Summary

[0005a] In one aspect, there is provided a method for producing a population of conditionally immortalized adult stem cells comprising: providing one or more adult stem cells with a) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation; and b) an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis; and wherein the Myc-fusion polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, and wherein the Myc-fusion polypeptide is continuously provided to the cells for at least 24 hours; wherein the Bcl-2 domain polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, so as to produce a population of conditionally immortalized adult stem cells; and wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain.

[0005b] In one aspect, there is provided a method for producing a population of conditionally immortalized adult stem cells comprising: providing one or more adult stem cells with a) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation, wherein the Myc-fusion polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml; and b) an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis, wherein the Bcl-2 domain polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml; and wherein the Myc-fusion polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours; wherein the Bcl-2 domain polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, so as to produce a population of conditionally immortalized adult stem cells; and wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain.

[0006] In some embodiments, 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 embodiments 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 embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once an hour. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc

polypeptide are supplied no more frequently than once every two hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every three hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every four hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every five hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every six hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every seven hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every eight hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every nine hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 10 hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 11 hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 12 hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 13 hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 14 hours. In some embodiments, the Bcl-2 domain polypeptide and/or the Myc polypeptide are supplied no more frequently than once every 15 hours. In some embodiments, the Bcl-2

WHAT IS CLAIMED IS:

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

providing one or more adult stem cells with

a) an exogenously synthesized Myc polypeptide that promotes one or more of cell survival or proliferation; and

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

wherein the Myc polypeptide is provided to the one or more adult stem cells at intervals of at least about 72 hours; and

wherein 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.

2. The method of claim 1, wherein the Myc polypeptide is provided at intervals at least about 48 hours.

3. The method of claim 1, wherein the Myc polypeptide is provided at intervals at least about 24 hours.

4. The method of claim 1, wherein the Myc polypeptide is provided continuously.

5. The method of any one of claims 1-4, wherein the Bcl-2 domain polypeptide is provided at intervals at least about 72 hours.

6. The method of any one of claims 1-4, wherein the Bcl-2 domain polypeptide is provided at intervals at least about 48 hours.

7. The method of any one of claims 1-4, wherein the Bcl-2 domain polypeptide is provided at intervals at least about 24 hours.

8. The method of any one of claims 1-4, wherein the Bcl-2 domain polypeptide is provided continuously.

9. The method of any one of claims 1-8, wherein the Myc polypeptide is one or more of n-Myc, c-Myc, l-Myc, v-Myc, or s-Myc.

10. The method of any one of claims 1-9, wherein the Myc polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml.

11. The method of any one of claims 1-9, wherein the Myc polypeptide is provided at a concentration of about 5 ug/ml.

12. The method of any one of claims 1-11, wherein the Bcl-2 domain polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml.

13. The method of any one of claims 1-11, wherein the Bcl-2 domain polypeptide is provided at a concentration of about 10 ug/ml.

14. The method of any one of claims 1-13, wherein the Bcl-2 domain polypeptide includes BH1, BH2, BH3, and BH4.

15. The method of any one of claims 1-14, wherein the one or more Bcl-2 domain polypeptide is one or more of Bcl-2, Bcl-w, Bcl-X, Bcl-XL, Mcl-1.

16. The method of any one of claims 1-14, wherein the one or more Bcl-2 domain polypeptide is Bcl-2.

17. The method of any one of claims 1-16, wherein one or more of the Myc polypeptide or the Bcl-2 polypeptide includes a protein transduction domain.

18. The method of claim 17, wherein the protein transduction domain is Tat.

19. The method of claim 17, wherein the protein transduction domain is EPTD.

20. The method of claim 17, wherein the protein transduction domain is vpr.

21. The method of any one of claims 1-20, wherein the one or more adult stem cells are cultured in media comprising IL3, IL6, and stem cell factor.

22. The method of any one of claims 1-20, wherein the one or more adult stem cells are cultured in media comprising IL3, IL6, stem cell factor, thrombopoeitin, and Flt3-L.

23. The method of any one of claims 1-20, wherein the one or more adult stem cells are cultured in media comprising IL3, IL6, stem cell factor, thrombopoeitin, and Flt3-L, and GM-CSF.

24. The method of any one of claims 1-23, 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.

25. The method of any one of claims 1-24, wherein the one or more adult stem cells are one or more hematopoietic adult stem cells.

26. The method of claim 25, wherein the hematopoietic adult stem cells are characterized by one or more of cell surface phenotype, in vitro differentiation capacity, ability to reconstitute the hematopoietic lineages in vivo following irradiation, or ability to be transplanted in a serial manner.

27. The method of any one of claims 1-26, wherein the one or more hematopoietic adult stem cells are isolated from one or more of cord blood, placenta, bone marrow, peripheral blood, mobilized peripheral blood, or adipose tissue.

28. The method of any one of claims 1-26, wherein the one or more hematopoietic adult stem cells are derived from embryonic stem cells or induced pluripotent stem cells.

29. The method of any one of claims 1-28, wherein the one or more adult stem cells are human cells.

30. The method of any one of claims 1-28, wherein the one or more adult stem cells are non-human animal cells.

31. A Myc fusion protein comprising:

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.

32. The Myc fusion protein of Claim 31, that has a half-life longer than about 60 minutes.

33. The Myc fusion protein of claim 31 that has a half-life of about 48 hours.

34. The Myc fusion protein of claim 31, wherein the Myc fusion protein is detectable up to about 72 hours.

35. The Myc fusion protein of claim 31, wherein the Myc fusion protein is detectable up to about 48 hours.

36. The Myc fusion protein of any one of claims 31-34, wherein the Myc fusion protein is transportable into a nucleus in a cell.

37. The Myc fusion protein of any one of claims 31-35, wherein the protein transduction domain is Tat.

38. The Myc fusion protein of any one of claims 31-35, wherein the protein transduction domain is Vpr.

39. The Myc fusion protein of any one of claims 31-35, wherein the protein transduction domain is EPTD.

40. The Myc fusion protein of any one of claims 31-38, wherein the Myc fusion protein has an order of components of:

a) the protein transduction domain connected in frame to the Myc polypeptide,

b) the Myc polypeptide connected in frame to the V5 domain, and

c) the V5 domain connected in frame to the six histidine epitope tag.

41. The Myc fusion protein of any one of claims 31-38, wherein the Myc fusion protein has an order of components:

a) the Myc polypeptide connected in frame to the protein transduction domain,

b) the protein transduction domain connected in frame to the V5 domain, and

c) the V5 domain connected in frame to the six histidine epitope tag.

42. A stem cell expansion media comprising IL3, IL6, stem cell factor, thrombopoeitin, Flt3-L, and GM-CSF.

43. The stem cell expansion media of Claim 42, further comprising a base media.

44. The stem cell expansion media of claim 43, further comprising:

a Myc polypeptide that promotes one or more of cell survival or proliferation.

45. The stem cell expansion media of any one of claims 41-44, further comprising:

a Bcl-2 domain polypeptide that inhibits apoptosis.

46. The stem cell expansion media of any one of claims 41-45, wherein the base media is one or more of StemSpan, Isco's media, RPMI, or DMEM.

47. A Myc fusion protein comprising:

a) a protein transduction domain

b) a Myc polypeptide that promotes one or more of cell survival or proliferation, wherein the Myc fusion protein half-life is longer than about 60 minutes.

48. A nucleic acid encoding the protein of any one of Claim 31-41, and 47.

49. A vector comprising the nucleic acid of Claim 48.

50. A cell comprising the vector of Claim 49.

51. The method of Claim 1, wherein, over a 8 hour period, no more than 1μg/ml of Myc polypeptide is provided.

52. The method of Claim 1, wherein, over a 16 hour period, no more than 1μg/ml of Myc polypeptide is provided.

53. The method of Claim 1, wherein, over a 24 hour period, no more than 1μg/ml of Myc polypeptide is provided.

54. The method of Claim 1, wherein producing the population occurs within a gas permeable container.

55. A Myc fusion protein comprising:

a protein transduction domain;

a Myc polypeptide that promotes one or more of cell survival or proliferation; and

a short peptide domain.

56. The Myc fusion of Claim 55, wherein the short peptide domain is selected from at least one of HA tags, FLAG tag, CBP, CYD, StrepII, or HPC.

57. A gas permeable vessel containing:

a stem cell expansion media comprising: IL3, IL6, stem cell factor, thrombopoeitin, Flt3-L, and GM-CSF;

a Myc polypeptide that promotes one or more of cell survival or proliferation; and

a Bcl-2 domain polypeptide that inhibits apoptosis.

58. The gas permeable container of Claim 57, wherein the container is a bag or a flask.

WHAT IS CLAIMED IS:

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

providing one or more adult stem cells with

a) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation; and

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

wherein the Myc-fusion polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, and wherein the Myc-fusion polypeptide is continuously provided to the cells for at least 24 hours;

wherein the Bcl-2 domain polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, so as to produce a population of conditionally immortalized adult stem cells; and wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain.

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 l, 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 l, 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 l, wherein the Bcl-2 domain polypeptide includes BH1, BH2, BH3, and BH4.

7. The method of claim l, 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 l, 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 1, wherein the protein transduction domain is Tat.

11. The method of claim 1, wherein the protein transduction domain is EPTD.

12. The method of claim 1, wherein the protein transduction domain is vpr.

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

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

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

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

18. The method of claim 1, wherein producing the population occurs within a gas permeable container.

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 detectable in the cells 48 hours after being provided to the one or more adult stem cells.

21. 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.

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

providing one or more adult stem cells with

a) an exogenously synthesized Myc-fusion polypeptide that promotes one or more of cell survival or proliferation, wherein the Myc-fusion polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml; and

b) an exogenously synthesized Bcl-2 domain polypeptide that inhibits apoptosis, wherein the Bcl-2 domain polypeptide is provided at a concentration of about 1 ug/ml to about 50 ug/ml; and

wherein the Myc-fusion polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours;

wherein the Bcl-2 domain polypeptide is provided to the one or more adult stem cells at intervals of at least 24 hours, so as to produce a population of conditionally immortalized adult stem cells; and wherein the Myc-fusion polypeptide comprises a Myc polypeptide fused to a protein transduction domain.

23. The method of claim 22, 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.

24. The method of claim 22, 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.

25. The method of claim 22, wherein the one or more Bcl-2 domain polypeptide is Bcl-2.

66. The method of claim 22, wherein the Bcl-2 polypeptide includes a protein transduction domain.

67. The method of claim 22, wherein the protein transduction domain is Tat.

68. The method of claim 22, wherein the Myc polypeptide is c-Myc and the protein transduction domain is Tat.

69. The method of claim 22, wherein the one or more adult stem cells are one or more hematopoietic adult stem cells.