

ENGINEERED CELLS FOR THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/950,063, filed December 18, 2019, U.S. Provisional Application No. 63/025,735, filed May 15, 2020, and U.S. Provisional Application No. 63/115,592, filed November 18, 2020, the contents of all of which are hereby incorporated herein in their entirety.

BACKGROUND

[0002] There remains a need for engineered cells for therapeutic interventions, as well as for methods of culturing stem cells, such as embryonic stem cells and induced pluripotent cells, such that pluripotency is maintained.

SUMMARY

[0002a] In one aspect, provided herein is 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).

[0002b] In another aspect, provided herein is 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).

[0002c] In another aspect, provided herein is 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.

[0002d] In another aspect, provided herein is a differentiated cell, (i) wherein the differentiated cell is a daughter cell of the pluripotent human stem cell as described herein, or (ii) wherein the differentiated cell is a daughter cell of the human iPSC as described herein, or (iii) wherein the differentiated cell is a daughter cell of the pluripotent human stem as described herein.

[0002e] In another aspect, provided herein is a method of making the cell as described herein, 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.

[0002f] In another aspect, provided herein is a method of culturing a pluripotent human stem cell, comprising culturing the stem cell in a medium comprising activin.

[0002g] In another aspect, provided herein is 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)

[0002h] In another aspect, provided herein is 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 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 (TGFβRII).

[0002i] In yet another aspect, provided herein is use of the cell as described herein 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.

[0003] In one aspect, the disclosure features 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 an agonist of the TGF beta signaling pathway, or a genomic edit that results in a loss of function of adenosine A2a receptor (ADORA2A). In some embodiments, the stem cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway and a genomic edit that results in a loss of function of ADORA2A.

[0004] In some embodiments, the stem cell comprises a genomic edit that results in a loss of function of a TGF beta receptor or a dominant-negative variant of a TGF beta receptor. In some embodiments, the TGF beta receptor is a TGF beta receptor II (TGFβRII).

[0005] In some embodiments, 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.

[0006] In some embodiments, the disclosure features a differentiated cell, wherein the differentiated cell is a daughter cell of a pluripotent human stem cell described herein. In some embodiments, the differentiated cell is an immune cell. In some embodiments, the