is the use of systemic immunosuppressive medications, which reduce the efficacy of the graft and substantially increase the risk of infections.

[0007] Advances in understanding immune surveillance, as well as the ability to genetically modify cells, such as SCs, is allowing the generation of cells that avoid immune rejection. However, there is an ongoing need to develop improved technologies for cell transplantation therapies.

SUMMARY

[0008] The present disclosure provides genetically modified SCs, as well as methods for their generation and use to treat diseases, such as type 1 diabetes (T1D).

[0009] Accordingly, in examples, the disclosure provides a method of generating a genetically modified SC. In one example, the method includes: a) modifying a SC to reduce expression relative to a wild-type SC of HLA-I, HLA-II, or a combination thereof; and b) introducing exogenous constructs to express immune evasion genes comprising CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and/or HLA-E-single chain trimer. In one aspect, the present disclosure provides a method of generating a stem cell (SC) comprising: a) modifying a SC to reduce expression relative to a wild-type SC of HLA-I, HLA-II, or a combination thereof; and b) introducing exogenous constructs to express immune evasion genes comprising CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

[0009a] In another aspect, the present disclosure provides a stem cell (SC) produced by the method as described herein. In another aspect, the present disclosure provides a use of the SC as described herein, or progeny of the SC as described herein, in the preparation of a medicament for treating a disease or disorder in a subject in need thereof. In another aspect, the present disclosure provides a β cell produced by the method as described herein.

[0009b] In another aspect, the present disclosure provides a use of the β cell as described herein in the preparation of a medicament for treatment of type 1 diabetes (T1D) in a subject, comprising that the β cell is to be administered to the subject, thereby treating T1D in the subject. In another aspect, the present disclosure provides a stem cell (SC), wherein: (i) expression of HLA-I and HLA-II is abrogated; and (ii) the SC is genetically modified to express CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

[0009c] In a further aspect, the present disclosure provides a cell line derived from the SC as described herein. In a further aspect, the present disclosure provides a cell or tissue generated by the method SC as described herein.

[0010] In some examples, the immune evasion genes include CR1 and CD24 and optionally one or more of CD47, CD55, CD46, CD59 and/or HLA-E-single chain trimer.

[0011] In some examples, the SCs may further be modified to express one or more of PDL1 and/or HLA-G-single chain trimer.

[0012] In some examples, the immune evasion genes include CR1, CD24, CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

[0013] In another example, the disclosure provides a genetically modified SC generated by the method of the disclosure.

[0014] In yet another example, the disclosure provides a modified SC wherein: (i) expression of HLA-I and HLA-II is abrogated; and (ii) the SC is genetically modified to express CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and/or HLA-E-single chain trimer.

[0015] In still another example, the disclosure provides a cell line derived from a genetically modified SC of the disclosure.

[0016] In another example, the disclosure provides a differentiated cell or tissue generated by differentiating a genetically modified SC of the disclosure. In various examples, the cell or tissue is microglia, retinal pigmented epithelia, astrocytes, oligodendrocytes, hepatocytes, podocytes, keratinocytes, cardiomyocytes, dopaminergic neurons, cortical

neurons, sensory neurons, NGN2-directed neurons, interneurons, basal forebrain cholinergic neurons, pancreatic beta cells, neural stem cells, natural killer cells, regulatory T cells, lung cell lineages, kidney cell lineages or blood cell lineages.

[0017] In another example, the disclosure provides a β cell generated by differentiating a genetically modified SC of the disclosure.

[0018] In yet another example, the disclosure provides a method of treating a disease or disorder in a subject in need thereof with a genetically modified SC, or progeny of a genetically modified SC of the disclosure.

[0019] In another example, the disclosure provides a method of treating T1D in a subject by administering a genetically modified SC or β cell of the present disclosure to the subject, thereby treating T1D in the subject.

BRIEF DESCRIPTION OF THE FIGURES

[0020] **Figure 1A** depicts generation of an HLA-deficient hES cell line. Shown is a genome editing workflow. Cas9 and three gRNAs targeted to genes essential for HLA expression were nucleofected into H1 hES cells. Two rounds of subcloning and MiSeq™ analysis yield clonal mutant cell lines.

[0021] **Figure 1B** depicts generation of an HLA-deficient hES cell line. Shown is an example of MiSeq™ analysis of targeted genes. Frameshift mutations were introduced in 5 of 6 alleles.

[0022] **Figure 1C** depicts generation of an HLA-deficient hES cell line. Graphical data is shown of WT or HLA-KO hES cells that were stained for HLA-I expression with or without IFNg-treatment. HLA-I expression was absent in b2m- and TAP1-deficient cells.

[0023] **Figure 2A** illustrates data showing that cord blood-humanized mice fail to reject xenogeneic teratomas. Shown is data representative of splenic chimerism of NSG-W41 mice 20 weeks after transplantation of cord blood CD34+ cells.

[0024] **Figure 2B** illustrates data showing that cord blood-humanized mice fail to reject xenogeneic teratomas. Graphical data is shown of teratoma growth in humanized or control NSG-41 recipients following transplantation of unmodified or HM-KO hES cells.

[0025] **Figure 3** is a graphical illustration showing that expressing immune evasion genes allows teratoma growth in immune-competent mice. HLAI/IIKO hES cells were lentivirally transduced with the listed mouse immune evasion genes. Approximately 30% of cells were infected with any given lentivirus, leading to a relatively low frequency of cells expressing all 4 genes. These or control cells were transplanted in bulk into 5 WT C57B16/N mice and

What is claimed is:

1. A method of generating a stem cell (SC) comprising:

a) modifying a SC to reduce expression relative to a wild-type SC of HLA-I, HLA-II, or a combination thereof; and

b) introducing exogenous constructs to express immune evasion genes comprising CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

2. The method of claim 1, wherein the immune evasion genes comprise CR1 and CD24.

3. The method of claim 1, wherein the immune evasion genes comprise CR1, CD24 and one or more of CD46, CD47, CD55, CD59 and HLA-E-single chain trimer.

4. The method of claim 1, wherein the immune evasion genes comprise CR1, CD24, CD47, CD55, CD46, CD59 and optionally HLA-E-single chain trimer.

5. The method of any of claims 1-4, further comprising introducing exogenous constructs to express one or more of PDL1 and HLA-G-single chain trimer.

6. The method of claim 1, wherein expression of HLA-I is reduced by abrogating expression of TAP1 or β2M.

7. The method of claim 1, wherein expression of HLA-II is reduced by abrogating expression of CD74 and CIITA.

8. The method of claim 1, wherein modifying comprises genome editing using CRISPR/Cas9 targeted mutation.

9. The method of claim 1, wherein introducing exogenous constructs is performed using an adeno-associated virus (AAV) construct.

10. The method of claim 9, wherein the AAV construct is a modified AAVS construct that targets a site of endogenous AAV integration located in an intronic region of PPPR12C.

11. The method of claim 1, wherein introducing exogenous constructed is performed by lentiviral transduction.

12. The method of any of claims 1-11, further comprising differentiating the SC to a β cell.

13. A stem cell (SC) produced by the method of any of claims 1-11.

14. The SC of claim 13, wherein:

(i) expression of HLA-I and HLA-II is abrogated; and

(ii) the SC expresses CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

15. The SC of claim 14, wherein the SC further expresses PDL1 and HLA-G-single chain trimer.

16. The SC of any of claims 13-15, wherein the SC is a mouse or human SC.

17. The SC of claim 16, wherein the SC is an embryonic SC or induced pluripotent SC.

18. Use of the SC of any of claims 13-17, or progeny of the SC of any of claims 13-17, in the preparation of a medicament for treating a disease or disorder in a subject in need thereof.

19. The use of claim 18, wherein the disease or disorder is an autoimmune disease or a neurodegenerative disease.

20. The use of claim 18, wherein the disease or disorder is cancer.

21. The use of claim 18, wherein the disease or disorder is type 1 diabetes.

22. A β cell produced by the method of claim 12.

23. Use of the β cell of claim 22 in the preparation of a medicament for treatment of type 1 diabetes (T1D) in a subject, comprising that the β cell is to be administered to the subject, thereby treating T1D in the subject.

24. A stem cell (SC), wherein:

(i) expression of HLA-I and HLA-II is abrogated; and

(ii) the SC is genetically modified to express CR1 and/or CD24 and optionally one or more of CD47, CD55, CD46, CD59 and HLA-E-single chain trimer.

25. The SC of claim 24, wherein the SC is further genetically modified to express PDL1 and HLA-G-single chain trimer.

26. The SC of any of claims 24-25, wherein the SC is a mouse or human SC.

27. The SC of claim 26, wherein the SC is an embryonic SC or induced pluripotent SC.

28. A cell line derived from the SC of any of claims 24-27.

29. The method of any of claims 1-11, further comprising differentiating the SC to produce a differentiated cell or tissue.

30. The method of claim 29, wherein the cell or tissue is selected from the group consisting of microglia, retinal pigmented epithelia, astrocytes, oligodendrocytes, hepatocytes, podocytes, keratinocytes, cardiomyocytes, dopaminergic neurons, cortical neurons, sensory neurons, NGN2-directed neurons, interneurons, basal forebrain cholinergic neurons, pancreatic beta cells, neural stem cells, natural killer cells, regulatory T cells, lung cell lineages, kidney cell lineages and blood cell lineages.

31. A cell or tissue generated by the method of claim 29.

32. The cell or tissue of claim 31, wherein the cell or tissue is selected from the group consisting of microglia, retinal pigmented epithelia, astrocytes, oligodendrocytes, hepatocytes, podocytes, keratinocytes, cardiomyocytes, dopaminergic neurons, cortical neurons, sensory neurons, NGN2-directed neurons, interneurons, basal forebrain cholinergic neurons, pancreatic beta cells, neural stem cells, natural killer cells, regulatory T cells, lung cell lineages, kidney cell lineages and blood cell lineages.