

CLAIMS

1. A pluripotent human stem cell, wherein the stem cell comprises:
 - (i) a genomic edit that results in loss of function of Cytokine Inducible SH2 Containing Protein (CISH) and
 - (ii) a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII), or a genomic edit that results in a loss of function of adenosine A2a receptor (ADORA2A).
2. The pluripotent human stem cell of claim 1, wherein the stem cell comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII) and a genomic edit that results in a loss of function of ADORA2A; and/or
wherein the stem cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.
3. A human induced pluripotent stem cell (iPSC), wherein the iPSC comprises a genomic edit that results in a loss of function of adenosine A2a receptor (ADORA2A).
4. The human iPSC of claim 3, wherein the iPSC comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII) or a genomic edit that results in loss of function of Cytokine Inducible SH2 Containing Protein (CISH), and optionally
wherein the iPSC comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII) and a genomic edit that results in loss of function of CISH; and/or
wherein the iPSC expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.
5. A pluripotent human stem cell, wherein the stem cell comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII); and optionally
further comprising a loss of function of an antagonist of interleukin signaling; and/or
wherein the stem cell further comprises a genomic modification that results in the loss of function of an antagonist of interleukin signaling; and/or
wherein the antagonist of interleukin signaling is an antagonist of the IL-15 signaling pathway and/or of the IL-2 signaling pathway; and/or

comprising a loss of function of Cytokine Inducible SH2 Containing Protein (CISH); and optionally

wherein the stem cell comprises a genomic modification that results in the loss of function of CISH; and/or

wherein the stem cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.

6. A differentiated cell,

(i) wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of any one of claims 1-2, or

(ii) wherein the differentiated cell is a daughter cell of the human iPSC of any one of claims 3-4, or

(iii) wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of claim 5.

7. The differentiated cell of claim 6, wherein the differentiated cell is an immune cell; or wherein the differentiated cell is a lymphocyte; or wherein the differentiated cell is a natural killer cell; or wherein the stem cell is a human induced pluripotent stem cell (iPSC), and wherein the differentiated daughter cell is an iNK cell.

8. The cell of any of one of the preceding claims, wherein the cell comprises one or more additional genomic edits.

9. The cell of any one of claims 1-4, claim 6 parts (i), and (ii), and claims 7-8, wherein: the genomic edit resulting in loss of function of CISH was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

the genomic edit resulting in loss of function of TGF β RII was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

the genomic edit resulting in loss of function of ADORA2A was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163; and/or wherein:

the genomic edit resulting in loss of function of CISH was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting

domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

the genomic edit resulting in loss of function of TGF β RII was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

the genomic edit resulting in loss of function of ADORA2A was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163.

10. A method of making the cell of any one of claims 1-4, and claim 6, parts(i) and (ii) and claims 7-9, the method comprising contacting the cell with one or more of:

(i) an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155 and 1162;

an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157 and 1161; and/or

an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163; or

(ii) a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159 and 1163.

11. The cell of claim 9 or the method of claim 10, wherein the RNA-guided nuclease is a Cas12a variant and optionally

wherein the Cas12a variant comprises one or more amino acid substitutions selected from M537R, F870L, and H800A; or

wherein the Cas12a variant comprises amino acid substitutions M537R, F870L, and H800A; or

wherein the Cas12a variant comprises the amino acid sequence of SEQ ID NO: 1148; and/or

comprising contacting the cell with:

(i) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1155 or 1162; a guide RNA comprises a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1157 or 1161; and a guide RNA comprises a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1159 or 1163; and

(ii) an RNA-guided nuclease comprising the amino acid sequence of one of SEQ ID NO: 1144-1151 (or a portion thereof).

12. A method of culturing a pluripotent human stem cell, comprising culturing the stem cell in a medium comprising activin.

13. The method of claim 12, wherein the pluripotent human stem cell is an embryonic stem cell or an induced pluripotent stem cell; and/or

wherein the pluripotent human stem cell is genetically engineered to knock out a gene encoding TGF β RII; and/or

wherein the activin is activin A; and/or

wherein the medium does not comprise TGF β ; and/or

wherein the culturing is performed for a defined period of time (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 days, or more); and/or

wherein at one or more times during or following the culturing step, the pluripotent human stem cell maintains pluripotency (e.g., exhibits one or more pluripotency markers); and optionally

wherein at one or more times during or following the culturing step, the pluripotent human stem cell expresses a detectable level of one or more of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog, or

wherein at a time during or following the culturing step, the pluripotent human stem cell is differentiated into cells of endoderm, mesoderm, and/or ectoderm lineage; and optionally

wherein the pluripotent human stem cell, or its progeny, is further differentiated into a natural killer (NK) cell.

14. A cell culture comprising (i) a pluripotent human stem cell and (ii) a cell culture medium comprising activin, wherein the pluripotent human stem cell comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII).

15. A method of increasing a level of iNK cell activity comprising:
(i) providing a pluripotent human stem cell comprising a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII); and
(ii) differentiating the pluripotent human stem cell into an iNK cell,
wherein the iNK cell has a higher level of cell activity as compared to an iNK cell not comprising a genomic edit that results in a loss of function of TGF beta receptor II (TGF β RII).

16. The method of claim 15, wherein the iNK is differentiated from a pluripotent human stem cell cultured in a medium comprising activin; and/or
further comprising culturing the pluripotent human stem cell in a medium comprising activin before and/or during the differentiating step.

17. Use of the cell of any one of claims 7-10 in the manufacture of a medicament for the treatment of a subject having or at risk of cancer, wherein the medicament is to be administered to the subject, thereby treating the cancer in the subject.