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 the steps of: a) modifying a SC to (i) abrogate expression of TAP1 or β2M or (ii) abrogate expression of CD74 or CIITA; and b) introducing exogenous constructs to express one or more immune evasion genes which inhibit complement activation and deposition and one or more immune evasion genes which inhibit natural killer (NK) cell activation.

[0009a] 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 one or more immune evasion genes which inhibit complement activation and deposition, and one or more immune evasion genes which inhibit natural killer (NK) cell activation.

[0009b] 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 β cell derived from the SC as described herein. In yet 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 an autoimmune disease or a neurodegenerative disease in a subject in need thereof. In a further 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 cancer in a subject in need thereof. In a further 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 type 1 diabetes in a subject in need thereof. In a

further aspect, the present disclosure provides a use of the β cell as described herein in the preparation of a medicament for treating type 1 diabetes in a subject in need thereof.

[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

What is claimed is:

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

a) modifying a SC to (i) abrogate expression of TAP1 or β2M or (ii) abrogate expression of CD74 or CIITA; and

b) introducing exogenous constructs to express one or more immune evasion genes which inhibit complement activation and deposition and one or more immune evasion genes which inhibit natural killer (NK) cell activation.

2. The method of claim 1, wherein the one or more immune evasion genes which inhibit complement activation and deposition comprise at least one of CD46, CD55, and CD59.

3. The method of claim 1, wherein the one or more immune evasion genes which inhibit complement activation and deposition comprise at least CR1.

4. The method of claim 1, wherein the one or more immune evasion genes which inhibit NK cell activation comprise at least one of HLA-E single chain trimer and HLA-G single chain trimer.

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

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

7. The method of claim 6, wherein the AAV construct is a modified AAVS construct that targets a site of endogenous AAV integration located in an intronic region of PPPR12C.

8. The method of claim 1, wherein introducing exogenous constructs is performed by lentiviral transduction.

9. The method of any of claims 1-8, further comprising a step of differentiating the SC to a β cell.

10. A stem cell (SC), wherein:

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

(ii) the SC is genetically modified to express one or more immune evasion genes which inhibit complement activation and deposition, and one or more immune evasion genes which inhibit natural killer (NK) cell activation.

11. The SC of claim 10, wherein the one or more immune evasion genes which inhibit complement activation and deposition comprise at least one of CD46, CD55, and CD59.

12. The SC of claim 10, wherein the one or more immune evasion genes which inhibit complement activation and deposition comprise at least CR1.

13. The SC of claim 10, wherein the one or more immune evasion genes which inhibit NK cell activation comprise at least one of HLA-E single chain trimer and HLA-G single chain trimer.

14. The SC of any of claims 10-13, wherein the SC is a mouse or human SC.

15. The SC of claim 14, wherein the SC is an embryonic SC or induced pluripotent SC.

16. A cell line derived from the SC of any of claims 10-15.

17. A β cell derived from the SC of any of claims 10-15.

18. Use of the SC of any of claims 10-15, or progeny of the SC of any of claims 10-15, in the preparation of a medicament for treating an autoimmune disease or a neurodegenerative disease in a subject in need thereof.

19. Use of the SC of any of claims 10-15, or progeny of the SC of any of claims 10-15, in the preparation of a medicament for treating cancer in a subject in need thereof.

20. Use of the SC of any of claims 10-15, or progeny of the SC of any of claims 10-15, in the preparation of a medicament for treating type 1 diabetes in a subject in need thereof.

21. Use of the β cell of claim 17 in the preparation of a medicament for treating type 1 diabetes in a subject in need thereof.

22. The method of any of claims 1-8, further comprising a step of differentiating the SC to produce a differentiated cell or tissue.

23. The method of claim 22, 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.