performed on TILs unmodified or modified with the engineered TGFβR1R2-IL9R system cultured under repeated antigen stimulation (i.e. "STI") and in the presence of IL-2. When TGFβ1 was absent (see the left panel), such cultured TILs proliferated well and there was substantially no difference between the modified and the unmodified T cells. When TGFβ1 was present (see the right panel), whereas TGFβ1 strongly inhibited proliferation of unmodified TILs under such culturing condition, such inhibitory effects of TGFβ1 was remarkably alleviated or reduced in the TGFβR1R2-IL9R modified TILs.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit

the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

1. An engineered transforming growth factor beta (TGFβ) receptor chain, encoding a transmembrane polypeptide when expressed in an immune cell, wherein the transmembrane polypeptide comprises an extracellular portion and an intracellular portion, operably connected by a transmembrane portion, wherein:

the extracellular portion comprises an extracellular domain (ECD) of any one of TGFβR1 or TGFβR2, or a functional portion or a functional variant thereof; and

the intracellular portion comprises an intracellular domain (ICD) of any one of IL9R or IL2Rγ, or a functional portion or a functional variant thereof.

2. The engineered TGFβ receptor chain of claim 1, wherein the transmembrane portion comprises a transmembrane domain (TM) of any one of TGFβR1, TGFβR2, IL2Rγ, IL4R, IL9R, IL7R, IL15R, IL10R, IL21R, CD28, CTLA4, PD1, TIM3, TIGIT, CD4, CD8, or 4-1BB, etc., or a functional portion or a functional variant thereof.

3. The engineered TGFβ receptor chain of claim 1, wherein the transmembrane portion comprises a transmembrane domain (TM) of one of TGFβR1, TGFβR2, IL2Rγ or IL9R, or a functional portion or a functional variant thereof.

4. The engineered TGFβ receptor chain of any one of claims 1-3, wherein:

the extracellular portion comprises an ECD of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and

the intracellular portion comprises an ICD of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 3.

5. The engineered TGFβ receptor chain of claim 4, wherein the transmembrane portion comprises a transmembrane domain (TM) of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 5.

6. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 9.

7. The engineered TGFβ receptor chain of claim 4, wherein the transmembrane portion comprises a transmembrane domain (TM) of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 7.

8. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 10.

9. The engineered TGFβ receptor chain of any one of claims 1-3, wherein:

the extracellular portion comprises an ECD of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and

the intracellular portion comprises an ICD of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

10. The engineered TGFβ receptor chain of claim 7, wherein the transmembrane portion comprises a transmembrane domain (TM) of TGFβR1, comprising a sequence having at least 70% identity to SEQ ID NO: 5.

11. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 11.

12. The engineered TGFβ receptor chain of claim 7, wherein the transmembrane portion comprises a transmembrane domain (TM) of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 8.

13. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 12.

14. The engineered TGFβ receptor chain of claim 1, wherein:

the extracellular portion comprises an ECD of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and

the intracellular portion comprises an ICD of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 3.

15. The engineered TGFβ receptor chain of claim 10, wherein the transmembrane portion comprises a transmembrane domain (TM) of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 6.

16. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 13.

17. The engineered TGFβ receptor chain of claim 10, wherein the transmembrane portion comprises a transmembrane domain (TM) of IL9R, comprising a sequence having at least 70% identity to SEQ ID NO: 7.

18. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 14.

19. The engineered TGFβ receptor chain of claim 1, wherein:

the extracellular portion comprises an ECD of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and

the intracellular portion comprises an ICD of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

20. The engineered TGFβ receptor chain of claim 13, wherein the transmembrane portion comprises a transmembrane domain (TM) of TGFβR2, comprising a sequence having at least 70% identity to SEQ ID NO: 6.

21. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 15.

22. The engineered TGFβ receptor chain of claim 13, wherein the transmembrane portion comprises a transmembrane domain (TM) of IL2Rγ, comprising a sequence having at least 70% identity to SEQ ID NO: 8.

23. The engineered TGFβ receptor chain of claim 5, comprising a sequence having at least 70% identity to SEQ ID NO: 16.

24. An engineered transforming growth factor beta (TGFβ) receptor system, comprising at least one engineered TGFβ receptor chain, each based on the engineered TGFβ receptor chain according to any one of claims 1-23.

25. The engineered TGFβ receptor system of claim 24, comprising a first engineered TGFβ receptor chain and a second engineered TGFβ receptor chain, wherein:

an extracellular portion of the first engineered TGFβ receptor chain and an extracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, and an ECD of TGFβR2 or a functional portion or a functional variant thereof, or vice versa; and

an intracellular portion of the first engineered TGFβ receptor chain and an intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ICD of IL9R or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, or vice versa.

26. The engineered TGFβ receptor system of claim 24 or claim 25, wherein:

the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprise:

an ECD of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and

an ICD of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 3;

and

the extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprise:

an ECD of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and

an ICD of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 4.

27. The engineered TGFβ receptor system of claim 26, wherein:

the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 5; and

the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 6.

28. The engineered TGFβ receptor system of claim 27, wherein:

the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 9; and

the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 15.

29. The engineered TGFβ receptor system of claim 26, wherein:

the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 7; and

the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 8.

30. The engineered TGFβ receptor system of claim 29, wherein:

the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 10; and

the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 16.

31. The engineered TGFβ receptor system of claim 24 or claim 25, wherein:

the extracellular portion and the intracellular portion of the first engineered TGFβ receptor chain respectively comprise:

an ECD of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 1; and

an ICD of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 4;

and

the extracellular portion and the intracellular portion of the second engineered TGFβ receptor chain respectively comprise:

an ECD of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 2; and

an ICD of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 3.

32. The engineered TGFβ receptor system of claim 31, wherein:

the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of TGFβR1 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 5; and

the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of TGFβR2 or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 6.

33. The engineered TGFβ receptor system of claim 32, wherein:

the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 11; and

the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 13.

34. The engineered TGFβ receptor system of claim 31, wherein:

the transmembrane portion of the first engineered TGFβ receptor chain comprises a TM of IL2Rγ or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 8; and

the transmembrane portion of the second engineered TGFβ receptor chain comprises a TM of IL9R or a functional portion or a functional variant thereof, comprising a sequence having at least 70% identity to SEQ ID NO: 7.

35. The engineered TGFβ receptor system of claim 34, wherein:

the first engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 12; and

the second engineered TGFβ receptor chain comprises a sequence having at least 70% identity to SEQ ID NO: 14.

36. The engineered TGFβ receptor system of any one of claims 24-35, wherein: when the engineered TGFβ receptor system is expressed in an immune cell, the immune cell stimulated with TGFβ exhibits an activation of the JAK-STAT signaling pathway compared to when TGFβ stimulation is absent.

37. The engineered TGFβ receptor system of claim 36, wherein when the engineered TGFβ receptor system is expressed in an immune cell, the immune cell stimulated with TGFβ exhibits increased phosphorylation of at least one of STAT1, STAT3 or STAT5 compared to when TGFβ stimulation is absent.

38. The engineered TGFβ receptor system of claim 37, wherein when the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 and/or STAT5 in the immune cell stimulated with TGFβ is at least 50% more than that when TGFβ stimulation is absent.

39. The engineered TGFβ receptor system of claim 38, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 and/or STAT5 in the immune cell stimulated with TGFβ is at least 2.5 fold that when TGFβ stimulation is absent.

40. The engineered TGFβ receptor system of claim 39, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when the engineered TGFβ receptor system is expressed in an immune cell, a phosphorylation level of STAT3 in the immune cell stimulated with TGFβ is at least 4.0 fold that when TGFβ stimulation is absent.

41. The engineered TGFβ receptor system of any one of claims 24-35, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the presence of IL-2, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

42. The engineered TGFβ receptor system of any one of claims 24-35, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a slower reduction in proliferation in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

43. The engineered TGFβ receptor system of any one of claims 24-35, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a better viability in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

44. The engineered TGFβ receptor system of claim 43, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2, the population of immune cells stimulated with TGFβ have a viability of at least 50% 12 days after IL-2 is withdrawn.

45. The engineered TGFβ receptor system of claim 44, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2,

the population of immune cells stimulated with TGFβ have a viability of at least 65% 12 days after IL-2 is withdrawn.

46. The engineered TGFβ receptor system of any one of claims 24-35, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

47. The engineered TGFβ receptor system of claim 46, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 300 fold.

48. The engineered TGFβ receptor system of claim 47, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when the engineered TGFβ receptor system is expressed in an immune cell cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 500 fold.

49. The engineered TGFβ receptor system of any one of claims 24-35, wherein when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2 and under repeated antigen stimulation, the population of immune cells stimulated with TGFβ exhibit an enhanced viability compared to when the engineered TGFβ receptor system is absent in the immune cell.

50. The engineered TGFβ receptor system of claim 49, wherein when the engineered TGFβ receptor system is expressed in a population of immune cells cultured in the absence of IL-2 and under repeated antigen stimulation, the population of immune cells stimulated with TGFβ have a viability of at least 70% 9 days after IL-2 is withdrawn.

51. A vector system, comprising at least one vector that encodes the engineered TGFβ receptor system according to any one of claims 24-50, wherein when the vector system is transduced in an immune cell, the immune cell is capable of activating JAK-STAT signaling upon TGFβ stimulation.

52. The vector system of claim 51, wherein the engineered TGFβ receptor system comprises a first engineered TGFβ receptor chain and a second engineered TGFβ receptor chain, wherein an extracellular portion of the first engineered TGFβ receptor chain and an extracellular portion of the second engineered TGFβ receptor chain respectively comprise an ECD of TGFβR1 or a functional portion or a functional variant thereof, and an ECD of TGFβR2 or a functional portion or a functional variant thereof, or vice versa; and an intracellular portion of the first engineered TGFβ receptor chain and an intracellular portion of the second engineered TGFβ receptor chain respectively comprise an ICD of IL9R or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, or vice versa.

53. The vector system of claim 52, wherein the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain in the engineered TGFβ receptor system are encoded in one vector.

54. The vector system of claim 53, wherein the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are encoded within one open reading frame (ORF) in the one vector.

55. The vector system of claim 54, wherein the ORF comprises a polynucleotide encoding a self-cleaving peptide, operably connecting a polynucleotide encoding the first engineered TGFβ receptor chain and a polynucleotide encoding the second engineered TGFβ receptor chain, wherein the self-cleaving peptide is selected from T2A, P2A, F2A, or E2A.

56. The vector system of claim 52, comprising two vectors, encoding the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain, respectively.

57. The vector system of any one of claims 52-56, wherein a first polynucleotide encoding a first signal peptide is operably connected to a polynucleotide encoding the first engineered TGFβ receptor chain, and a second polynucleotide encoding a second signal peptide is operably connected to a polynucleotide encoding the second engineered TGFβ receptor chain, wherein the first signal peptide and the extracellular portion of the first engineered TGFβ receptor chain are derived from a same protein, and the second signal peptide and the extracellular portion of the second engineered TGFβ receptor chain are derived from a same protein.

58. An immune cell, expressing the engineered TGFβ receptor system according to any one of claims 24-50.

59. The immune cell of claim 58, wherein: when stimulated with TGFβ, the immune cell exhibits an activation of the JAK-STAT signaling pathway compared to when TGFβ stimulation is absent.

60. The immune cell of claim 59, wherein when stimulated with TGFβ, the immune cell exhibits increased phosphorylation of at least one of STAT1, STAT3 or STAT5 compared to when TGFβ stimulation is absent.

61. The immune cell of claim 60, wherein when the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 and/or STAT5 in the immune cell is at least 50% more than that when TGFβ stimulation is absent.

62. The immune cell of claim 61, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 and/or STAT5 in the immune cell is at least 2.5 fold that when TGFβ stimulation is absent.

63. The immune cell of claim 62, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when the immune cell is stimulated with TGFβ, a phosphorylation level of STAT3 in the immune cell is at least 4.0 fold that when TGFβ stimulation is absent.

64. The immune cell of any one of claims 58-63, wherein when cultured in the presence of IL-2, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

65. The immune cell of any one of claims 58-63, wherein when cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a slower reduction in proliferation in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

66. The immune cell of any one of claims 58-63, wherein when cultured in the absence of IL-2, the immune cell stimulated with TGFβ exhibits a better viability in the presence of TGFβ1 compared to when the engineered TGFβ receptor system is absent in the immune cell.

67. The immune cell of claim 66, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when cultured in the absence of IL-2, a population of the immune cell stimulated with TGFβ has a viability of at least 50% 12 days after IL-2 is withdrawn.

68. The immune cell of claim 67, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of

TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when cultured in the absence of IL-2, a population of the immune cell stimulated with TGFβ has a viability of at least 65% 12 days after IL-2 is withdrawn.

69. The immune cell of any one of claims 58-63, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ exhibits an enhanced proliferation capability compared to when the engineered TGFβ receptor system is absent in the immune cell.

70. The immune cell of claim 69, wherein the extracellular portion and the transmembrane portion of any one of the first engineered TGFβ receptor chain and the second engineered TGFβ receptor chain are derived from a same protein, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 300 fold.

71. The immune cell of claim 70, wherein the extracellular portion, the transmembrane portion and the intracellular portion of the first engineered TGFβ receptor chain comprises an ECD of TGFβR1 or a functional portion or a functional variant thereof, a TM of TGFβR1 or a functional portion or a functional variant thereof, and an ICD of IL9R or a functional portion or a functional variant thereof; and the extracellular portion, the transmembrane portion and the intracellular portion of the second engineered TGFβ receptor chain comprises an ECD of TGFβR2 or a functional portion or a functional variant thereof, a TM of TGFβR2 or a functional portion or a functional variant thereof, and an ICD of IL2Rγ or a functional portion or a functional variant thereof, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, the immune cell stimulated with TGFβ expands by at least 500 fold.

72. The immune cell of any one of claims 58-63, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, a population of the immune cell stimulated with

TGFβ exhibit an enhanced viability compared to when the engineered TGFβ receptor system is absent in the immune cell.

73. The immune cell of claim 72, wherein when cultured in the absence of IL-2 and under repeated antigen stimulation, a population of the immune cells stimulated with TGFβ have a viability of at least 70% 9 days after IL-2 is withdrawn.

74. The immune cell of any one of claims 58-73, wherein the immune cell is a T cell, a natural killer (NK) cell, or a tumor infiltrating lymphocyte (TIL).

75. The immune cell of claim 74, wherein the immune cell further expresses a target-recognizing receptor capable of binding a target molecule on a target cell.

76. The immune cell of claim 75, wherein the target-recognizing receptor is selected from a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-TCR.

77. A method for treating a tumor in a patient in need thereof, comprising administering a population of the immune cell according to any one of claims 58-77.

78. The method of claim 77, wherein the immune cell further expresses a target-recognizing receptor capable of binding a target molecule on a target cancer cell within the tumor, wherein the target-recognizing receptor is selected from a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-TCR.

79. The method of claim 77 or claim 78, wherein the tumor is a solid tumor characterized to have a high level of TGFβ expression therewithin.

80. The method of claim 79, wherein the solid tumor is from liver cancer (HCC), breast cancer, prostate cancer, gliomas, bladder cancer, or thymic epithelial tumor.