

What Is Claimed Is:

1. — A population of genetically engineered hematopoietic cells, comprising:

(i) — a first group of genetically engineered hematopoietic cells, which have genetic editing in a first gene encoding a first lineage-specific cell-surface antigen, wherein the first group of genetically engineered hematopoietic cells (a) have reduced or eliminated expression of the first lineage-specific cell-surface antigen or (b) express a mutant of the first lineage-specific cell-surface antigen; and

(ii) — a second group of genetically engineered hematopoietic cells, which have genetic editing in a second gene encoding a second lineage-specific cell-surface antigen, wherein the second group of genetically engineered hematopoietic cells (a) have reduced or eliminated expression of the second lineage-specific cell-surface antigen or (b) express a mutant of the second lineage-specific cell-surface antigen;

wherein the first group of genetically engineered hematopoietic cells overlaps with the second group of genetically engineered hematopoietic cells; and

wherein the first and second lineage-specific cell-surface antigens are selected from the group consisting of:

(i) — CD19 and CD33;

(ii) — CD33 and CD123; and

(iii) — CD33 and CLL-1.

2. — A population of genetically engineered hematopoietic cells, wherein one or more cells of the population:

(i) — have reduced or eliminated expression of a first lineage-specific cell-surface antigen relative to a wild-type counterpart cell, or express a mutant of the first lineage-specific cell-surface antigen, wherein the first lineage-specific cell-surface antigen is expressed in a primary cancer in a subject; and

(ii) ~~have reduced or eliminated expression of a second lineage-specific cell-surface antigen relative to a wild-type counterpart cell, or express a mutant of the second-lineage-specific cell-surface antigen, wherein the second lineage-specific cell-surface antigen is expressed in a relapsed cancer in the subject.~~

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3. ~~The population of genetically engineered hematopoietic cells of claim 2, wherein the first lineage-specific cell-surface antigen is CD19 and the second lineage-specific cell-surface antigen is CD33.~~

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4. ~~A population of genetically engineered hematopoietic cells, wherein one or more cells of the population:~~

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(i) ~~have reduced or eliminated expression of a first lineage-specific cell-surface antigen relative to a wild-type counterpart cell, or express a mutant of the first lineage-specific cell-surface antigen, wherein the first lineage-specific cell-surface antigen is expressed in a first sub-population of cancer cells in a subject; and~~

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(ii) ~~have reduced or eliminated expression of a second lineage-specific cell-surface antigen relative to a wild-type counterpart cell, or express a mutant of the second-lineage-specific cell-surface antigen, wherein the second lineage-specific cell-surface antigen is expressed in a second sub-population of cancer cells in the subject.~~

5. ~~The population of genetically engineered hematopoietic cells of claim 4, wherein the first lineage-specific cell-surface antigen is CD33 and the second lineage-specific cell-surface antigen is CD123 or CLL-1.~~

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6. ~~The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein at least 40% of copies of the gene encoding the first lineage-specific cell-surface antigen has genetic editing and at least 40% of copies of the gene~~

~~encoding the second lineage-specific cell surface antigen have genetic editing.~~

5 ~~7.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein surface levels of the first lineage-specific cell surface antigen in the population are less than 40% of surface levels of the first lineage-specific cell surface antigen in wild-type counterpart cells.~~

10 ~~8.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein surface levels of the second lineage-specific cell surface antigen in the population are less than 40% of surface levels of the second lineage-specific cell surface antigen in wild-type counterpart cells.~~

15 ~~9.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein one or both of: the genetic editing of the gene encoding the first lineage-specific cell surface antigen comprises a frameshift mutation, and the genetic editing of the second gene comprises a frameshift mutation.~~

20 ~~10.——The population of genetically engineered hematopoietic cells of any of the preceding claims, which are capable of growing in culture by at least 2-fold over 8 days.~~

25 ~~11.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein surface levels of the second lineage-specific cell surface antigen in the population are less than 50%, 40%, 30%, 20%, 10%, 5%, 2%, or 1% of surface levels of the second lineage-specific cell surface antigen in wild-type counterpart cells.~~

~~12.——The population of genetically engineered hematopoietic cells of any of the preceding claims, which are capable of engraftment~~

30 ~~13.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein the hematopoietic cells are hematopoietic stem cells (HSCs).~~

~~14.——The population of genetically engineered hematopoietic cells of any of the preceding claims, wherein the CD33 pseudogene is not modified in one or more (e.g., at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or all) of the cells of the population.~~

~~15.——A method of supplying hematopoietic cells to a subject (e.g., a subject having a hematopoietic malignancy), comprising:~~

~~(a) providing a population of genetically engineered hematopoietic cells of any one of claims 1-14; and~~

~~(b) administering the population of genetically engineered hematopoietic cells to the subject,~~

~~thereby supplying the hematopoietic cells to the subject.~~

~~16.——A method of treating a hematopoietic malignancy, comprising:~~

~~-administering to a subject in need thereof a population of genetically engineered hematopoietic cells of any one of claims 1-14;~~

~~administering to the subject an effective amount of a first immunotherapeutic agent that targets the first lineage-specific cell surface antigen, and~~

~~administering to the subject an effective amount of a second immunotherapeutic agent that targets the second lineage-specific cell surface antigen.~~

~~17.——The method of claim 16, wherein the first immunotherapeutic agent is administered when the subject has a primary cancer, and the second immunotherapeutic is administered when the subject has a relapsed cancer or cancer that is resistant to the first immunotherapeutic agent.~~

~~18.—The method of claim 16, wherein the subject has a cancer that comprises a first sub-population of cancer cells that express the first lineage-specific cell-surface antigen and a second sub-population of cancer cells that express the second lineage-specific cell-surface antigen.~~

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~~19.—The method of claim 18, wherein the first lineage-specific cell-surface antigen is CD33 and the second lineage-specific cell-surface antigen is CD123 or CLL-1.~~

Claims

1. A pharmaceutical composition comprising:
 - (a) a plurality of hematopoietic cells (HCs), wherein the plurality of HCs comprises
 - (i) a genetically engineered gene encoding CD33 that is engineered to have reduced or eliminated expression of CD33, and
 - (ii) a genetically engineered gene encoding CLL1 that is engineered to have reduced or eliminated expression of CLL1; and
 - (b) a therapeutically effective amount of at least one agent comprising an anti-CD33 binding domain or an anti-CLL1 binding domain.
2. The pharmaceutical composition according to claim 1 for use in therapy.
3. Use of the pharmaceutical composition according to claim 1 in the manufacture of a medicament for treating a hematopoietic malignancy in a subject.
4. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the at least one agent comprises an anti-CLL1 binding domain.
5. The pharmaceutical composition or the use according to claim 4, wherein the at least one agent comprises a T cell comprising a CAR comprising the anti-CLL1 binding domain, or an antibody or fragment thereof comprising the anti-CLL1 binding domain.
6. The pharmaceutical composition or the use according to claim 5, wherein the at least one agent further comprises an anti-CD33 binding domain.
7. The pharmaceutical composition or the use according to claim 6, wherein the at least one agent comprises a T cell comprising a CAR comprising the anti-CD33 binding domain, or an antibody or fragment thereof comprising the anti-CD33 binding domain.
8. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the genetically engineered gene encoding CLL1 or CD33 comprises a frameshift mutation.

9. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the genetically engineered HCs are genetically engineered using a CRISPR system comprising a guide nucleic acid.
10. The pharmaceutical composition or the use according to claim 9, wherein the guide nucleic acid comprises a sequence according to SEQ ID NO: 67 or a complement thereof.
11. The pharmaceutical composition or the use according to claim 9, wherein the guide nucleic acid comprises a sequence according to SEQ ID NO: 137 or a complement thereof.
12. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein exon 3 of the genetically engineered gene encoding CD33 is genetically engineered.
13. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein exon 4 of the genetically engineered gene encoding CLL1 is genetically engineered.
14. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the plurality of genetically engineered HCs retains the capacity to differentiate normally compared to a population of HCs that are not genetically engineered.
15. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein each cell of the plurality of genetically engineered HCs comprises the genetically engineered gene encoding CD33 and the genetically engineered gene encoding CLL1.
16. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the plurality of genetically engineered HCs comprises HCs with reduced or no binding to the anti-CD33 binding domain and reduced or no binding to the anti-CLL1 binding domain.

17. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the plurality of genetically engineered HCs is capable of expanding by at least 2-fold over 8 days in culture.
18. The use according to claim 3, wherein the plurality of HCs is obtained from a method comprising providing HCs from a biological sample from the subject, and genetically engineering the HCs from the biological sample from the subject, thereby forming the plurality of genetically engineered HCs.
19. The pharmaceutical composition according to claim 1 or claim 2, or the use according to claim 3, wherein the plurality of HCs comprises HCs obtained from a healthy donor, or descendants thereof.
20. A method of preparing a population of genetically engineered hematopoietic cells (HCs), the method comprising:
- (a) providing a population of HCs obtained from a human subject, wherein the population of HCs or descendants thereof express CD33 and CLL1; and
 - (b) genetically modifying the population of HCs such that
 - (i) a gene encoding CD33 is engineered to have reduced or eliminated expression of CD33; and
 - (ii) a gene encoding CLL1 is engineered to have reduced or eliminated expression of CLL1.