

CLAIMS

1. A composition comprising two or more immune cells, each immune cell comprising:
 - a) a different chimeric antigen receptor (CAR) that targets an antigen selected from the group consisting of CD5 and CD7, wherein the immune cells comprise mutations that reduce or eliminate expression of the targeted antigens, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge , a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain; and
 - b) one or more mutations that reduce or eliminate the expression of an immunogenic polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1.
2. A composition comprising:
 - at least three immune cells, each comprising a different chimeric antigen receptor (CAR), wherein one chimeric antigen receptor targets CD3, one targets CD5, and a third targets CD7, wherein the cells further comprise mutations that reduce or eliminate the expression of the targeted antigens; and further comprise one or more mutations that reduce or eliminate the expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge , a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain; or
 - at least two immune cells, each comprising a different chimeric antigen receptor (CAR), wherein one chimeric antigen receptor targets CD3 and another targets CD5, wherein the cells further comprise mutations that reduce or eliminate the expression of the targeted antigens; and further comprise one or more mutations that reduce or eliminate the expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge , a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain.

3. The composition of claim 1 or 2, wherein the mutation is a C to T or A to G mutation that silences the gene or introduces a stop codon into the gene;
wherein the mutation introduces a premature stop codon or alters a splice donor or acceptor site;
wherein the mutation is generated by a base editor comprising a deaminase domain;
wherein the mutation is generated by a base editor comprising a deaminase domain that is a cytidine or an adenosine deaminase; and/or
wherein the mutation reduces expression of an encoded polypeptide by 50% or more relative to a corresponding control cell lacking the mutation.
4. The composition of any one of claims 1-3, wherein the immune cells are fratricide resistant;
wherein the immune cells have increased anti-neoplasia activity;
wherein the immune cells comprise no detectable translocations;
wherein the immune cells comprise less than 1% indels;
wherein the immune cell is a mammalian cell, a human cell, or a rodent cell; and/or
wherein the immune cell is a cytotoxic T cell, a regulatory T cell, a T helper cell, a dendritic cell, a B cell, a NK cell, or a progenitor thereof, or a hematopoietic stem cell.
5. A pharmaceutical composition comprising an effective amount of the composition of any one of claims 1-4 and a pharmaceutically acceptable excipient.
6. An *in vitro* or *ex vivo* method for producing a CAR-expressing immune cell or population of CAR-expressing immune cells having reduced immunogenicity, the method comprising:
a) contacting a target polynucleotide in an immune cell or population of immune cells with a base editor system comprising a guide polynucleotide and a base editor comprising a fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase domain or a base editor system comprising a guide polynucleotide and a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase domain;
b) introducing by nucleobase modification using the base editor system a mutation that reduces or eliminates the expression of a CD5 antigen;

c) introducing by nucleobase modification using the base editor system a mutation that reduces or eliminates the expression of at least one polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1 into the immune cells; and

d) expressing in the immune cell or population of immune cells a CAR that targets the CD5 antigen, thereby producing a population of CAR-expressing immune cells having reduced immunogenicity, wherein the CAR comprises a CD8a hinge, a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3ζ signaling domain.

7. The method of claim 6, wherein the CAR that targets the CD5 antigen comprises or consists of an amino acid sequence selected from the following:

a)

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1 MEFGLSWLFLVAILKGVQCIDAMGNIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNW 60
61 VKQAPGKGLRWMGWINTHTGEPTYADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFC 120
121 TRRGYDWYFDVWGAGTTVTVSSGGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVTITC 180
181 KASQDINSYLSWFHHKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLDYEDM 240
241 GIYYCQQYDESPWTFGGGKLEMGSGDPATTTTPAPRPPTPAPTIASQPLSLRPEACRPA 300
301 AGGAVHTRGLDFACDFWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPR 360
361 RGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK 420
421 RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTAT 480
481 KDTYDALHMQALPPR 495 (SEQ ID NO: 4); and
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b)

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1 MEFGLSWLFLVAILKGVQCIDAMGNIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNW 60
61 VKQAPGKGLRWMGWINTHTGEPTYADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFC 120
121 TRRGYDWYFDVWGAGTTVTVSSGGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVTITC 180
181 KASQDINSYLSWFHHKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLDYEDM 240
241 GIYYCQQYDESPWTFGGGKLEMGSGDPATTTTPAPRPPTPAPTIASQPLSLRPEACRPA 300
301 AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT 360
361 TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR 420
421 DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKD 480
481 DALHMQALPPRX 494 (SEQ ID NO: 7).
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8. The method of claim 6 or 7, wherein the immune cell produced by the method exhibits fratricide resistance and/or increased anti-neoplasia activity as compared to a corresponding control cell;
- wherein the immune cell produced by the method comprises no detectable translocations;
- wherein the immune cell produced by the method comprises less than 1% indels; and/or
- wherein the immune cell produced by the method comprises less than 5% of non-target edits or off-target edits.
9. The method of claim 6 or 7,
- wherein the mutation is in an exon;
- wherein the mutation results in a premature stop codon that reduces or eliminates protein expression;
- wherein the mutation is in a splice donor site or a splice acceptor site; or
- wherein the mutation reduces expression of an encoded polypeptide by at least 50% or more relative to a corresponding control cell lacking the mutation.
10. The method of any one of claims 6-9, wherein the deaminase is an adenosine or cytidine deaminase.
11. The method of claim 9, wherein the guide polynucleotide comprises a sequence selected from those provided at **Table 26**;
- wherein the guide polynucleotide comprises a nucleic acid sequence selected from AGCGACUGCAGAAAGAAGAG (SEQ ID NO: 1) or CAUACCAGCUGAGCCGUCCG (SEQ ID NO: 2);
- wherein each of the one or more guide nucleic acid sequences targets the napDNAbp to a *CD3e*, *CD5*, *FAS*, *LAG-3*, *CD52*, *TRAC*, *B2M*, *CIITA*, *TRBC1*, *TRBC2* and/or a *PDC1/PD-1* gene, or regulatory element; or
- wherein the base editor and one or more guide nucleic acid sequences are introduced into the immune cell via electroporation, nucleofection, cationic lipid-mediated methods, viral transduction, or a combination thereof.

12. The method of any one of claims 6-11, further comprising expanding the immune cell in culture to generate a population of immune cells;
wherein expression of the antigen or polypeptide in the population of immune cells is reduced in at least 50% of the population of immune cells; and/or
further comprising depleting TCR α/β ⁺ cells from the population of modified immune cells.
13. A CAR-expressing immune cell having reduced immunogenicity produced by the method of any one of claims 6-12.
14. A pharmaceutical composition comprising the immune cell of claim 13.
15. A pharmaceutical composition comprising an effective amount of two or more immune cells, each immune cell expressing a different chimeric antigen receptor that targets an antigen expressed by a neoplastic cell of a subject, selected from the group consisting of CD5 and CD7, wherein the immune cell comprises one or more mutations that reduce or eliminate expression of the antigen, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge, a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain, and wherein each of the immune cells further comprises one or more mutations that reduce or eliminate expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1, for use in treating a neoplasia in a subject.
16. Use of two or more immune cells, each immune cell expressing a different chimeric antigen receptor that targets an antigen expressed by a neoplastic cell of the a subject, selected from the group consisting of CD5 and CD7, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge, a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain, in the preparation of a medicament for treating a neoplasia in a subject, wherein the neoplasia is a T-cell acute lymphoblastic leukemia, an acute myelogenous leukemia, or a lymphoma, wherein the immune cell comprises one or more mutations that reduce or eliminate expression of the antigen, and each of the immune cells further comprises one or more

mutations that reduce or eliminate expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1.

17. The composition for use of claims 14 or the use of claim 16, wherein the subject is selected as having a neoplasia expressing an antigen selected from the group consisting of CD5 and CD7.

18. A pharmaceutical composition comprising three or more immune cells, each immune cell expressing a different chimeric antigen receptor that targets CD3, CD5, and CD7 antigens, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge, a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain, wherein the immune cells comprise one or more mutations that reduce or eliminate expression of the targeted antigen, and one or more mutations that reduce or eliminate expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1 for use in treating cancer in a selected subject, wherein the subject is selected by characterizing the cancer as expressing CD3, CD5, and CD7 antigens.

19. Use of three or more immune cells, each immune cell expressing a different chimeric antigen receptor that targets CD3, CD5, and CD7 antigens, wherein the CAR targeting the CD5 antigen comprises a CD8a hinge, a CD28 or CD8a transmembrane domain, a CD28 or 4-1BB secondary signaling domain, and a CD3 ζ signaling domain, in the preparation of a medicament for treating cancer in a selected subject, wherein the immune cells comprise one or more mutations that reduce or eliminate expression of the targeted antigen, and one or more mutations that reduce or eliminate expression of a polypeptide selected from the group consisting of CD3e, TRAC, LAG-3, FAS, CIITA, TRBC1, TRBC2, CD52, B2M, and PD1, and wherein the subject is selected by characterizing the cancer as expressing CD3, CD5, and CD7 antigens.

20. The pharmaceutical composition of claim 18 or the use of claim 19, wherein the cancer expressing CD3, CD5, and CD7 antigens is T-ALL.

21. The pharmaceutical composition for use of claim 18 or 20, or the use of claim 19 or 20, wherein the immune cells are cytotoxic T cells, regulatory T cells, T helper cells, dendritic cells, B cells, or NK cells;

wherein the subject has been previously treated with lymphodepletion;

which optionally comprises cyclophosphamide, fludarabine, and/or alemtuzumab (Cy/Flu/Campath) to be administered;

wherein the subject is refractory to chemotherapy or has a high tumor burden; or

wherein the subject is subsequently to be treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT).

22. The composition or pharmaceutical composition of any one of claims 1-5, 15, 17, 18, 20-21; the use of claims 16-17, 19-21; or the method of any one of claims 6-12, wherein the immune cell is derived from a single human donor;

wherein the immune cell is autologous to the subject;

wherein the immune cell is allogenic to the subject;

wherein the subject is a mammalian subject;

wherein the subject is a human or rodent subject; or

wherein the subject is a pediatric human subject.

23. A kit comprising the composition or the pharmaceutical composition of any one of claims 1-5, 15, 17, 18 or 20-21, for use in the treatment of cancer.