

2018-0228842 A1) in their entireties. The contact of engineered T cells that express the orthogonal CD122 with a corresponding orthogonal ligand cognate for such orthogonal CD122 (“IL2 ortholog”) facilitates specific activation of such engineered T cells that express the orthogonal CD122. In particular this orthogonal IL2 receptor ligand complex provides for selective expansion of cells engineered to express the orthogonal receptor in a mixed population of cells, in particular a mixed population of T cells.

SUMMARY OF THE INVENTION

[0013] The present disclosure provides methods and compositions useful in the practice of adoptive cell therapy.

[0013a] In one aspect, provided herein is an orthogonal mammalian CAR-T cell, the cell comprising:

(A) a first nucleic acid sequence encoding a signal peptide and an orthogonal receptor, the orthogonal receptor comprising an extracellular domain, a transmembrane domain and intracellular domain, wherein:

(i) the extracellular domain of the orthogonal receptor polypeptide comprises a polypeptide of the formula:

Ala-Val-Asn-Gly-Thr-Ser-Gln-Phe-Thr-Cys-Phe-Tyr-
Asn-Ser-Arg-Ala-Asn-Ile-Ser-Cys-Val-Trp-Ser-Gln-
Asp-Gly-Ala-Leu-Gln-Asp-Thr-Ser-Cys-Gln-Val-His-
Ala-Trp-Pro-Asp-Arg-Arg-Arg-Trp-Asn-Gln-Thr-Cys-
Glu-Leu-Leu-Pro-Val-Ser-Gln-Ala-Ser-Trp-Ala-Cys-
Asn-Leu-Ile-Leu-Gly-Ala-Pro-Asp-Ser-AA70-Lys-Leu-
AA73-Thr-Val-Asp-Ile-Val-Thr-Leu-Arg-Val-Leu-Cys-
Arg-Glu-Gly-Val-Arg-Trp-Arg-Val-Met-Ala-Ile-Gln-
Asp-Phe-Lys-Pro-Phe-Glu-Asn-Leu-Arg-Leu-Met-Ala-
Pro-Ile-Ser-Leu-Gln-Val-Val-His-Val-Glu-Thr-His-Arg-
Cys-Asn-Ile-Ser-Trp-Glu-Ile-Ser-Gln-Ala-Ser-AA133-
AA134-Phe-Glu-Arg-His-Leu-Glu-Phe-Glu-Ala-Arg-
Thr-Leu-Ser-Pro-Gly-His-Thr-Trp-Glu-Glu-Ala-Pro-
Leu-Leu-Thr-Leu-Lys-Gln-Lys-Gln-Glu-Trp-Ile-Cys—
Leu-Glu-Thr-Leu-Thr-Pro-Asp-Thr-Gln-Tyr-Glu-Phe-
Gln-Val-Arg-Val-Lys-Pro-Leu-Gln-Gly-Glu-Phe-Thr-
Thr-Trp-Ser-Pro-Trp-Ser-Gln-Pro-Leu-Ala-Phe-Arg-
Thr-Lys-Pro-Ala-Ala-Leu-Gly-Lys-Asp-Thr

wherein:

AA70 = Gln or Tyr;

AA73 = Thr, Asp or Tyr;

AA133 = His, Asp, Glu or Lys; and/or

AA134 = Tyr, Phe, Glu or Arg;

and

(ii) the intracellular [and transmembrane] domain[s] of the receptor polypeptide is [are] selected from the group consisting of the [and transmembrane] domain[s] of human CD122, the intracellular [and transmembrane] domain[s] of human IL4 receptor Type II receptor subunit a (hIL4Ra) (SEQ ID NO:9), the intracellular [and transmembrane] domain[s] of human IL-7 receptor subunit a (hIL7Ra) (SEQ ID NO:11), the intracellular [and transmembrane] domain[s] of human IL9 receptor (hIL9R) (SEQ ID NO:13), and the human IL21 receptor (hIL21R) (SEQ ID NO:15); and

(B) a second nucleic acid sequence encoding a signal peptide and a chimeric antigen receptor (CAR), the CAR comprising an antigen binding domain (ABD), an optional hinge region, a transmembrane domain and an intracellular signaling domain (ISD), wherein:

(i) the antigen binding domain of the CAR is a polypeptide that specifically binds a tumor antigen selected from the group consisting of CD123, CD19, CD20, BCMA, CD22, CD30, CD70, GPC3, Lewis Y, GD3, GD3, mesothelin, ROR CD44, CD171, EGP2, EphA2, ErbB2, ErbB3/4, FAP, FAR IL11Ra, PSCA, PSMA, NCAM, HER2, NY-ESO-1, MUC1, CD123, FLT3, B7-H3, CD33, IL1RAP, CLL1 (CLEC12A), PSA, CEA, VEGF, VEGF-R2, CD22, ROR1, mesothelin, c-Met, Glycolipid F77, FAP, EGFRvIII, MAGE A3, 5T4, WT1, KG2D ligand, a folate receptor (FRa), and Wnt1 antigens; and,

(ii) the intracellular signaling domain of the CAR comprises a signaling domain selected from the group consisting of human CD3 ζ - chain, the Fc ϵ R1 γ chain, the Fc ϵ R1 β chains, the MB1 (Ig α) chain, the B29 (Ig β) chain, the CD3 δ polypeptide, the CD3 Δ polypeptide and CD3 ϵ polypeptide, and optionally further comprises a co-stimulatory domain selected from the group consisting of the CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 co-stimulatory domains.

[0013b] In one aspect, provided herein is a method of selectively activating an orthogonal mammalian CAR T cell comprising contacting the orthogonal mammalian CAR T cell of any of claims 1-23 with an hIL2 ortholog (hoIL2), the hoIL2 polypeptide having at least 95% identity with a polypeptide of the formula [1]:

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-(AA9)_i-T10-Q11-L12-(AA13)-(AA14)-(AA15)-(AA16)-L17-(AA18)-(AA19)-(AA20)-L21-(AA22)-(AA23)-I24-L25-N26-(AA27)-I28-N29-N30-Y31-K32-N33-P34-K35-

L36-T37-(AA38)-(AA39)-L40-T41-(AA42)-K43-F44-Y45-M46-P47-K48-K49-
A50-(AA51)-E52-L53-K54-(AA55)-L56-Q57-C58-L59-E60-E61-E62-L63-K64-
P65-L66-E67-E68-V69-L70-N71-L72-A73-(AA74)-S75-K76-N77-F78-H79- [1]
(AA80-(AA81)-P82-R83-D84-(AA85)-(AA86)-S87-N88-(AA89)-N90-(AA91)-
5 (AA92)-V93-L94-E95-L96-(AA97)-G98-S99-E100-T101-T102-F103-(AA104)-
C105-E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-E116-
F117-L118-N119-R120-W121-I122-T123-F124-(AA125)-(AA126)-S127-I128-
I129-(AA130)-T131-L132-T133

wherein:

- 10 AA1 is A (wild type) or deleted;
AA2 is P (wild type) or deleted;
AA3 is T (wild type), C, A, G, Q, E, N, D, R, K, P, or deleted;
AA4 is S (wild type) or deleted;
AA5 is S (wild type) or deleted;
- 15 AA6 is S (wild type) or deleted;
AA7 is T (wild type) or deleted;
AA8 is K (wild type) or deleted;
AA9 is K (wild type) or deleted;
AA13 is Q (wild type), W or deleted;
- 20 AA14 is L (wild type), M, W or deleted;
AA15 is E (wildtype), K, D, T, A, S, Q, H or deleted;
AA16 is H (wildtype), N or Q or deleted;
AA18 is L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;
AA19 is L (wildtype), A, V, I or deleted;
- 25 AA20 is D (wildtype), T, S M L, or deleted;;
AA22 is Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, F or
deleted;
AA23 is M (wild type), A,W,H,Y,F,Q, S, V, L, T, or deleted;
AA27 IS G (wildtype), K, S or deleted;
- 30 AA38 is R (wild type), W or G;
AA39 is M (wildtype), L or V;
AA42 is F (wildtype) or K;
AA51 is T (wildtype), I or deleted
AA55 is H (wildtype) or Y;
- 35 AA74 is Q (wild type), N, H, S;
AA80 is L (wild type), F or V;
AA81 is R (wild type), I, D, Y, T or deleted
AA85 is L (wild type) or V;
AA86 is I (wild type) or V;
- 40 AA88 is N (wildtype), E or Q or deleted;
AA89 is I (wild type) or V;
AA91 is V (wild type), R or K;
AA92 is I (wild type) or F;
AA97 is K (wild type) or Q;
- 45 AA104 is M (wild type) or A;
AA109 is D (wildtype), C or a non-natural amino acid with an activated side chain;
AA113 is T (wild type) or N;
AA125 is C (wild type), A or S;

AA126 is Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T; and
AA130 is S (wild type), T or R.

[0013c] In one aspect, provided herein is a cell product substantially enriched for a
population of activated orthogonal immune cells the product obtained by a process
5 comprising the steps of:

- (a) Isolating a quantity of immune cells from a mammalian subject;
- (b) Contacting said isolated quantity of isolated immune cells with a nucleic acid
sequence under conditions for the uptake of said nucleic acid sequence by the
isolated immune cells, said nucleic acid sequence encoding a transmembrane
10 receptor, said transmembrane receptor comprising an intracellular signaling
domain in operable communication with an extracellular domain, said
extracellular domain of said receptor comprising the ECD of a an orthogonal
hCD122 or a functional fragment thereof;
- (c) Contacting the isolated quantity of cells from step (b) ex vivo with a quantity
15 of a orthogonal ligand sufficient to induce proliferation of cells transduced by
the contacting of step (b), said contacting being applied for a period of time to
such that the transduced cells comprise at least 20% of the cells of the
population.

[0013d] In one aspect, provided herein is a human orthogonal CD19 CAR-T cell comprising,
20 the cell comprising:

(a) a first nucleic acid sequence encoding an orthogonal CD122 receptor
polypeptide, the nucleic acid sequence encoding a polypeptide of the amino acid sequence:

AVNGTSQFTCFYNSRANISCVWSQDQALQDTSCQVHAWPDRRRWNQTCELLPVS
25 QASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMA
PISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEAPLLTLKQ
KQEWICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDTIP
WLGHLVLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSOLSSEH
GGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLS
30 SNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSS
PQPLQPLSGEDDAYCTFSPSRDLLLLFSPSLGPGSPSTAPGGSGAGEERMPPS
LQERVPRDWDQPPLGPPTPGVPDLVDFQPPPELVLRAGEEVPDAGPREGVSEF
WSRPPGQGEFRALNARLPLNTDAYLSLQELQGDPTHLV (SEQ ID NO:
29); and

(b) a second nucleic acid sequence encoding a signal peptide and a CD19 CAR,
the nucleic acid sequence encoding a polypeptide of the amino acid sequence:

MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQDISKY
LNWYQQKPDGTVKLLIYHTSRHSGVPSRFSGSGSGTDYSLTISNLEQEDIATY
40 FCQQGNTLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPS
QSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRITI
IKDNSKSQVFLKMNSLQTDDAIYYCAKHHYYGGSYAMDYWGQTSVTVSSAAA
IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWVLVVVGGVLACYS
LLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSR
45 VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPFMGGKPRRKNPQ

wherein the first and second nucleic acid sequences are operably linked to one or more
5 expression control elements functional in a human immune cell such that the orthogonal
CD122 receptor polypeptide and the CD19 CAR are expressed by the cell and the
extracellular domains of the orthogonal CD122 receptor polypeptide and the CD19 CAR
presented are on the cell surface.

[0014] In some embodiments, the present disclosure provides orthogonal receptors. In some
10 embodiments, the orthogonal receptor is an orthogonal CD122. In some embodiments, the
orthogonal receptor is an orthogonal human CD122 (hCD122). In some embodiments, the
orthogonal receptor is an orthogonal CD122 comprising at least one STAT3 binding motifs.

[0015] In some embodiments, the present disclosure provides a recombinant vector
comprising a nucleic acid sequence encoding an orthogonal hCD122 receptor.

15 [0016] In some embodiments, the present disclosure provides a recombinant vector
comprising a nucleic acid sequence encoding an orthogonal hCD122 receptor and a CAR. In
some embodiments the CAR.

[0017] In some embodiments, the present disclosure provides recombinant mammalian
immune cell comprising a nucleic acid sequence encoding an orthogonal hCD122 receptor
20 and a nucleic acid sequence encoding a CAR.

[0018] In some embodiments, the present disclosure provides orthologs that are cognate
ligands for an orthogonal receptor. In some embodiments, the orthologs are IL2 orthologs.
In some embodiments, the IL2 orthologs are ligands for an orthogonal CD122 receptor. In
some embodiments, the IL2 orthologs are cognate ligands for a transmembrane receptor
25 protein comprising the extracellular domain of an orthogonal hCD122 receptor. In some
embodiments, the IL2 orthologs are cognate ligands for a transmembrane receptor protein
comprising the extracellular domain of an orthogonal hCD122 receptor comprising amino
acid substitutions at positions H133 and Y134. In some embodiments, the IL2 orthologs are
ligands for an orthogonal hCD122 comprising amino acid substitutions at positions H133 and
30 Y134. In some embodiments, the IL2 orthologs are ligands for an orthogonal hCD122
comprising the amino acid substitutions H133D and Y134F.

engineer the cell to include a “kill switch” or employ immunodepletive or immunosuppressive treatment regimens; a cell for response to a without hancing the proliferation of adoptively transferred cells in a mammalian subject following administration of the cell product without significant toxicity;

- 5 • compositions and methods that avoid the need for prior immunodepletion of the the subject prior to adoptive cell therapy;
- pharmaceutical formulations of orthogonal ligands, particularly orthogonal ligands having extended duration of action enabling less frequent dosing of the supportive orthogonal ligand;
- 10 • compositions and methods that provide demonstrable therapeutic anti-tumor efficacy superior to existing cell therapy agents for similar indications; and
- compositions and methods that when combined with supplementary therapeutic agents provide enhanced anti-tumor efficacy.

[0204] A series of experiments were performed to demonstrate the utility and functionality of the compositions and methods of the present disclosure, in particular in the treatment of a mammalian subject suffering from a neoplastic disease condition in a mammalian subject suffer orthogonal system in the context of adoptive cell therapy. The utility of the compositions and methods of the disclosure were evaluated in a series of experiments as more fully detailed in the attached examples and the data provided in the attached figures.

20 [0205] Briefly, a series of in vitro and in vivo experiments were conducted using a representative human IL2 ortholog of Formula 1, an hIL2 variant containing the set of amino acid substitutions: [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A], numbered in accordance with wt hIL2 and having the amino acid sequence:

25 PTSSSTKKTQLQLSPLLKAILNGINNYKNPKLTRM
 LTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK
 NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIV
 EFLNRWITFCQSIISTLT (SEQ ID NO: 23)

also sometimes referred to herein as “orthoIL2” and “hoIL2”.

[0206] To confirm the selective binding properties of STK-007, a Biacore surface plasmon resonance study was performed to confirm that STK-007 retains substantial binding to the CD25 (IL2Ra) and CD132 (IL2Rg) components of the IL2 receptor and exhibits specific binding to hoRb while wt hIL2 does not significantly bind to hoRb. Briefly, C-terminal HIS-

LVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYL
SLQELQGQDPHTLV (SEQ ID NO: 29)

also sometimes referred to herein as “hoCD122” or “hoRb” (human ortho receptor beta). The extracellular domain of the hoRb receptor comprises two amino acid substitutions H133D and Y134F (numbered in accordance with wt hCD122).

[0208] The exemplary CAR used in these studies is an anti-CD19 chimeric antigen receptor comprising the FMC63 anti-CD19 scFv as the antigen binding domain, the CD28 transmembrane and co-stimulatory domain and the CD3z domain (Figure 2, Panel A) and having the amino acid sequence:

10 MLLLVTSLLLCELPHPAFLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWY
QQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
PYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIIKDNSKSQVFLKMNSL
15 QTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTII
HVKGKHLCPSPLPFGPSKPFVWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDY
MNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 30)

[0209] A nucleic acid sequence was synthesized encoding: the CD19_28z CAR, a T2A peptide and the hoRb sequence

20 MLLLVTSLLLCELPHPAFLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWY
QQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
PYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIIKDNSKSQVFLKMNSL
25 QTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTII
HVKGKHLCPSPLPFGPSKPFVWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDY
MNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPGSGEGRGSLTTCGDVEENPGMAAPALSWRLPLL
30 ILLLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWN
QTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFEN
LRLMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEAPLLTLK
QKQEWICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDTIPWLG
HLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKW
35 LSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLSCTFN
QGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYC
TFPSRDDLLLFSPSLLGGPSPSTAPGGSGAGEERMPPSLQERVPRDWDQPPLGPPTP
GVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA
YLSLQELQGQDPHTLV (SEQ ID NO: 31)

5 QGTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFWVLVV
 VGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDF
 AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
 PP (SEQ ID NO: 32)

[0237] CD19_{4-1bbz}: a construct comprising a CD8a receptor signal peptide, FMC63 scFv, CD8 hinge and transmembrane domain, a 4-1BB hinge and costimulatory domain and CD3 zeta:

10 MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
 QQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT
 LPYTFGGGKLEITGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVS
 LPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIIKDNSKSQVFLKMNSL
 QTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTPAPRPPTPAPTIASQPLSL
 RPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
 FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELN
 15 LGREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
 GKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 33)

[0238] In an alternative embodiment, the CD19 CAR comprises the amino acid sequence:

20 MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
 YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQG
 NTLPTYTFGGGKLEITGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVS
 GVSLLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIIKDNSKSQVFLK
 MNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTPAPRPPTPAPTIAS
 QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR
 KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQN
 25 QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
 GMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 33)

[0239] In an alternative embodiment, the CD19 CAR comprises the amino acid sequence:

30 MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
 YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN
 TLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTV
 SGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIIKDNSKSQVFLK
 MNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKS
 35 NGTIIHVKGKHLCPSPFLPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSR
 LLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
 ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
 RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 30)

BCMA CARs:

40 **[0240]** The present disclosure provides methods and compositions for treating a subject suffering from multiple myeloma by the administration of a plurality of engineered T cells

PFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 34)

which can be co-expressed with the ortho CD122 reeptor using a T2a linker with an amino acid sequence of:

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGRSLRLSCAASGFTFSNYAMSW
VRQAPGKGLGWVSGISRSAGENTYYADSVKGRFTISRDNKNTLYLQMNSLRDEDTAV
YYCARSPAHYYGGMDVWGQGTITVTVSSASGGGGSGGRASGGGGSDIVLTQSPGTLSL
SPGERATLSCRASQSISSSFLAWYQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGT
DFTLTISRLEPEDSAVYYCQQYHSSPSWTFGQGTKLEIKTTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMA
APALSWRLPLLLILLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDQALQDTSC
QVHAWPDRRRWNQTCCELLPVSQASWACNLILGAPDSQKLTIVDIVTLRVLCREGVRW
RVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLS
PGHTWEEAPLLTLKQKQEWICLETLPDQYEFQVRVKPLQGEFTTWSPWSQPLAFR
TKPAALGKDTIPWLGHLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSK
FFSQLSSEHGGDVQKWLSSPFPSSSPGGLAPEISPLEVLERDKVTQLLLQQDKVP
EPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPT
GSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPS
LQERVPRDWPQPLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFPWSR
PPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 35)

[0249] GSI5021_41bbz CAR:

MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSRLRLSCAASEHTFSSHVMGW
FRQAPGKERESVAVIGWRDISTSYADSVKGRFTISRDNKNTLYLQMNSLKPEDTAV
YYCAARRIDAADFDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQAGGSLRLSCAASG
RTFTMGWFRQAPGKEREFVAAISLSPTLAYYAESVKGRFTISRDNKNTVVLQMNSL
KPEDTALYYCAADRKSVMISIRPDYWGQGTQVTVSSSTSTTPAPRPPTPAPTIASQPL
SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQGGQNQLYNE
LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
RRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 36)

which can be co-expressed with the ortho CD122 reeptor using a T2a linker with an amino acid sequence of:

MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSRLRLSCAASEHTFSSHVMGWFRQAP
GKERESVAVIGWRDISTSYADSVKGRFTISRDNKNTLYLQMNSLKPEDTAVYYCAARRIDA
ADFDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQAGGSLRLSCAASGRTFTMGWFRQAPGKE
REFVAAISLSPTLAYYAESVKGRFTISRDNKNTVVLQMNSLKPEDTALYYCAADRKSVMIS
RPDYWGQGTQVTVSSSTSTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEG
GCELRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGS
LLTCGDVE

ENPGPMAAPALSWRLPLLILLLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDG
 ALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVL
 CREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASDFFERHLE
 FEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPTDQYEFQVRVKPLQGEFTTWSPW
 5 SQPLAFRTKPAALGKDTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKC
 NTPDP SKFFS QLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLL
 LQODKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDE
 GVAGAPTGSSPQPLQPLSGEDDAYCTFP SRDDLLLFSPSLLGGPSPSTAPGGSGAG
 EERMPPSLQERVPRDWDPPQLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREG
 10 VSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO:
 37)

[0250] B2121 BCMA_41bbz) CAR

15 MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHL
 IHWYQQKPGQPPTLLIQLASNVQTVPARFSGSGSRDTFLTIDPVEEDDVAVYYCL
 QSRTIPRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKIS
 CKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSAST
 20 AYLQINNLYEDTATYFCALDYSYAMDYWGQGTSTVTVSSAAATTTAPRPPTPAPT
 ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRG
 RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN
 QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
 MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 38)

25 which can be co-expressed with the ortho CD122 reeceptor using a T2a linker with an amino acid
 sequence of:

30 MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSRLRLSCAASEHTFSSHVMGW
 FRQAPGKERESVAVIGWRDISTSYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAV
 YYCAARRIDAADFDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQAGGSLRLSCAASG
 RTFTMGWFRQAPGKEREFVAAISLPTLAYYAESVKGRFTISRDNAKNTVVLQMNSL
 KPEDTALYYCAADRKSVMISIRPDYWGQGTQVTVSSSTSTTTPAPRPPTPAPT
 35 SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLL
 YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNE
 LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
 RRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLTTCGDVEENPGPMAAP
 ALSWRLPLLILLLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQV
 40 HAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
 MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPG
 HTWEEAPLLTLKQKQEWICLETLTPTDQYEFQVRVKPLQGEFTTWSPWSQPLAFRTK
 PAALGKDTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDP SKFF
 S QLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQODKVPEP
 ASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGS
 45 SPQPLQPLSGEDDAYCTFP SRDDLLLFSPSLLGGPSPSTAPGGSGAGEERMPPSLQ
 ERVPRDWDPPQLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFPWSRPP
 GQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 37)

[0251] BMCA10_41bbz CAR

50 MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFALS NHGMSW
 VRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNARNLTLYLQMNSLRPEDTAIY
 YCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGG

GSDIQLTQSPSSLSASVGDRTTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
 SGVPSRFSGSGSGTDFTLTISSLPEDFATYYCQQSYSTPYTFGQGTKVEIKTTTPA
 PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
 LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSA
 DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPFEMGGKPRRKNPQEGLYNELQKD
 KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
 NO: 39)

which can be co-expressed with the ortho CD122 receptor using a T2a linker with an amino acid sequence of:

MALPVTALLLP LALLLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFALS NHGMSW
 VRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSTNTLYLQMNSLRPEDTAIY
 YCSAHGSEDVWGQGTITVTVSSASGGGSGGRASGGGGSDIQLTQSPSSLSASVGD
 VTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI
 SSLQPEDFATYYCQQSYSTPYTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPE
 ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQ
 PFMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGR
 REEYDVLDKRRGRDPFEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKG
 HDGLYQGLSTATKDTYDALHMQALPPRSGEGRGSLLTCGDVEENPGPMAAPALSWR
 LPLLIILLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDGALQDTSQVHAWPD
 RRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQD
 FKFPEENLRMAPISLQVHVHETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEE
 APLLTLLKQKQEWICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALG
 KDTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDP SKFFS QLSS
 EHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSS
 NHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPL
 QPLSGEDDAYCTFPSRDDLLLFSPSLGGSPPSTAPGGSGAGEERMPPSLQERVPR
 DWDPQPLGPPTPGVPDLVDFQPPPELVLRAGEEVPDAGPREGVSPFWSRPPGQGEF
 RALNARLP LN TDAYLSLQELQGQDP THLV (SEQ ID NO: 40)

GD2 CARs

[0252] The present disclosure provides methods and compositions for treating a subject suffering from a neoplastic disease of neuroectodermal origin (including human neuroblastoma and melanoma) or high risk osteosarcoma by the administration of a plurality of engineered T cells expressing an orthogonal CD122 polypeptide and a chimeric antigen receptor (CAR) the extracellular domain of said CAR specifically binds to GD2 and the contemporaneous combination administration of orthogonal IL2 ligand of Formula 1 and the subsequent administration to said subject of a maintenance therapy comprising the periodic administration of an orthogonal IL2 ligand of Formula 1. The present disclosure provides methods and compositions for preventing the metastasis or relapse of a neoplastic disease of neuroectodermal origin (including human neuroblastoma and melanoma) by the administration of a plurality of engineered T cells expressing an orthogonal CD122 polypeptide and a chimeric antigen receptor (CAR) the extracellular domain of said CAR specifically binds to BCMA and the contemporaneous combination administration of

methods and compositions of the present disclosure enable the clinician to maintaining the CARs in an active state for a prolonged period of time enabling much greater exposure (“Area Under the Curve” or AUC) than conventional CAR T cell therapies and thus providing a means to treat cancers that have previously been resistant to CAR T therapy, particularly solid tumors that require prolonged exposure to the CAR for effective treatment and to achieve as extravasation and penetration into the solid tumor environment. Conventional non-orthogonal CAR T cells are unable to provide the duration of exposure required without excessive toxicity or multiple dosing of the CAR T cell.

[0257] The present disclosure provides a method of treating prostate cancer, an optionally preventing relapse or recurrence, the method comprising the administration of a therapeutically effective amount of an orthogonal PSMA CAR T cell in combination with an orthogonal ligand of Formula 1.

[0258] In some embodiments, the present disclosure provides an orthogonal PSMA CAR T cell. comprising a PSMA CAR is PSMA_28z: An anti-PSMA CAR comprising a CD8a signal peptide, a deimmunized J591 scFv, a AAA spacer, CD28 hinge/transmembrane/co-stimulatory domain and CD3zeta:

```
MALPVTALLLPLALLLHAARPD IQMTQSPSSSLSTSVGDRVTLTCKASQDVGTAVDW
YQKPGSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISLQPEDFADYYCQQY
NSYPLTFGPGTKVDIKGSTSGGGSGGGSGGGSEVQLVQSGPEVKKPGATVKISC
KTSGYTFTEYTIHWVKQAPGKGLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDT
AYMELSSLRSED TAVYYCAAGWNFDYWGGTLLTVSSAAAIEVMYPPPYLDNEKSN
GTIIHVKGKHLCPSPLPFGPSKPFVVLVVGGVLACYSLLVTVAFIIFWVRSKRSR
LLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 41)
```

which can be co-expressed with the ortho CD122 receptor using a T2a linker with an amino acid sequence of:

```
MALPVTALLLPLALLLHAARPD IQMTQSPSSSLSTSVGDRVTLTCKASQDVGTAVDWY
QKPGSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISLQPEDFADYYCQQYNS
YPLTFGPGTKVDIKGSTSGGGSGGGSGGGSEVQLVQSGPEVKKPGATVKISCKTS
GYTFTEYTIHWVKQAPGKGLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDTAYME
LSSLRSED TAVYYCAAGWNFDYWGGTLLTVSSAAAIEVMYPPPYLDNEKSNGTIIH
VKGKHLCPSPLPFGPSKPFVVLVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDY
MNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR
REEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
HDGLYQGLSTATKDTYDALHMQALPPRSGEGRGSLLTCGDVEENPGPMAAPALSWR
LPLILLPLATSWASAVNGTSQFTCFYNSRANISCVWSQDQALQDTSCQVHAWPD
RRRWNTCELLPVSQASWACNLILGAPDSQKLTTVDIVTL
```

RVLCREGVRWRVMAIQDFKPFENLRMAPISLQVVHVETHRCNISWEISQASDFFER
HLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLPDTQYEFQVRVKPLQGEFTTW
SPWSQPLAFRTKPAALGKDTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKV
LKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVT
QLLLQQDKVPEPASLSSNHSLSCTFTNQGYFFFHLPDALEIEACQVYFTYDPYSEED
PDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPPSRDDLLLFSPSLLGGPSPPSTAPGGS
GAGEERMPPSLQERVPRDWDPPQLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGP
REGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID
NO: 42)

[0259] In some embodiments, the PSMA CAR is PSMA_4-1BBz: a deimmunized J591 scfv;
CD8a signal peptide, CD8 hinge and transmembrane domain and 4-1bb costimulatory
domain and CD3z:

MALPVTALLPLALLLHAARPDQMTQSPSSSLSTSVGDRVTLTCKASQDVGTAVDWY
QQKPGSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISSLPEDFADYYCQQYNS
YPLTFPGTKVDIKGSTSGGGSGGGSGGGSSSEVQLVQSGPEVKKPGATVKISCKTS
GYTFTEYTIHWVKQAPGKLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDTAYME
LSSLRSED TAVYYCAAGWNFDYWGQGTLLTVSSTTTPAPRPPTPAPTIASQPLSLRP
EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEGGCEL RVKF SRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH
DGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 43)

which can be co-expressed with the ortho CD122 receptor using a T2a linker with an amino acid
sequence of:

MALPVTALLPLALLLHAARPDQMTQSPSSSLSTSVGDRVTLTCKASQDVGTAVDWY
QQKPGSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISSLPEDFADYYCQQYNS
YPLTFPGTKVDIKGSTSGGGSGGGSGGGSSSEVQLVQSGPEVKKPGATVKISCKTS
GYTFTEYTIHWVKQAPGKLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDTAYME
LSSLRSED TAVYYCAAGWNFDYWGQGTLLTVSSTTTPAPRPPTPAPTIASQPLSLRP
EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEGGCEL RVKF SRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH
DGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMAAPALSWRL
PLLI LLLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQD GALQDTSCQVHAWPDR
RRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF
KPFENLRMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEA
PLTLKQKQEWICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGK
DTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTDPDSKFFSQLSSE
HGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSN
HSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQ
PLSGEDDAYCTFPPSRDDLLLFSPSLLGGPSPPSTAPGGS GAGEERMPPSLQERVPRD
WDPPQLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSPWSRPPGQGEFR
ALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 44)

GPC3 CARs

[0260] In some embodiments, the present disclosure provides an orthogonal GPC3 CAR T cell. In some embodiments, the methods and compositions of the present disclosure are useful in the treatment of GPC3 expressing cancers including but not limited to liver cancer. Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths in the world. Glypican-3, a cell-surface glycoprotein, is overexpressed in HCC tissues but not in the healthy adult liver and as such provides a useful targeting domain for the ABD of the CAR. A variety of GPC targeting domains may be employed in the construction of a CAR which may be incorporated into an orthogonal cell for use in combination with a IL2 ortholog of Formula 1 for use in the treatment of liver cancer. Examples GPC3 CARs which may be used in the preparation of an orthogonal GPC3 CAR T cell include but are not limited to a CAR GPC3_28z having the amino acid sequence:

```
DVVMTQSPFLSLPVTGPGEPAISICRSSQSLVHSNANTYLHWYLQKPGQSPQLLIYKVS
NRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGTKLEIKRG
GGGSGGGGSGGGGSGVQLVQSGAEVKKPGASVKVSCKASGYTFDYEMHWVRQAPGQ
GLEWMGALDPKTDGTAYSQKFKGRVTLTADESTSTAYMELSSLRSEDTAVYYCTRFY
SYTYWGQGTTLTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDFWVLVVGGLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHY
QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKQHDGLYQGLSTATKD
TYDALHMQALPPR (SEQ ID NO: 45)
```

and GPC3_4-1BB having the amino acid sequence:

```
DVVMTQSPFLSLPVTGPGEPAISICRSSQSLVHSNANTYLHWYLQKPGQSPQLLIYKVS
NRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGTKLEIKRG
GGGSGGGGSGGGGSGVQLVQSGAEVKKPGASVKVSCKASGYTFDYEMHWVRQAPGQ
GLEWMGALDPKTDGTAYSQKFKGRVTLTADESTSTAYMELSSLRSEDTAVYYCTRFY
SYTYWGQGTTLTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDIYIWAPLAGTCGVLLLSLVITKGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP
EEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
PQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKQHDGLYQGLSTATKDTYDAL
HMQALPPR (SEQ ID NO: 46)
```

[0261] In some embodiments, the present disclosure provides an orthogonal orthogonal HPV-16 E6 TCR cell for use in the treatment of HPV related tumors. Examples of HPV-16 E6 CARs which may be incorporated into the an orthogonal cell of the present disclosure include but are not limited to a CAR having the sequence :

```
MALEHLLIIILWMQLTWVSGQQLNQSPQSMFIQEGEDVSMNCTSSSIFNTWLW
YKQDPGEGPVLLIALYKAGELTSNGRLTAQFGITRKDSFLNISASIPSDVGI
YFCAGHPSSNSGYALNFGKGTSLLVTPDIQNPEPAVYQLKDPRSQDSTLCFLF
TDFDSQINVPKTMESGTFITDKTVLDMKAMDSKNGAIAWSNQTSTFCQDIF
KETNATYPSSDVPCDATLTEKSFETDMNL
```

NFQNLSVMGLRILLKLVAGFNLLMTLRLWSSRAKRSGSGATNFSLLKQAGDV
 EENPGPMGTSLLCWVVLGFLGTDHTGAGVSQSPRYKVTKRGQDVALRCDPIS
 GHVSLYWYRQALGQGPEFLTYFNIEAQQDKSGLPNDRFSAERPEGSISTLTI
 QRTEQRDSAMYRCASSSQTGARTDTQYFGPGTRTLTVLEDLRNVTTPPKVSLFE
 PSKAEIANKQKATLVCLARGFFPDHVELSWWVNGKEVHSGVSTDPQAYKESN
 YSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDKWPEGSPKPVTONISA
 EAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTLVVMAMVKRK
 NS (SEQ ID NO: 47)

[0262] Or:

MAPGLLCWALLCLLGAGLVDAVGTQSPHTLIKTRGQQVTLRCSPKSGHDTVSWYQQA
 LGQGPQFIFQYEEEEERQGNFPDRFSGHQFPNYSSELNVNALLGDSALYLCASSL
 GWRGGRYNEQFFGPGTRTLTVLEDLRNVTTPPKVSLFEPSKAEIANKQKATLVCLARGF
 FPDHVELSWWVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQ
 VQFHGLSEEDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLG
 KATLYAVLVSTLVVMAMVKRKNSRAKRSGSGATNFSLLKQAGDVEENPGPMWGVLL
 YVSMKMGTTGQNIQPTTEMTATEGAIVQINCTYQTSGFNGLFWYQQHAGEAPTFLS
 YNVLDGLEEKGRFSSFLSRSGKYSYLLKELQMKDSASYLCASVDGNNRLAFGKGNQ
 VVVIPNIQNPEPAVYQLKDPQSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDM
 KAMDSKSNAGIAWSNQTSTFCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLFQ
 NLLVIVLRILLKLVAGFNLLMTLRLWSS (SEQ ID NO: 48)

GD2 CARs for Neuroblastoma

[0263] GD2 is highly expressed by almost all neuroblastomas, by most melanomas and retinoblastomas, and by many Ewing sarcomas and, to a more variable degree, by small cell lung cancer, gliomas, osteosarcomas, and soft tissue sarcomas. Consequently, GD2 was identified as a target for CAR T cell therapy and a variety of GD2 CARs are known in the art. However, persistence remains an issue in the treatment of GD2 expressing tumors. Consequently, the ability to extend the duration of action of orthogonal immune cells by the administration of an ortho ligand would be of significant benefit in the development of GD2 cell therapeutics. In some embodiments, the present invention provides an orthoGD2 targeted immune cell. In one embodiment, targeting domain of the orthogonal GD2 immune cell, e.g. orthogonal GD2 CAR-T cells, incorporates the 14g2a scFv which has the amino acid sequences:

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHRNGNTYLHWYLQKPGQSPKLLIHKVS
 NRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPPLTFGAGTKLELGG
 GSGGGGSGGGGSGGGGSEVQLLQSGPELEKPGASVMISCKASGSSFTGYNMNWVRQ
 NIGKSLEWIGAIDPYYGGTSYNQKFKGRATLTVDKSSSTAYMHLKSLTSEDSAVYYC
 VSGMEYWGQGTSTVTVSS (SEQ ID NO: 49)

Making Cell A More Homogenous Cell Product:

[0264] The present disclosure provide methods of preparing a cell product comprising an engineered immune cell species, wherein the engineered cell immune species is an immune cell that that has been recombinantly modified to express a receptor comprising the

embodiments, the orthologs of the present disclosure are hIL2 orthologs that are cognate ligands of a receptor comprising the extracellular domain of a hCD122 molecule comprising amino acid substitutions at position Y134F. In some embodiments, the orthologs of the present disclosure are hIL2 orthologs that are cognate ligands of a receptor comprising the extracellular domain of a hCD122 molecule comprising amino acid substitutions at position Y134F the ICD of which comprises one or more STAT3 binding motifs.

[0275] In some embodiments, the orthologs of the present disclosure are hIL2 orthologs that are cognate ligands of a receptor comprising the extracellular domain of a hCD122 molecule comprising amino acid substitutions at position Y134 the ICD of which comprises one or more STAT3 binding motifs. In some embodiments, the orthologs of the present disclosure are hIL2 orthologs that are cognate ligands of receptor comprising the extracellular domain of orthogonal human CD122 comprising the amino acid substitutions H133D and Y134F. In some embodiments, the orthologs of the present disclosure are hIL2 orthologs that are cognate ligands of and orthogonal human CD122 comprising the amino acid substitutions H133D and Y134F.

[0276] IL2 Orthologs (FORMULA #1): In one embodiment, the present disclosure provides an hIL2 ortholog, the amino acid sequence of which has at least 80%, 90%, 95%, 98%, 99% or 100% identity to polypeptide of the Formula #1 (SEQ ID NO: 50):

<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 4em; vertical-align: middle;">[</div> <div style="display: inline-block; vertical-align: middle; text-align: left;"> <p>(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-(AA9)_i-T10-Q11-L12-(AA13)-(AA14)-(AA15)-(AA16)-L17-(AA18)-(AA19)-(AA20)-L21-(AA22)-(AA23)-I24-L25-N26-(AA27)-I28-N29-N30-Y31-K32-N33-P34-K35-L36-T37-(AA38)-(AA39)-L40-T41-(AA42)-K43-F44-Y45-M46-P47-K48-K49-A50-(AA51)-E52-L53-K54-(AA55)-L56-Q57-C58-L59-E60-E61-E62-L63-K64-P65-L66-E67-E68-V69-L70-N71-L72-A73-(AA74)-S75-K76-N77-F78-H79-(AA80)-(AA81)-P82-R83-D84-(AA85)-(AA86)-S87-N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-E95-L96-(AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-E116-F117-L118-N119-R120-W121-I122-T123-F124-(AA125)-(AA126)-S127-I128-I129-(AA130)-T131-L132-T133</p> </div> <div style="font-size: 4em; vertical-align: middle;">]</div> </div>	[1]
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wherein:

AA1 is A (wild type) or deleted;

AA2 is P (wild type) or deleted;

AA3 is T (wild type), C, A, G, Q, E, N, D, R, K, P, or deleted;

AA4 is S (wild type) or deleted;

AA5 is S (wild type) or deleted;
 AA6 is S (wild type) or deleted;
 AA7 is T (wild type) or deleted;
 AA8 is K (wild type) or deleted;
 AA9 is K (wild type) or deleted;
 AA13 is Q (wild type), W or deleted;
 AA14 is L (wild type), M, W or deleted;
 AA15 is E (wildtype), K, D, T, A, S, Q, H or deleted;
 AA16 is H (wildtype), N or Q or deleted;
 AA18 is L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;
 AA19 is L (wildtype), A, V, I or deleted;;
 AA20 is D (wildtype), T, S M L, or deleted;;
 AA22 is Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, F or deleted;
 AA23 is M (wild type), A,W,H,Y,F,Q, S, V, L, T, or deleted;
 AA27 IS G (wildtype), K, S or deleted;
 AA38 is R (wild type), W or G;
 AA39 is M (wildtype), L or V;
 AA42 is F (wildtype) or K;
 AA51 is T (wildtype), I or deleted
 AA55 is H (wildtype) or Y;
 AA74 is Q (wild type), N, H, S;
 AA80 is L (wild type), F or V;
 AA81 is R (wild type), I, D, Y, T or deleted
 AA85 is L (wild type) or V;
 AA86 is I (wild type) or V;
 AA89 is I (wild type) or V;
 AA91 is V (wild type), R or K;
 AA92 is I (wild type) or F;
 AA97 is K (wild type) or Q;
 AA104 is M (wild type) or A;
 AA109 is D (wildtype), C or a non-natural amino acid with an activated side chain;
 AA113 is T (wild type) or N;

an IL2 ortholog polypeptide. As alluded to above, fusion proteins which comprise an albumin binding domain (ABD) polypeptide sequence and an hIL2 ortholog polypeptide can, for example, be achieved by genetic manipulation, such that the nucleic acid coding for HSA, or a fragment thereof, is joined to the nucleic acid coding for the one or more IL2 ortholog sequences. In some embodiments, the albumin-binding peptide comprises the amino acid sequence DICLPRWGCLW (SEQ ID NO: 22).

[0303] The IL2 ortholog polypeptide can also be conjugated to large, slowly metabolized macromolecules such as proteins; polysaccharides, such as sepharose, agarose, cellulose, or cellulose beads; polymeric amino acids such as polyglutamic acid, or polylysine; amino acid copolymers; inactivated virus particles; inactivated bacterial toxins such as toxoid from diphtheria, tetanus, cholera, or leukotoxin molecules; inactivated bacteria, dendritic cells, thyroglobulin; tetanus toxoid; Diphtheria toxoid; polyamino acids such as poly(D-lysine:D-glutamic acid); VP6 polypeptides of rotaviruses; influenza virus hemagglutinin, influenza virus nucleoprotein; Keyhole Limpet Hemocyanin (KLH); and hepatitis B virus core protein and surface antigen. Such conjugated forms, if desired, can be used to produce antibodies against a polypeptide of the present disclosure.

[0304] In some embodiments, the IL2 ortholog is conjugated (either chemically or as a fusion protein) with an XTEN which provides extended duration of action akin to PEGylation and may be produced as a recombinant fusion protein in *E. coli*. XTEN polymers suitable for use in conjunction with the IL2 orthologs of the present disclosure are provided in Podust, *et al.* (2016) “*Extension of in vivo half-life of biologically active molecules by XTEN protein polymers*”, J Controlled Release 240:52-66 and Haeckel et al. (2016) “*XTEN as Biological Alternative to PEGylation Allows Complete Expression of a Protease- Activatable Killin-Based Cytostatic*” PLOS ONE | DOI:10.1371/journal.pone.0157193 June 13, 2016. The XTEN polymer may fusion protein may incorporate a protease sensitive cleavage site between the XTEN polypeptide and the IL2 ortholog such as an MMP-2 cleavage site.

[0305] Additional candidate components and molecules for conjugation include those suitable for isolation or purification. Particular non-limiting examples include binding molecules, such as biotin (biotin-avidin specific binding pair), an antibody, a receptor, a ligand, a lectin, or molecules that comprise a solid support, including, for example, plastic or polystyrene beads, plates or beads, magnetic beads, test strips, and membranes.

[0306] In some embodiments, the IL-2 mutein also may be linked to additional therapeutic agents including therapeutic compounds such as anti-inflammatory compounds or antineoplastic agents, therapeutic antibodies (e.g. Herceptin), immune checkpoint

components and molecules. The linker molecules are generally about 6-50 atoms long. The linker molecules can also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Suitable linkers can be readily selected and can be of any suitable length, such as 1 amino acid (e.g., Gly), 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, 30-50 or more than 50 amino acids. Examples of flexible linkers include glycine polymers (G)_n, glycine-serine polymers, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Glycine and glycine-serine polymers are relatively unstructured, and therefore can serve as a neutral tether between components. Further examples of flexible linkers include glycine polymers (G)_n, glycine-alanine polymers, alanine-serine polymers, glycine-serine polymers. Glycine and glycine-serine polymers are relatively unstructured, and therefore may serve as a neutral tether between components. A multimer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, or 30-50) of these linker sequences may be linked together to provide flexible linkers that may be used to conjugate a heterologous amino acid sequence to the polypeptides disclosed herein.

[0322] Further, such linkers may be used to link the IL2 ortholog to additional heterologous polypeptide components as described herein, the heterologous amino acid sequence may be a signal sequence and/or a fusion partner, such as, albumin, Fc sequence, and the like.

[0323] In one embodiment of the disclosure, the IL2 ortholog is a human IL2 ortholog of the structure:



wherein n = 0 or 1, or



wherein n = 0 or 1, or

[0324] In another embodiment of the invention, the IL2 ortholog is a human IL2 ortholog of the structure

40kD-PEG-(linker)_n-PTSSSTKKTQLQLSOLLVLLKAILNGINNYKNPKLTRM
LTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
KGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 23)

wherein n = 0 or 1.

Acylation

reference. Particular transition metal chelating polypeptides useful in the practice of the present invention are peptides comprising 3-6 contiguous histidine residues (SEQ ID NO: 51) such as a six-histidine peptide (His)₆ (SEQ ID NO: 52) and are frequently referred to in the art as “His-tags.”

Targeted IL2 Ortholog Fusion Proteins:

[0338] In some embodiments, the IL2 ortholog is provided as a fusion protein with a polypeptide sequence (“targeting domain”) to facilitate selective binding to particular cell type or tissue expressing a cell surface molecule that specifically binds to such targeting domain, optionally incorporating a linker molecule of from 1-40 (alternatively 2-20, alternatively 5-20, alternatively 10-20) amino acids between the IL2 ortholog sequence and the sequence of the targeting domain of the fusion protein.

[0339] In other embodiments, a chimeric polypeptide including a orthogonal IL-2 and an antibody or antigen-binding portion thereof can be generated. The antibody or antigen-binding component of the chimeric protein can serve as a targeting moiety. For example, it can be used to localize the chimeric protein to a particular subset of cells or target molecule. Methods of generating cytokine-antibody chimeric polypeptides are described, for example, in U.S. Pat. No. 6,617,135.

[0340] In some embodiments, the targeting domain of the IL2 ortholog fusion protein specifically binds to a cell surface molecule of a tumor cell. In one embodiment wherein the ECD of the CAR of a CAR-T cell specifically binds to CD-19, the IL2 ortholog may be provided as a fusion protein with a CD-19 targeting moiety. For example, in one embodiment wherein the ECD of the CAR of a CAR-T cell is an scFv molecule that provides specific binding to CD-19, the IL2 ortholog is provided as a fusion protein with a CD-19 targeting moiety such as a single chain antibody (e.g., an scFv or VHH) that specifically binds to CD-19.

[0341] In some embodiments, the fusion protein comprises an IL-2 mutein and the anti-CD19 sdFv FMC63 (Nicholson, et al. (1997) Mol Immunol 34: 1157–1165). Similarly, in some embodiments wherein the ECD of the CAR of a CAR-T cell specifically binds to BCMA, the IL2 ortholog is provided as a fusion protein with a BCMA targeting moiety, such as antibody comprising the CDRs of anti-BCMA antibodies as described in Kalled, *et al.* (United States Patent 9,034,324 issued May 9, 2015) or antibodies comprising the CDRs as described in Brogdon, et al., (United States Patent No 10,174,095 issued January 8, 2019). In some embodiments the IL2 ortholog is provided as a fusion protein with a GD2 targeting moiety, such as an antibody comprising the CDRs of described in Cheung, et al., (United States

are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be encoded and most preferably that the wild type IL2 signal sequence be used. Alternatively, heterologous mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. When the recombinant host cell is a yeast cell such as *Saccharomyces cerevisiae*, the alpha mating factor secretion signal sequence may be employed to achieve extracellular secretion of the IL2 ortholog into the culture medium as described in Singh, United States Patent No. 7,198,919 B1 issued April 3, 2007.

[0359] In the event the IL2 ortholog to be expressed is to be expressed as a chimera (e.g., a fusion protein comprising an IL2 ortholog and a heterologous polypeptide sequence), the chimeric protein can be encoded by a hybrid nucleic acid molecule comprising a first sequence that encodes all or part of the IL2 ortholog and a second sequence that encodes all or part of the heterologous polypeptide. For example, subject IL2 orthologs described herein may be fused to a hexa-histidine tag (SEQ ID NO: 52) to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells. By first and second, it should not be understood as limiting to the orientation of the elements of the fusion protein and a heterologous polypeptide can be linked at either the N-terminus and/or C-terminus of the IL2 ortholog. For example, the N-terminus may be linked to a targeting domain and the C-terminus linked to a hexa-histidine tag (SEQ ID NO: 52) purification handle.

[0360] The complete amino acid sequence of the polypeptide (or fusion/chimera) to be expressed can be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for IL2 ortholog can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Codon Optimization:

[0361] In some embodiments, the nucleic acid sequence encoding the recombinant protein (IL2 ortholog, orthogonal CD122, or CAR) may be “codon optimized” to facilitate expression in a particular host cell type. Techniques for codon optimization in a wide variety of expression systems, including mammalian, yeast and bacterial host cells, are well known in the art and there are online tools to provide for a codon optimized sequences for expression in a variety of host cell types. See e.g. Hawash, et al., (2017) 9:46-53 and Mauro and Chappell in Recombinant Protein Expression in Mammalian Cells: Methods and Protocols, edited by

involved in the binding of the hIL2 to hCD122 include but are not limited to amino acids R41, R42, Q70, K71, T73, T74, V75, S132, H133, Y134, F135, E136, and/or Q214. In some embodiments, the orthogonal CD122 comprises a one or more substitutions or deletions of amino acids R41, R42, Q70, K71, T73, T74, V75, S132, H133, Y134, F135, E136, and/or Q214. In some embodiments, the orthogonal CD122 comprises one or more substitutions or deletions of the amino acids Q70, T73, H133, and/or Y134. In some embodiments, the orthogonal CD122 comprises one or more substitutions or deletions of the amino acids H133 and/or Y134. In some embodiments, the orthogonal CD122 comprises one or more substitutions or deletions of the amino acids H133 and/or Y134. In some embodiments, the orthogonal CD122 comprises one or more substitutions or deletions of the amino acids H133 and Y134.

[0394] A receptor polypeptide comprising an extracellular domain, a transmembrane domain and an intracellular domain, the extracellular domain of said polypeptide comprising an amino acid sequence of the following structure:

Ala-Val-Asn-Gly-Thr-Ser-Gln-Phe-Thr-Cys-Phe-Tyr—Asn-Ser-Arg-Ala-Asn-Ile-Ser-Cys-Val-Trp-Ser-Gln-Asp-Gly-Ala-Leu-Gln-Asp-Thr-Ser-Cys-Gln-Val-His-Ala-Trp-Pro-Asp-Arg-Arg-Arg-Trp-Asn-Gln-Thr-Cys-Glu-Leu-Leu-Pro-Val-Ser-Gln-Ala-Ser-Trp-Ala-Cys-Asn-Leu-Ile-Leu-Gly-Ala-Pro-Asp-Ser-AA70-Lys-Leu-AA73-Thr-Val-Asp-Ile-Val-Thr-Leu-Arg-Val-Leu-Cys-Arg-Glu-Gly-Val-Arg-Trp-Arg-Val-Met-Ala-Ile-Gln-Asp-Phe-Lys-Pro-Phe-Glu-Asn-Leu-Arg-Leu-Met-Ala-Pro-Ile-Ser-Leu-Gln-Val-Val-His-Val-Glu-Thr-His-Arg-Cys-Asn-Ile-Ser-Trp-Glu-Ile-Ser-Gln-Ala-Ser-AA133-AA134-Phe-Glu-Arg-His-Leu-Glu-Phe-Glu-Ala-Arg-Thr-Leu-Ser-Pro-Gly-His-Thr-Trp-Glu-Glu-Ala-Pro-Leu-Leu-Thr-Leu-Lys-Gln-Lys-Gln-Glu-Trp-Ile-Cys—Leu-Glu-Thr-Leu-Thr-Pro-Asp-Thr-Gln-Tyr-Glu-Phe-Gln-Val-Arg-Val-Lys-Pro-Leu-Gln-Gly-Glu-Phe-Thr-Thr-Trp-Ser-Pro-Trp-Ser-Gln-Pro-Leu-Ala-Phe-Arg-Thr-Lys-Pro-Ala-Ala-Leu-Gly-Lys-Asp-Thr (SEQ ID NO: 53)

wherein:

AA70 = Gln or Tyr;
AA73 = Thr, Asp or Tyr;
AA133 = His, Asp, Glu or Lys; and/or
AA134 = Tyr, Phe, Glu or Arg.

[0395] The ECD of the hCD122 receptor comprises several secondary structural features as summarized in the Table 3 below:

Table 3 Secondary Structural Features of hCD122 ECD		
Feature	Position In Precursor form of hCD122 (including 26AA signal sequence)	Position In Mature form of hCD122 (excluding signal sequence)

cyclin D1, and activating c-myc. Kallal, *et al, supra*. STAT3 can be activated through tyrosine phosphorylation by a variety of cytokines whose receptors share the gp130 chain, including IL-6 and IL21, oncostatin M (OSM) and leukemia inhibitory factor (LIF). STAT3 has roles in a variety of biological functions including oncogenesis, angiogenesis and tumor metastasis, and, anti-apoptosis. See e.g. Sun et al. (2006) FEBS Lett. 580(25):5880-4 and Fukada, *et al.* (1996) Immunity 5(5): 449-460. The incorporation of one or more functional STAT3 signaling motifs in the intracellular domain of the orthogonal CD122 upregulates anti-apoptotic factors in the modified cells expressing the orthogonal IL2 in response to binding of a cognate IL2 ortholog to the ECD of such STAT3 modified orthogonal CD122. Consequently, orthogonal cells which express an orthogonal CD122 comprising an intracellular domain incorporating one or more functional STAT3 domains have an enhanced survival and longer life and therefore a longer duration of action in vivo.

[0409] In some embodiments, the present disclosure provides a human orthogonal CD122 (comprising the intact STAT5 motif) has been modified to introduce one or more STAT3 binding motifs and the modified human CD122 so produced retains STAT5 recognition motif and gains one or more STAT3 binding motifs.

[0410] In some embodiments, the modified orthogonal CD122 may comprise one, two, three, or more STAT3 binding motifs. In some embodiments, the STAT3 recognition motif has an amino acid sequence of YX₁X₂Q (SEQ ID NO: 21). In some embodiments, X₁ is selected from the group consisting of L, R, F, M, and X₂ is selected from the group consisting of R, K, H, and P. In some embodiments, the STAT3 recognition motif has an amino acid sequence selected from the group consisting of: YLRQ (SEQ ID NO :24); YLKQ (SEQ ID NO: 25); YRHQ (SEQ ID NO: 26); YLRQ (SEQ ID NO: 24); YFKQ (SEQ ID NO: 28); YLPQ (SEQ ID NO: 16); YMPQ (SEQ ID NO: 17), and YDKPH (SEQ ID NO: 18).

[0411] In some embodiments, the one or more STAT3 binding motifs may be incorporated at the C-terminus of the orthogonal CD122 ICD or as an internal sequence of the ICD of the orthogonal CD122

[0412] In some cases, the modified human CD122 comprises one or more STAT3 binding motifs fused to the C-terminus of the intracellular domain of a human CD122. In some representative embodiments, the orthogonal CD122 comprises the addition of C-terminal STAT3 recognition domains resulting in orthogonal CD122 polypeptides of the structures:

Ortho-CD122-GGYLRQ ("GGYLRQ" disclosed as SEQ ID NO: 54);

Ortho-CD122-GGYLKQ ("GGYLKQ" disclosed as SEQ ID NO: 55);
Ortho-CD122-GGYRHQ ("GGYRHQ" disclosed as SEQ ID NO: 56);
Ortho-CD122-GGYLRQ ("GGYLRQ" disclosed as SEQ ID NO: 54);
Ortho-CD122-GGYFKQ ("GGYFKQ" disclosed as SEQ ID NO: 58);
Ortho-CD122-GGYLPQ ("GGYLPQ" disclosed as SEQ ID NO: 59);
Ortho-CD122-GGYMPQ ("GGYMPQ" disclosed as SEQ ID NO: 60); and
Ortho-CD122-GGYDKPH ("GGYDKPH" disclosed as SEQ ID NO: 61).

[0413] In some cases, the orthogonal CD122 comprises a STAT3 binding motif that is connected to the human CD122 through a linker. Linkers can be derived from naturally-occurring proteins or synthetic sequences. Methods for designing linkers are well-known in the art, for example, as disclosed in Chen et al. (2013) *Adv. Drug. Deliv. Rev.* 65(10):1357–1369, the relevant portion thereof is herein incorporated by reference. Suitable linkers can be readily selected and can be of any suitable length, such as 1 amino acid (e.g., Gly), 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, or 20-30 amino acids. Examples of flexible linkers include glycine polymers (G)_n, glycine-serine polymers, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Glycine and glycine-serine polymers are relatively unstructured, and therefore can serve as a neutral tether between components. Further examples of flexible linkers include glycine polymers (G)_n, glycine-alanine polymers, alanine-serine polymers, glycine-serine polymers. Glycine and glycine-serine polymers are relatively unstructured, and therefore may serve as a neutral tether between components. The modified orthogonal CD122 may comprise one or more STAT3 binding motifs and one or more linker sequences. Said linker sequences may connect the human CD122 and one of the STAT3 binding motifs or connect individual STAT3 binding motifs. The one or more linker sequence may have the same or different sequences.

[0414] In some embodiments, one or more STAT3 binding motifs are present as an internal (i.e., at neither C nor N terminus) sequence of the orthogonal CD122. A modified orthogonal CD122 of this configuration can be produced by identifying a suitable region within the orthogonal CD122 ICD amino acid sequence that can be mutated to create a STAT3 binding motif. In one embodiment, the naturally occurring ICD of the CD122 (which is typically present in the orthogonal CD122) possess sequences similar to the STAT3 binding motif that may readily be modified to create a STAT3 binding motif with minimal modification. For example a region comprising a four-nucleotide sequence that begins with a tyrosine residue. One such region in the native human CD122 encodes a sequence of YFTYDPYSEE (SEQ ID NO: 62), which is

located between position s355 and position 364 of the native human CD122 protein. In some embodiments, one or two of the YFTY (SEQ ID NO: 63), YDPY (SEQ ID NO: 64), or YSEE (SEQ ID NO: 65) comprised in this region are substituted with a STAT3 recognition motif to produce a modified human CD122 disclosed herein.

[0415] Such modified orthogonal CD122s are able to induce STAT3 and STAT5 signaling upon binding to a cognate IL2 ligand and the ability can be confirmed by e.g., monitoring the levels of phosphorylated STAT3 and STAT5 in response to contacting a cell expressing the orthogonal CD122 having the STAT modified ICD with a cognate IL2 ortholog. For example, the modified human CD122 can be introduced and expressed in T cells and antibodies that are specific to phospho-STAT5 and phosphor-STAT3 are used to detect the phosphorylation of STAT3 and STAT5. One exemplary method for detecting a recombinant protein's ability to induce STAT3 and STAT5 signaling is described in Kagoya et al. (2018) Nat Med. 24(3):352–359.

[0416] In some embodiments, this disclosure provides a method of stimulating an engineered cell expressing a modified human orthogonal CD122 comprising one or more STAT3 binding motifs, the method comprising contacting the engineered cell with a human IL2 ortholog which is a cognate ligand of the modified human orthogonal CD122 thereby stimulating the engineered cells. In some embodiments, this disclosure provides a method of increasing the intracellular levels of STAT3 and STAT5 in an engineered cell expressing a modified human orthogonal CD122 comprising one or more STAT3 binding motifs, the method comprising contacting the engineered cell with a human IL2 ortholog which is a cognate ligand of the modified human orthogonal CD122 such that the intracellular levels of STAT3 and STAT5 are increased in the engineered cell.

Method of Selective Activation of STAT5 STAT3

[0417] In some embodiments, this disclosure provides a method of stimulating an engineered T cell expressing a modified human orthogonal CD122 comprising one or more STAT3 binding motifs, the method comprising contacting the engineered T cell with a human IL2 ortholog which is a cognate ligand of the modified human orthogonal CD122 thereby stimulating the engineered T cells. In some embodiments, this disclosure provides a method of increasing the intracellular levels of STAT3 and STAT5 in an engineered T cell expressing a modified human orthogonal CD122 comprising one or more STAT3 binding motifs, the method comprising contacting the engineered T cell with a human IL2 ortholog which is a

[0493] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention.

Example 1. Generation of the human IL2 expression vector pcDNA3.1/hygro(+)-huIL2

[0494] The human IL2 DNA ORF (Genbank NM_000586.3) was synthesized (Life Technologies GeneArt Service, Carlsbad, CA), and amplified via PCR using Platinum SuperFi II DNA polymerase kit (item #12361050, ThermoFisher) following the manufacturer's protocol, and using primers:

5' TATAGTCAGCGCCACcCATGTACAGGATGCAACTCCTGTC 3' (SEQ ID NO: 66)

which incorporates and NheI restriction site, and

5' TATAGGGCCCTATCAAGTCAGTGTGAGATG 3' (SEQ ID NO: 67)

which incorporates an ApaI restriction site. The PCR fragment was visualized on a 1% agarose gel (item #54803, Lonza, Rockland, ME), excised from the gel and purified using a QIAquick PCR Purification kit (item #28106, Qiagen, Germany) according to the manufacturer's protocol.

[0495] The purified PCR fragment and mammalian expression vector pcDNA 3.1/Hygro(+) (#V87020, ThermoFisher) were digested with NheI and ApaI (#R0111S and #R0114L, New England Biolabs, Ipswich, MA) restriction enzymes. The expression vector was further treated with a Quick Dephosphorylation kit (#M0508L, New England Biolabs) according to the manufacturer's protocol. The PCR fragment was ligated into pcDNA 3.1/Hygro(+) using the Rapid DNA Ligation Kit (#11635379001, Sigma Aldrich, St. Louis, MO) following the manufacturer's protocol, transformed into One Shot TOP10 Chemically Competent E. coli (#C404006, Life Technologies, Carlsbad, CA), plated onto LB Agar plates containing 100ug/ml carbenicillin (#L1010, Teknova, Hollister, CA), and grown overnight at 37C.

[0496] The following day individual bacterial colonies were picked and used to start a 3ml bacterial culture in LB Broth (#10855-001, Life Technologies) with 100ug/ml ampicillin (#A9626, Teknova). The cultures were grown overnight at 37C.

[0497] The following day the E. coli were pelleted (6,000rpm, 10 minutes, tabletop centrifuge #5424, Eppendorf, Hauppauge, NY), and the DNA expression vector isolated using QIAprep Spin Miniprep Kit (#27106, Qiagen). The plasmid DNA was sequence verified (MCLab, South San Francisco, CA).

Example 2. Generation of the human IL2 ORTHO expression vector pcDNA3.1/hygro(+)-huIL2-ORTHO:

[0498] An expression vector which introduced six mutations into the human IL2 ORF (E35S, H36Q, L39V, D40L, Q42K and M43A; all numbering is based on the full length human IL2 ORF NM_000586.3 numbering) was assembled in substantial accordance with the teaching of Example 1 for the human IL2 expression vector in pcDNA3.1/Hygro(+), with the following exceptions: The initial template DNA used for PCR was synthesized with the E35S, H36Q, L39V, D40L, Q42K and M43A mutations.

Example 3. Introduction of mutations into pcDNA3.1/hygro(+)-huIL2 or back-mutations into pcDNA3.1/hygro(+)-huIL2 IRTHO expression vectors.

[0499] All mutations or back-mutations (reverting a mutation in *pcDNA3.1/hygro(+)-huIL2-ORTHO* back to match the wild type human IL2 ORF) were introduced into the *pcDNA3.1/Hygro(+)-huIL2* or *pcDNA3.1/Hygro(+)-huIL2-ORTHO* expression vectors using a Quik Change II Site Directed Mutagenesis Kit (#200524, Agilent Technologies, Santa Clara, CA) in substantial accordance with the manufacturer's protocol.

[0500] Table 5 lists the mutations generated, the template into which the mutation was introduced, and the primer sets used to introduce the mutation. The transformation of the Quik Change PCR reactions into *E. coli*, as well as the isolation and sequence analysis of the plasmid DNA, was performed using substantially the same protocol as in the generation of the pcDNA3.1/Hygro-huIL2 expression vector.

Table 7. QuikChange Mutagenesis and Sequence Information Regarding Mutations								
Peptide Name	35	36	39	40	42	43	*Templates	
Mature Peptide #	15	16	19	20	22	23	IL2: IL2 ORTHO:	pcDNA3.1/hygro(+)-huIL2 pcDNA3.1/Hygro(+)-huIL2 ORTHO
Wild Type human IL2 Residue	E	H	L	D	Q	M	Primer Set (5' → 3')	Template*
-QVLKA	E	Q	V	L	K	A	CAAAGAAAACACAGCTACAACTGGAGC AGTTACTGGTGCTCTTAAAGGC (SEQ ID NO: 68)	IL2 ORTHO

							GCCTTTAAGAGCACCAGTAACTGCTCCA GTTGTAGCTGTGTTTTCTTTG (SEQ ID NO: 69)	IL2 ORTHO
S-VLKA	S	H	V	L	K	A	CAGCTACAAGTGGAGCCATTTACTGGTGC TCTTAAA (SEQ ID NO: 70) TTTAAGAGCACCAGTAAATGGCTCAGTT GTAGCTG (SEQ ID NO: 71)	IL2 ORTHO
SQ-LKA	S	Q	L	L	K	A	CTGAGCCAGTTACTGCTGCTCTTAAAGG CC (SEQ ID NO: 72) GGCCTTTAAGAGCAGCAGTAACTGGCTC AG (SEQ ID NO: 73)	IL2 ORTHO
SQV-KA	S	Q	V	D	K	A	AACTGAGCCAGTTACTGGTGGATTTAAA GGCCATTTTGAATG (SEQ ID NO: 74) CATTCAAAATGGCCTTTAAATCCACCAG TAACTGGCTCAGTT (SEQ ID NO: 75)	IL2 ORTHO
SQVL-A	S	Q	V	L	Q	A	TGAGCCAGTTACTGGTGGCTCTTACAGGC CATTTTGA (SEQ ID NO: 76) TCAAAATGGCCTGTAAGAGCACCAGTA ACTGGCTCA (SEQ ID NO: 77)	IL2 ORTHO
SQVLK-	S	Q	V	L	K	M	CTGGTGGCTCTTAAAGATGATTTTGAATG GAATTAA (SEQ ID NO: 78) TTAATTCATTCAAAATCATCTTTAAGA GCACCAG (SEQ ID NO: 79)	IL2 ORTHO
S-----	S	H	L	D	Q	M	CACAGCTACAAGTGTGCGCATTTACTGCT GG (SEQ ID NO: 80) CCAGCAGTAAATGCGACAGTTGTAGCT GTG (SEQ ID NO: 81)	IL2
-Q-----	E	Q	L	D	Q	M	GCTACAAGTGGAGCAGTTACTGCTGGAT TTAC (SEQ ID NO: 82) GTAAATCCAGCAGTAACTGCTCCAGTTG TAGC (SEQ ID NO: 83)	IL2
--V---	E	H	V	D	Q	M	CTGGAGCATTTACTGGTGGATTTACAGA TGATTTTG (SEQ ID NO: 84) CAAAATCATCTGTAAATCCACCAGTAAA TGCTCCAG (SEQ ID NO: 85)	IL2
---L--	E	H	L	L	Q	M	GAGCATTTACTGCTGCTATTACAGATGA TTTTG (SEQ ID NO: 86) CAAAATCATCTGTAAATAGCAGCAGTAA ATGCTC (SEQ ID NO: 87)	IL2

---K-	E	H	L	D	K	M	CATTTACTGCTGGATTTAAAGATGATTT TGAATGG (SEQ ID NO: 88) CCATTCAAAATCATCTTTAAATCCAGCA GTAAATG (SEQ ID NO: 89)	IL2
----A	E	H	L	D	Q	A	CTGGAGCATTTACTGCTGGATTTACAGG CGATTTTGAATGGAATTAATAATTACA (SEQ ID NO: 90) TGTAATTATTAATTCCATTCAAAATCGC CTGTAAATCCAGCAGTAAATGCTCCAG (SEQ ID NO: 91)	IL2
SQ---	S	Q	L	D	Q	M	CAAAGAAAACACAGCTACAACTGAGCC AGTTACTGCTGGATTTACAGATG (SEQ ID NO: 92) CATCTGTAAATCCAGCAGTAACTGGCTC AGTTGTAGCTGTGTTTTCTTTG (SEQ ID NO: 93)	IL2
SQVL--	S	Q	V	L	Q	M	CCATTCAAAATCATCTGTAAGAGCACCA GTAAGTGGCTCAGTTGTAGCTG (SEQ ID NO: 27) CAACTGAGCCAGTTACTGGTGCTCTTAA AGGCCATTTTGAATGGAATTAATAATTA CAAG (SEQ ID NO: 57)	IL2

(Figure 2) were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0507] To compare the effect of each IL2 variant upon NKL cell and NKL hoRB cell proliferation, celltiterglo values for cells treated with the supernatants were compared to those obtained for control cells treated with growth medium alone, with 293T supernatant from empty-vector transfection, wild-type IL2 transfection, or supernatant from human orthogonal IL2 transfection. The data from these experiments is presented in Figures 2 and 3 of the accompanying drawings.

Example 7 Efficacy of orthoCAR T cells In Disseminated RAJI-luc lymphoma mouse model

[0508] The efficacy of orthogonal CAR-T cells in combination with a cognate orthogonal ligand was evaluated in a mouse leukemia model as follows. The CD19 chimeric antigen receptor protein used in this study (hereinafter referred to as “CD19_28z”) had the following structure: a GMCSF receptor signal peptide, the FMC63 anti-CD19 scFv, an AAA linker, a CD28 hinge/transmembrane/costimulatory domain and a CD3zeta. The amino acid sequence of the CD19_28z is as follows:

```
MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF
GGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYG
VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIY
YCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCP S
PLFPGPSKPFVWL VVVGGVLACYSLLVTVAFI IFWVRSKRSRL LHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDA
LHMQUALPPR (SEQ ID NO: 30)
```

[0509] The orthogonal T cells used in the conduct of this study were generated by techniques known in the art by transfecting isolated T cells with a lentiviral vector the foregoing CD19_28z CAR, T2A linker polypeptide and human orthogonal CD122 orthogonal receptor (hoCD122) having the amino acid sequence:

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MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF
GGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYG
VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIY
YCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCP S
PLFPGPSKPFVWL VVVGGVLACYSLLVTVAFI IFWVRSKRSRL LHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSR
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VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE
 LQKDKMAEAYSEIGMKGERRRRGKGHDLGLYQGLSTATKDTYDALHMQALPPGSGEGRGSL
 TCGDVEENPGPMAAPALSWRLPLLI LLLPLATSWASAAVNGTSQFTCFYNSRANISCVWS
 QDQALQDTSQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVL
 CREGVRWRVMAIQDFKPFENLRMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEA
 RTLSPGHTWEEAPLLTLKQKQEWICLETLTPTDQYEFQVRVKPLQGEFTTWSQPLAF
 RTKPAALGKDTIPWLGHLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDPSKFF
 SQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASL
 SSNHSLSLTCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQ
 PLSGEDDAYCTFP SRDDLLLFSPSLGGSPSPSTAPGGSGAGEERMPPSLQERVPRDWD
 PQLGPPTPGVPDLVDFQPPPELVLRAGEEVPDAGPREGVSPWSPRPPGQGEFRALNARL
 PLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 31)

allowing the use of a single lentiviral plasmid to generate the orthogonal CAR-T cells. The resulting orthogonal CAR-T cells were designated CD19_28z orthoCAR T cells also referred to SYNCAR-001.

[0510] Healthy donor primary blood mononuclear cells (PBMCs) were isolated from leukopaks via Ficoll-Paque separation (Global Life Sciences Solutions). CD4 and CD8 T cells were stained with CD4 and CD8 microbeads and isolated using MACS magnetic separation columns (Miltenyi Biotec). Cells were stimulated with anti-CD3 (clone OKT3, Miltenyi Biotec) and anti-CD28 antibody (clone CD28.2, BD Biosciences). 48h post-stimulation, cells were transduced with lentivirus and maintained in complete OpTmizer T cell media (Gibco) with wild type IL-2 (Miltenyi Biotec). 48 hours post-transduction, cells were washed and either maintained in WT IL-2 containing media or switched to STK-009 containing media. Cells were expanded and maintained at 1E6 cells/ml during the remainder of manufacturing. Cell counts and viability were performed using a Vi-Cell XR (Beckman Coulter). On day 14, cells were frozen down in CryoStor CS10 cell freezing medium (STEMCELL).

[0511] The orthogonal cognate ligand for the hoCD122 receptor used in these studies was a compound of the Formula 1 described as STK-009 described in the specification comprising a monopegylated 40kD branched (2x20kD) PEG molecule with an aldehyde linker a 40kDa 2-arm branched PEG-aldehyde the 40 kDa PEG-aldehyde comprising two 20kDa linear PEG molecules (Sunbright® GL2-400AL3, NOF America Corporation, One North Broadway, White Plains, NY 10601 USA).

[0512] Female NOD *scid* gamma (NSG) mice aged 6 to 8 weeks were obtained from The Jackson Laboratory, 600 Main Street, Bar Harbor, ME USA 04609). Prior to initiation of the

WHAT IS CLAIMED:

1. An orthogonal mammalian CAR-T cell, the cell comprising:

(A) a first nucleic acid sequence encoding a signal peptide and an orthogonal receptor, the orthogonal receptor comprising an extracellular domain, a transmembrane domain and intracellular domain, wherein:

(i) the extracellular domain of the orthogonal receptor polypeptide comprises a polypeptide of the formula:

Ala-Val-Asn-Gly-Thr-Ser-Gln-Phe-Thr-Cys-Phe-Tyr-Asn-Ser-Arg-Ala-Asn-Ile-Ser-Cys-Val-Trp-Ser-Gln-Asp-Gly-Ala-Leu-Gln-Asp-Thr-Ser-Cys-Gln-Val-His-Ala-Trp-Pro-Asp-Arg-Arg-Arg-Trp-Asn-Gln-Thr-Cys-Glu-Leu-Leu-Pro-Val-Ser-Gln-Ala-Ser-Trp-Ala-Cys-Asn-Leu-Ile-Leu-Gly-Ala-Pro-Asp-Ser-AA70-Lys-Leu-AA73-Thr-Val-Asp-Ile-Val-Thr-Leu-Arg-Val-Leu-Cys-Arg-Glu-Gly-Val-Arg-Trp-Arg-Val-Met-Ala-Ile-Gln-Asp-Phe-Lys-Pro-Phe-Glu-Asn-Leu-Arg-Leu-Met-Ala-Pro-Ile-Ser-Leu-Gln-Val-Val-His-Val-Glu-Thr-His-Arg-Cys-Asn-Ile-Ser-Trp-Glu-Ile-Ser-Gln-Ala-Ser-AA133-AA134-Phe-Glu-Arg-His-Leu-Glu-Phe-Glu-Ala-Arg-Thr-Leu-Ser-Pro-Gly-His-Thr-Trp-Glu-Glu-Ala-Pro-Leu-Leu-Thr-Leu-Lys-Gln-Lys-Gln-Glu-Trp-Ile-Cys—Leu-Glu-Thr-Leu-Thr-Pro-Asp-Thr-Gln-Tyr-Glu-Phe-Gln-Val-Arg-Val-Lys-Pro-Leu-Gln-Gly-Glu-Phe-Thr-Thr-Trp-Ser-Pro-Trp-Ser-Gln-Pro-Leu-Ala-Phe-Arg-Thr-Lys-Pro-Ala-Ala-Leu-Gly-Lys-Asp-Thr

wherein:

AA70 = Gln or Tyr;

AA73 = Thr, Asp or Tyr;

AA133 = His, Asp, Glu or Lys; and/or

AA134 = Tyr, Phe, Glu or Arg;

and

(ii) the intracellular [and transmembrane] domain[s] of the receptor polypeptide is [are] selected from the group consisting of the [and transmembrane] domain[s] of human CD122, the intracellular [and transmembrane] domain[s] of human IL4 receptor Type II receptor subunit a (hIL4Ra) (SEQ ID NO:9), the intracellular [and transmembrane] domain[s] of human IL-7 receptor subunit a (hIL7Ra) (SEQ ID NO:11), the intracellular [and transmembrane] domain[s] of human IL9 receptor (hIL9R) (SEQ ID NO:13), and the human IL21 receptor (hIL21R) (SEQ ID NO:15); and

(B) a second nucleic acid sequence encoding a signal peptide and a chimeric antigen receptor (CAR), the CAR comprising an antigen binding domain (ABD), an optional hinge region, a transmembrane domain and an intracellular signaling domain (ISD), wherein:

- (i) the antigen binding domain of the CAR is a polypeptide that specifically binds a tumor antigen selected from the group consisting of CD123, CD19, CD20, BCMA, CD22, CD30, CD70, GPC3, Lewis Y, GD3, GD3, mesothelin, ROR CD44, CD171, EGP2, EphA2, ErbB2, ErbB3/4, FAP, FAR IL11Ra, PSCA, PSMA, NCAM, HER2, NY-ESO-1, MUC1, CD123, FLT3, B7-H3, CD33, IL1RAP, CLL1 (CLEC12A), PSA, CEA, VEGF, VEGF-R2, CD22, ROR1, mesothelin, c-Met, Glycolipid F77, FAP, EGFRvIII, MAGE A3, 5T4, WT1, KG2D ligand, a folate receptor (FRa), and Wnt1 antigens; and,
- (ii) the intracellular signaling domain of the CAR comprises a signaling domain selected from the group consisting of human CD3 ζ -chain, the Fc ϵ R1 γ chain, the Fc ϵ R1 β chains, the MB1 (Ig α) chain, the B29 (Ig β) chain, the CD3 δ polypeptide, the CD3 Δ polypeptide and CD3 ϵ polypeptide, and optionally further comprises a co-stimulatory domain selected from the group consisting of the CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 co-stimulatory domains.

2. The orthogonal mammalian CAR-T cell of claim 1 wherein the extracellular domain of the receptor comprises the amino acid sequence:

```
AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVS
QASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRIMA
PISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEAPLLTLKQ
KQEWICLETLPDTQYEFQVRVKPLQGEFTTWSQPLAFRTKPAALGKDT
(SEQ ID NO:6)
```

3. The orthogonal mammalian CAR-T cell of claim 2 wherein the intracellular domain of the receptor is selected from the group consisting of the intracellular domain of human CD122, the intracellular domain of human IL4 receptor Type II receptor subunit a (hIL4Ra) (SEQ ID NO:9), the intracellular domain of human IL-7 receptor subunit a (hIL7Ra) (SEQ ID NO:11), the intracellular domain of human IL9 receptor (hIL9R) (SEQ ID NO:13), and the intracellular domain of human IL21 receptor (hIL21R) (SEQ ID NO:15).

4. The orthogonal mammalian CAR-T cell of claim 3 wherein the intracellular domain of the receptor is the intracellular domain of human CD122.

5. The orthogonal mammalian CAR-T cell of claim 4 wherein the receptor polypeptide is a polypeptide having the amino acid sequence:

AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVS
QASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMA
PISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEAPLLTLKQ
KQEWICLETLTPTDQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDTIP
WLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFS QLSSEH
GGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLS
SNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTSS
PQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPS
LQERVPRDWDQPPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFP
WSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTH (SEQ ID NO: 7).

6. The orthogonal mammalian CAR-T cell of claim 5 wherein the intracellular domain of the receptor further comprises at least one STAT3 signaling motif, the STAT3 signaling motif comprising a polypeptide of the formula YX_1X_2Q wherein: Y is the amino acid tyrosine, X_1 is selected from the group consisting of L, R, F, M, and X_2 is selected from the group consisting of R, K, H, and P.

7. The orthogonal mammalian CAR-T cell of claim 6 wherein the polypeptide of the formula YX_1X_2Q is a polypeptide selected from the group consisting of YLRQ (SEQ ID NO: 11); YLKQ (SEQ ID NO: 12); YRHQ (SEQ ID NO: 13); YLRQ (SEQ ID NO: 14); YFKQ (SEQ ID NO: 15); YLPQ (SEQ ID NO: 16); YMPQ (SEQ ID NO: 17), and YDKPH (SEQ ID NO: 18).

8. The orthogonal mammalian CAR-T cell of any one of claims 1-7 wherein the antigen binding domain selectively binds to CD19.

9. The orthogonal mammalian CAR-T cell of any one of claims 1-7 wherein the antigen binding domain of the CAR selectively binds to PSMA.

10. The orthogonal mammalian CAR-T cell of any one of claims 1-7 wherein the antigen binding domain of the CAR selectively binds to BCMA.

11. The orthogonal mammalian CAR-T cell of any one of claims 1-7 wherein the antigen binding domain of the CAR selectively binds to GPC3.

12. The orthogonal mammalian CAR-T cell of any one of claims 1-7 wherein the antigen binding domain of the CAR selectively binds to GD2.

13. The orthogonal mammalian CAR-T cell of claim 8 wherein the second nucleic acid sequence encodes a polypeptide consisting of a signal peptide and a CD19 CAR having a sequence selected from the group consisting of:

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLP
DYGVSWIRQPPRKGLEWLGVWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDD
TAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGK
HLCPSPLFPGPSKPFVWL VVGVLACYSLLVTVAFI IFWVRSKRSRLLHSDYMNMTPR
RPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLST
ATKDTYDALHMQALPP (SEQ ID NO: 32) ;

MALPVTALLLPLALLLHAARPD IQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGGTKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGV
SWIRQPPRKGLEWLGVWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIY
YCAKHYYYGGSYAMDYWGQTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT
QEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL
DKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATK
DTYDALHMQALPPR (SEQ ID NO: 33) ;

MALPVTALLLPLALLLHAARPD IQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGGTKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGV
SWIRQPPRKGLEWLGVWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIY
YCAKHYYYGGSYAMDYWGQTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT
QEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL
DKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATK
DTYDALHMQALPPR (SEQ ID NO: 33) ; and

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLP
DYGVSWIRQPPRKGLEWLGVWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDD
TAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGK
HLCPSPLFPGPSKPFVWL VVGVLACYSLLVTVAFI IFWVRSKRSRLLHSDYMNMTPR
RPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLST
ATKDTYDALHMQALPPR (SEQ ID NO: 30) .

14. The orthogonal mammalian CAR-T cell of claim 9 wherein the second nucleic acid sequence encodes a polypeptide consisting of a signal peptide and PSMA CAR, the polypeptide selected from the group consisting of:

MALPVTALLLPLALLLHAARPDIQMTQSPSSLSTSVGDRVTLTCKASQDVGTAVDWYQQ
KPGPSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISLQPEDFADYYCQQYNSYPLT
FGPGTKVDIKGSTSGGGSGGGSGGGGSSEVQLVQSGPEVKKPGATVKISCKTSGYTFTE
YTIHWVKQAPGKGLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDYAMELSSLRSED
TAVYYCAAGWNFDYWGGTLLTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP
LFPGPSKPFVWL VVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTY
DALHMQUALPPR (SEQ ID NO: **41**); and

MALPVTALLLPLALLLHAARPDIQMTQSPSSLSTSVGDRVTLTCKASQDVGTAVDWYQQ
KPGPSPKLLIYWASTRHTGIPSRFSGSGSGTDFTLTISLQPEDFADYYCQQYNSYPLT
FGPGTKVDIKGSTSGGGSGGGSGGGGSSEVQLVQSGPEVKKPGATVKISCKTSGYTFTE
YTIHWVKQAPGKGLEWIGNINPNNGGTTYNQKFEDKATLTVDKSTDYAMELSSLRSED
TAVYYCAAGWNFDYWGGTLLTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA
VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDG
CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYD
ALHMQUALPPR (SEQ ID NO: **43**).

15. The orthogonal mammalian CAR-T cell of claim 10 wherein the second nucleic acid sequence encodes a polypeptide consisting of a signal peptide and BCMA CAR, the polypeptide selected from the group consisting of:

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGRSRLRLSCAASGFTFSNYAMSWV
RQAPGKGLGWVSGISRSGENTYYADSVKGRFTISRDN SKNTLYLQMNSLRDEDTAVYY
CARSPAHYYGGMDVWGQGTITVTVSSASGGGGSGGRASGGGGSDIVLTQSPGTLISLSPG
ERATLSCRASQSISSSFLAWYQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGTDFTL
TISRLEPEDSAVYYCQQYHSSPSWTFGGGTGLEIKTTTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFK
QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGR
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGH
DGLYQGLSTATKDTYDALHMQUALPPR (SEQ ID NO: **34**);

MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSRLRLSCAASEHTFSSHVMGWFR
QAPGKERESVAVIGWRDISTSYADSVKGRFTISRDN AKKTLTYLQMNSLKPEDTAVYYCA
ARRIDAADFDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQAGGSLRLSCAASGRTFTMG
WFRQAPGKEREFVAAISLPTLAYYAESVKGRFTISRDN AKNTVVLQMNSLKPEDTALY
YCAADRKSVM SIRPDYWGGTQVTVSSTSTTTPAPRPPTPAPTIASQPLSLRPEACRPA
AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
TKDTYDALHMQUALPPR (SEQ ID NO: **36**);

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIH
WYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRT
IPRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGY
TFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNL
KYEDTATYFCALDYSYAMDYWGQTSVTVSSAAATTPAPRPPTPAPTIASQPLSLRPE
ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY
DVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQ
GLSTATKDTYDALHMQALPPR (SEQ ID NO: **38**); and

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFALSNGMSWVR
RAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSTNTLYLQMNSLRPEDTAIYYCSA
HGGESDVWGQGTITVTVSSASGGGGSGGRASGGGGSDIQLTQSPSSLSASVGDRVITTCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF
ATYYCQQSYSTPYTFGGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV
HTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE
DGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD
PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTY
DALHMQALPPR (SEQ ID NO: **39**).

16. The orthogonal mammalian CAR-T cell of claim 11 wherein the second nucleic acid sequence encodes a signal peptide and a GPC3 CAR, the GPC3 CAR selected from the group consisting of:

DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNANTYLHWYLQKPGQSPQLLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGTKLEIKRGGGGS
GGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAPGQGLEWMG
ALDPKTGDTAYSQKFKGRVTLTADESTSTAYMELSSLRSED TAVYYCTRFYSYTYWGQG
TLVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVV
GGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY
RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: **45**); and

DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNANTYLHWYLQKPGQSPQLLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGTKLEIKRGGGGS
GGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAPGQGLEWMG
ALDPKTGDTAYSQKFKGRVTLTADESTSTAYMELSSLRSED TAVYYCTRFYSYTYWGQG
TLVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNE
LQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
NO: **46**)

17. The orthogonal mammalian CAR-T cell of claim 12 wherein the second nucleic acid sequence encodes a signal peptide and a GD2 CAR, wherein the antigen binding domain of the GD2 CAR has the amino acid sequence:

DVVMQTPLSLPVSLGDQASISCRSSQSLVHRNGNTYLHWYLOKPGQSPKLLIHKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPPLTFGAGTKLELGSGSG
GGGSGGGSGGGGSEVQLLQSGPELEKPGASVMISCKASGSSFTGYNMNWVRQNIGKSL
EWIGAIDPYYGGTSYNQKFGRATLTVDKSSSTAYMHLKSLTSEDSAVYYCVSGMEYWG
QGTSVTVSS (SEQ ID NO: 49)

18. The orthogonal mammalian CAR-T cell of any one of claims 1-17 wherein the T cell is selected from naïve CD8⁺ T cells, cytotoxic CD8⁺ T cells, naïve CD4⁺ T cells, helper T cells, e.g. T_H1, T_H2, T_H9, T_H11, T_H22, T_{FH}; regulatory T cells, e.g. T_R1, Tregs, inducible Tregs; memory T cells, e.g. central memory T cells, effector memory T cells, NK cells, tumor infiltrating lymphocytes (TILs).

19. The orthogonal mammalian CAR-T cell of any one of claims 1-18 wherein the first and second nucleic acid sequences are encoded on a single vector or on separate vectors.

20. The orthogonal mammalian CAR-T cell of any one of claims 1-19 wherein the first and second nucleic acid sequences are encoded on a single vector and the first and second nucleic acid sequences are operably linked to a single expression control element.

21. The orthogonal mammalian CAR-T cell of claim 20 wherein the vector is a lentiviral vector or a retroviral vector.

22. The orthogonal mammalian CAR-T cell of claim 21 wherein the first and second nucleic acid sequences are linked via a T2A polypeptide.

23. The orthogonal mammalian CAR-T cell of claim 22 wherein the first and second nucleic acid sequences linked by the T2A polypeptide encode a polypeptide of the sequence:

MLLLVTSLLLCELPHPAFLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKP
DGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGT
KLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ
PPRKGLEWLGVWIGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYY
GGSYAMDYWGQTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLFPGPSKPF
WVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDF
AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGKPRRKNPQEG
LYNELQDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPGSGEGRGS
LLTCGDVEENPGPMAAPALSWRLPLLILLPLATSWASAAVNGTSQFTCFYNSRANISCVWS

QD GALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCR
 EGVRWRVMAIQDFKPFENLRMAPISLQVVHVETHRCNISWEISQASDFFERHLEFEARTLS
 PGHTWEEAPLLTLKQKQEWICLETLTPTDQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAA
 LGKDTIPWLGHLLVGLSGAFGFIIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFS QLSSEHG
 GDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLSLTSF
 TNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTF
 PSRDDLLLFSPSLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPPQPLGPPTPGVPDLV
 DFQPPPELVLRAGEEVPDAGPREGVSPFWSRPPGQGEFRALNARLP LNTDAYLSLQELQGG
 DPTHLV (SEQ ID NO: 31)

24. A method of selectively activating an orthogonal mammalian CAR T cell comprising contacting the orthogonal mammalian CAR T cell of any of claims 1-23 with an hIL2 ortholog (hoIL2), the hoIL2 polypeptide having at least 95% identity with a polypeptide of the formula [1]:

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-(AA9)_i-T10-Q11-L12-
 (AA13)-(AA14)-(AA15)-(AA16)-L17-(AA18)-(AA19)-(AA20)-L21-(AA22)-
 (AA23)-I24-L25-N26-(AA27)-I28-N29-N30-Y31-K32-N33-P34-K35-L36-T37-
 (AA38)-(AA39)-L40-T41-(AA42)-K43-F44-Y45-M46-P47-K48-K49-A50-(AA51)-
 E52-L53-K54-(AA55)-L56-Q57-C58-L59-E60-E61-E62-L63-K64-P65-L66-E67- [1]
 E68-V69-L70-N71-L72-A73-(AA74)-S75-K76-N77-F78-H79-(AA80)-(AA81)-P82-
 R83-D84-(AA85)-(AA86)-S87-N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-E95-
 L96-(AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-E106-Y107-A108-
 (AA109)-E110-T111-A112-(AA113)-I114-V115-E116-F117-L118-N119-R120-
 W121-I122-T123-F124-(AA125)-(AA126)-S127-I128-I129-(AA130)-T131-L132-
 T133

wherein:

AA1 is A (wild type) or deleted;
 AA2 is P (wild type) or deleted;
 AA3 is T (wild type), C, A, G, Q, E, N, D, R, K, P, or deleted;
 AA4 is S (wild type) or deleted;
 AA5 is S (wild type) or deleted;
 AA6 is S (wild type) or deleted;
 AA7 is T (wild type) or deleted;
 AA8 is K (wild type) or deleted;
 AA9 is K (wild type) or deleted;
 AA13 is Q (wild type), W or deleted;
 AA14 is L (wild type), M, W or deleted;
 AA15 is E (wildtype), K, D, T, A, S, Q, H or deleted;
 AA16 is H (wildtype), N or Q or deleted;
 AA18 is L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;
 AA19 is L (wildtype), A, V, I or deleted;

AA20 is D (wildtype), T, S M L, or deleted;;
 AA22 is Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, F or deleted;
 AA23 is M (wild type), A, W, H, Y, F, Q, S, V, L, T, or deleted;
 AA27 is G (wildtype), K, S or deleted;
 AA38 is R (wild type), W or G;
 AA39 is M (wildtype), L or V;
 AA42 is F (wildtype) or K;
 AA51 is T (wildtype), I or deleted
 AA55 is H (wildtype) or Y;
 AA74 is Q (wild type), N, H, S;
 AA80 is L (wild type), F or V;
 AA81 is R (wild type), I, D, Y, T or deleted
 AA85 is L (wild type) or V;
 AA86 is I (wild type) or V;
 AA88 is N (wildtype), E or Q or deleted;
 AA89 is I (wild type) or V;
 AA91 is V (wild type), R or K;
 AA92 is I (wild type) or F;
 AA97 is K (wild type) or Q;
 AA104 is M (wild type) or A;
 AA109 is D (wildtype), C or a non-natural amino acid with an activated side chain;
 AA113 is T (wild type) or N;
 AA125 is C (wild type), A or S;
 AA126 is Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T; and
 AA130 is S (wild type), T or R.

25. The method of claim 24 wherein the IL2 ortholog (hoIL2) is selected from the group of hIL2 orthologs having one of the following sets of amino acid modifications:

[E15D, H16N, L19V, D20L, Q22K, M23A];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [E15S-H16Q-L19V-D20L-Q22K-M23A L80F-R81D-L85V-I86V-I92F];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-L85V];
 [E15S-H16Q-L19V-D20L-M23A-Q126H];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-R38A-F42A-Y45A-E62A];
 [desAla1-E15S-H16Q-L19V-D20L];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-E15S-H16Q-L19V-D20L-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];

[desAla1-E15S-H16Q-L19V-D20L-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L- C125A-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125S-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S-Q126M]; and
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S-Q126M].
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L] ;
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L] ;
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-M23A]; and
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L].

26. The method of claim 25 wherein the hoIL2 is modified to extend the duration of action of the hoIL2 in vivo in a human subject.

27. The method of claim 26 wherein the IL2 ortholog is a human IL2 ortholog is a PEGylated hoIL2 of the structure:



wherein PEG is a linear or branched polyethylene glycol molecule having an average molecular weight from about 2,000 to about 80,000 daltons, alternatively about 2,000 to about 70,000 daltons, alternatively about 5,000 to about 50,000 daltons, alternatively about 10,000 to about 50,000 daltons, alternatively about 20,000 to about 50,000 daltons, alternatively about 30,000 to about 50,000 daltons, alternatively about 20,000 to about 40,000 daltons, alternatively about 30,000 to about 40,000; and $n = 0$ or 1 .

28. The method of claim 27 wherein the PEGylated hoIL2 ortholog is a compound of the formula:



wherein $n = 0$ or 1 .

29. The method of claim 28 wherein the IL2 ortholog is a human IL2 ortholog of the structure:

40kD-PEG-(linker)_n-PTSSSTKKTQLQLSQQLLVLLKAILNGINNYKNPKLT
RMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
KGSETTFMCEYADETATIVEFLNRWITFCQSIIISTLT (SEQUENCE ID: 23)

wherein 40kD-PEG is a branched polyethylene glycol having an average molecular weight of 40,000 daltons and $n = 1$.

30. A cell product substantially enriched for a population of activated orthogonal immune cells the product obtained by a process comprising the steps of:

- (a) Isolating a quantity of immune cells from a mammalian subject;
- (b) Contacting said isolated quantity of isolated immune cells with a nucleic acid sequence under conditions for the uptake of said nucleic acid sequence by the isolated immune cells, said nucleic acid sequence encoding a transmembrane receptor, said transmembrane receptor comprising an intracellular signaling domain in operable communication with an extracellular domain, said extracellular domain of said receptor comprising the ECD of a an orthogonal hCD122 or a functional fragment thereof;

- (c) Contacting the isolated quantity of cells from step (b) ex vivo with a quantity of a orthogonal ligand sufficient to induce proliferation of cells transduced by the contacting of step (b), said contacting being applied for a period of time to such that the transduced cells comprise at least 20% of the cells of the population.

31. A human orthogonal CD19 CAR-T cell comprising, the cell comprising:

- (a) a first nucleic acid sequence encoding an orthogonal CD122 receptor polypeptide, the nucleic acid sequence encoding a polypeptide of the amino acid sequence:

AVNGTSQFTCFYNSRANISCVWSQDQALQDTSCQVHAWPDRRRWNTCELLPVSQAS
WACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRMAPISLQV
VHVETHRCNISWEISQASDFFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLET
LTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDTIPWLGHLLVGLSGA
FGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSS
SFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLSCTFNQGYFFFH
LPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPPSRD
DLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPPQLGPPTPGVPDL
VDFQPPPELVLRAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSL
QELQGQDPHTLV (SEQ ID NO: 29); and

- (b) a second nucleic acid sequence encoding a signal peptide and a CD19 CAR, the nucleic acid sequence encoding a polypeptide of the amino acid sequence:

MLLLVTSLLLCELPHPAFLLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN
TLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTV
SGVSLPDYGVSWIRQPPRKGLEWLGVWGETTYNSALKSRLTIKDNSKSQVFLK
MNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAAEVVMYPPPYLDNEKS
NGTIIHVKGKHLCPSPFLFPGPSKPFVWLVVGGVLACYLLVTVAFIIFWVRSKRSR
LLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDRRGRDPGMPGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 30)

wherein the first and second nucleic acid sequences are operably linked to one or more expression control elements functional in a human immune cell such that the orthogonal CD122 receptor polypeptide and the CD19 CAR are expressed by the cell and the extracellular domains of the orthogonal CD122 receptor polypeptide and the CD19 CAR presented are on the cell surface.