Summary

[0005a] In one aspect, the present disclosure refers to a method for producing a population of conditionally immortalized adult stem cells comprising:

a) contacting one or more adult stem cells with

i) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation, wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain; and

ii) an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis; and

b) seeding the adult stem cells in a culture vessel so as to produce a population of conditionally immortalized adult stem cells,

wherein adult stem cells are contacted with the Myc-fusion polypeptide and the Bcl-2 domain polypeptide prior to seeding and wherein the Myc-fusion polypeptide and the Bcl-2 domain polypeptide are cultured with the cells in the culture vessel for at least 24 hours.

[0006] In some examples, a method for producing a population of conditionally immortalized adult stem cells is provided. The method can comprise providing one or more adult stem cells with an exogenously synthesized Myc polypeptide that promotes one or more of cell survival or proliferation and an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis. In some examples the Myc polypeptide is provided to the one or more adult stem cells at intervals of at least about 72 hours and the Bcl-2 domain polypeptide is provided to the one or more adult stem cells at intervals of at least about 96 hours, so as to produce a population of conditionally immortalized adult stem cells. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once an hour. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every two hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every three hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every four hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every five hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every six hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every seven hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every eight hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every nine hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 10 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 11 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 12 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 13 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 14 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 15 hours. In some examples, the Bcl-2

domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 16 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 17 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 18 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 19 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 20 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 21 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 22 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 23 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 24 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 36 hours. In some examples, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 48 hours.

[0007] In some examples, a Myc fusion protein is provided. The fusion protein comprises a protein transduction domain, a Myc polypeptide that promotes one or more of cell survival or proliferation, a V5 domain, and a six histidine epitope tag.

[0008] In some examples, a stem cell expansion media is provided. The expansion media comprises IL3, IL6, stem cell factor, thrombopoeitin, Flt3-L, and GM-CSF.

[0009] In some examples, a Myc fusion protein is provided. The Myc fusion protein comprises a protein transduction domain, a Myc polypeptide that promotes one or more of cell survival or proliferation. The Myc fusion protein half-life is longer than about 60 minutes.

[0010] In some examples, a nucleic acid encoding any of the proteins disclosed herein is provided.

[0011] In some examples, a vector comprising any of the nucleic acids provided herein is provided.

[0012] In some examples, a cell comprising any of the vectors or nucleic acids provided herein is provided.

Detailed Description of the Drawings

[0013] The features of various embodiments are set forth with particularity in the appended claims. A better understanding of the features and advantages of some of the present embodiments will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the various embodiments are utilized, and the accompanying drawings of which:

[0014] Figures **1A, 1B, 1C, 1D,** and **1E** show an illustrative embodiment of the generation and in vitro characterization of Tat fusion proteins. **Fig. 1A** shows an illustrative embodiment of a graphic representation of Tat-Myc and Tat-Bcl-2 fusion proteins including the location of the in frame protein transduction domain of HIV-1 Tat and the V5 and 6xHis tags. **Fig. 1B** shows an illustrative embodiment of the recombinant proteins following purification from E. coli, separation by SDS-PAGE, and staining with Coomassie. **Fig. 1C** shows an illustrative embodiment of a lawn of confluent 3T3 cells exposed to purified recombinant Tat-Myc, Tat-Bcl-2, or left untreated (NT) for two hours, and then fixed and stained with a monoclonal antibody to V5 and with a Hoechst 9934 nuclear stain. The Tat-Myc protein largely localized to the nuclear region in this timeframe, whereas the Tat-Bcl-2 remained in the cytoplasmic and perinuclear space. **Fig. 1D** shows an illustrative embodiment of a SDS-PAGE and western blot analysis (monoclonal antibodies to V5 and β-actin) of human cord blood derived HSCs pulsed with a single exposure of Tat-Myc for 1 hours, washed, and then lysed (at the indicated time points) to separate the plasma membrane and cytoplasmic fraction from the nuclear fraction. **Fig. 1E** shows an illustrative embodiment of a SDS-PAGE and western blot analysis (monoclonal antibodies to V5 and β-actin) of the nuclear fraction of human cord blood derived HSCs pulsed with a single exposure of Tat-Myc for 2 hours, washed, and then lysed (at the indicated time points) to separate the plasma membrane and cytoplasmic fraction from the nuclear fraction. The bulk of the protein is lost between 24 and 48 hours. There is no detectable protein left at any point after 72 hours.

[0015] Figures **2A, 2B, 2C,** and **2D** show an illustrative embodiment of a graphical representation showing that recombinant Tat-Myc and Tat-Bcl-2 are biologically active.

WHAT IS CLAIMED IS:

1. A method for producing a population of conditionally immortalized adult stem cells comprising:

a) contacting one or more adult stem cells with

i) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation, wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain; and

ii) an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis; and

b) seeding the adult stem cells in a culture vessel so as to produce a population of conditionally immortalized adult stem cells,

wherein adult stem cells are contacted with the Myc-fusion polypeptide and the Bcl-2 domain polypeptide prior to seeding and wherein the Myc-fusion polypeptide and the Bcl-2 domain polypeptide are cultured with the cells in the culture vessel for at least 24 hours.

2. The method of claim 1, wherein the Myc-fusion polypeptide is provided at intervals at least 72 hours; and the Bcl-2 domain polypeptide is at intervals of at least 72 hours.

3. The method of claim 1, wherein the Myc polypeptide of the Myc-fusion polypeptide is one or more of n-Myc, c-Myc, 1-Myc, v-Myc, or s-Myc.

4. The method of claim 1, wherein the Myc-fusion polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml.

5. The method of claim 1, wherein the Bcl-2 domain polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml.

6. The method of claim 1, wherein the Bcl-2 domain polypeptide includes BH1, BH2, BH3, and BH4.

7. The method of claim 1, wherein the one or more Bcl-2 domain polypeptide is one or more of Bcl-2, Bcl-w, Bcl-X, Bel-XL, Mcl-1.

8. The method of claim 1, wherein the one or more Bcl-2 domain polypeptide is Bcl-2.

9. The method of claim 1, wherein the Bcl-2 polypeptide includes a protein transduction domain selected from the group consisting of Tat, EPTD and vpr.

10. The method of claim 9, wherein the protein transduction domain is Tat.

11. The method of claim 9, wherein the protein transduction domain is EPTD.

12. The method of claim 9, wherein the protein transduction domain is vpr.

13. The method of claim 1, wherein the one or more adult stem cells are cultured in media comprising IL3, IL6, and stem cell factor.

14. The method of claim 1, wherein the one or more adult stem cells are cultured in media comprising IL3, IL6, stem cell factor, thrombopoietin, and Flt3-L, and GM-CSF.

15. The method of claim 1, wherein the one or more adult stem cells are expanded one or more of about 270 fold over about 28 days, about 150 fold over about 14 days, 100 fold over about 21 days, or about 85 fold over about 9 to 14 days.

16. The method of claim 1, wherein the one or more adult stem cells are one or more hematopoietic adult stem cells.

17. The method of claim 16, wherein the hematopoietic adult stem cells are human cord blood-derived hematopoietic stem cells, human G-CSF mobilized peripheral blood hematopoietic stem cells, or human bone marrow hematopoietic stem cells.

18. The method of claim 1, wherein, over a 8 hour period, no more than 1 µg/ml of Myc-fusion polypeptide is provided.

19. The method of claim 1, wherein the Myc-fusion polypeptide is provided at intervals at least 96 hours; and the Bcl-2 domain polypeptide is at intervals of at least 96 hours.

20. The method of claim 1, wherein the Myc-fusion polypeptide is provided at intervals at least 48 hours; and the Bcl-2 domain polypeptide is at intervals of at least 48 hours.