What is claimed is:

CLAIMS

1. A method comprising culturing pluripotent stem cells (PSCs) to produce a population of cells comprising oligodendrocyte progenitor cells (OPCs), wherein the PSCs express an engineered nucleic acid encoding SOX9 at a level sufficient to differentiate PSCs into OPCs in the absence of other transcription factors encoded by engineered nucleic acids.

2. The method of claim 1, wherein the engineered nucleic acid encoding SOX9 is the only transcription factor encoding by an engineered nucleic acid that is expressed by the PSCs.

3. The method of claim 1 or 2, wherein at least 30%, at least 40%, at least 50%, or at least 60% of the population of cells expresses O4 and/or NG2.

4. The method of any one of the preceding claims further comprising transfecting the PSCs with the engineered nucleic acid.

5. The method of claim 4, wherein the engineered nucleic acid is a PiggyBac vector.

6. The method of any one of claims 1-5, wherein the PSCs have a population average of less than 20 copies of the engineered nucleic acid encoding SOX9 per cell.

7. The method of any one of claims 1-5, wherein the PSCs have a population average of 5 to 20, 10 to 20, or 15 to 20 copies of the engineered nucleic acid encoding SOX9 per cell.

8. The method of any one of the preceding claims, wherein OPCs of the population of cells express at least one oligodendrocyte gene selected from the group consisting of Myelin Basic Protein (MBP), NK2 Homeobox 1 (NKX2-1), Myelin Oligodendrocyte Glycoprotein (MOG), Myelin Associated Oligodendrocyte Basic Protein (MOBP), OLIG2 (Oligodendrocyte Transcription Factor 2), Chondroitin Sulfate Proteoglycan 4 (CSPG4), Oligodendrocyte Transcription Factor 1 (OLIG1), SRY-Box Transcription Factor 8 (SOX8), SRY-Box Transcription Factor 10 (SOX10), Myelin Regulatory Factor (MYRF), Platelet Derived Growth Factor Receptor Alpha (PDGFRA), Matrix Metallopeptidase 15 (MMP15),

Proteolipid Protein 1 (PLP1), Transmembrane Protein 88B (TMEM88B), Ectonucleotide Pyrophosphatase/Phosphodiesterase 6 (ENPP6), and Neurofascin (NFASC).

9. The method of any one of the preceding claims, wherein at least one of the following pluripotency genes is down-regulated in OPCs of the population of cells Myelin Regulatory Factor (MYRF), POU Class 5 Homeobox 1 (POU5F1), Nanog Homeobox (NANOG), and SRY-Box Transcription Factor 2 (SOX2).

10. The method of any one of the preceding claims, wherein the PSCs are human PSCs.

11. The method of any one of the preceding claims, wherein the PSCs are induced PSCs (iPSCs).

12. The method of any one of the preceding claims, wherein the PSCs further express and an engineered nucleic acid encoding a cytokine selected from IL-10 and IFNβ.

13. The method of claim 12, wherein the PSCs further express an engineered nucleic acid encoding IL-10 and an engineered nucleic acid IFNβ.

14. The method of claim 12 or 13, wherein the OPCs of the population of cells express IL-10 and/or IFNβ.

15. The method of claim 14, wherein the OPCs secrete IL-10 and/or IFNβ.

16. The method of any one of the preceding claims further comprising culturing OPCs of the population of cells produce oligodendrocytes.

17. The method of claim 16, wherein the oligodendrocytes express at least one biomarker selected from the group consisting of 2',3'-Cyclic Nucleotide 3' Phosphodiesterase (CNPase), Proteolipid Protein 1 (PLP1), Galactosylceramidase (GALC), Myelin Oligodendrocyte Glycoprotein (MOG), Myelin Basic Protein (MBP), and Myelin Associated Glycoprotein (MAG).

18. A population of cells produced by the method of any one of the preceding claims.

19. A pharmaceutical composition comprising OPCs produced by the method of any one of the preceding claims.

20. A pharmaceutical composition comprising oligodendrocytes produced by the method of claim 16 or 17.

21. A method comprising administering to a subject having a demyelinating disorder the pharmaceutical composition of claim 19 or 20.

22. The method of claim 21, wherein the demyelinating disorder is selected from multiple sclerosis, transverse myelitis, metachromatic leukodystropy (MLD), and adrenoleukodystropy (ALD).

23. A pluripotent stem cell (PSC) comprising an engineered nucleic acid encoding SOX9 at a level sufficient to differentiate the PSC into an oligodendrocyte progenitor cell (OPC), in the absence of other transcription factors encoded by engineered nucleic acids.

24. The PSC of claim 23, wherein the engineered nucleic acid encoding SOX9 is the only transcription factor encoding by an engineered nucleic acid that is expressed by the PSCs.

25. A method comprising culturing a first population of the PSC of claim 23 or 24 with at least one other population of PSCs to produce multiple cell types, wherein the at least one other population of PSCs comprises an engineered nucleic acid encoding a lineage-specifying gene.

26. The method of claim 25, wherein the engineered nucleic acid encoding SOX9 and/or the engineered nucleic acid encoding a lineage-specifying gene is operably linked to an inducible promoter.

27. The method of claim 26 comprising culturing the first population of the PSC with the at least one other population of PSCs, and inducing expression of the engineered nucleic acid encoding SOX9 and/or the engineered nucleic acid encoding a lineage-specifying gene.

28. A method comprising culturing a first population of the PSC of claim 23 or 24 with at least one other population of PSCs to produce myelinated organoids, wherein the at least one other population of PSCs comprises unmodified PSCs.

29. The method of claim 28, wherein the engineered nucleic acid encoding SOX9 is operably linked to an inducible promoter.

30. The method of claim 26 comprising culturing the first population of the PSC with the at least one other population of PSCs, and inducing expression of the engineered nucleic acid encoding SOX9.

31. A method comprising: (a) contacting pluripotent stem cells (PSCs) with a transcription factor, wherein the transcription factor consists essentially of SOX9, or one or more copies of a nucleic acid encoding SOX9, and (b) culturing the PSCs to produce a population of cells comprising oligodendrocyte progenitor cells (OPCs).

32. A method comprising: (a) contacting pluripotent stem cells (PSCs) with a differentiation agent, wherein the differentiation agent consists essentially of SOX9, or one or more copies of a nucleic acid encoding SOX9, and (b) culturing the PSCs to produce a population of cells comprising oligodendrocyte progenitor cells (OPCs).

33. The method of claim 31 or 32, wherein the population of cells comprise at least 70% OPCs.

34. The method of any one of claims 31-33, wherein the PSCs of (a) are present in culture media.

35. The method of any one of claims 31-33, wherein the PSCs are human PSCs.

36. The method of any one of claims 31-34, wherein the PSCs are induced PSCs (iPSCs).

37. The method of any one of claims 31-35, wherein the OPCs express O4 and/or NG2.

38. The method of any one of claims 31-36, wherein an engineered nucleic acid encoding a cytokine selected from IL-10 and IFNβ is introduced into the PSCs of (a).

39. The method of claim 37, wherein an engineered nucleic acid encoding IL-10 and an engineered nucleic acid encoding IFNβ are introduced into the pluripotent stem cells.

40. The method of claim 38, wherein IL-10 and IFNβ are encoded by the same engineered nucleic acid.

41. The method of any one of claims 31-39, wherein the PSCs of (b) are cultured for 1 to 10 days.

42. The method of any one of claims 38-41, further comprising culturing the OPCs for 31 to 10 days.

43. The method of any one of claims 38-42, wherein the OPCs secrete IL-10.

44. The method of claim 13, wherein the level of IL-10 secreted by the OPCs is at least 50-fold higher, at least 100-fold, or at least 1,000-fold higher than control cells, wherein the control cells are (i) naturally-occurring OPCs, (ii) OPCs that are not engineered to express IL-10, and/or (iii) OPCs that are engineered to express IL-10 under an inducible promoter and are cultured in the absence of an inducing agent.

45. The method of any one of claims 31-44, wherein the differentiation agent consists essentially of 1 to 50 copies of the nucleotide sequence encoding SOX9.

46. The method of any one of claims 31-45, wherein the one or more engineered nucleic acid is operably linked to a promoter.

47. The method of claim 46, wherein the promoter is an inducible promoter.

48. The method of claim 47, wherein the PSCs of (b) are cultured in the presence of an agent that induces the inducible promoter to activate transcription of the transcription factors and/or cytokines.

49. The method of any one of claims 31-48, wherein the one or more engineered nucleic acid is present on a PIGGYBAC™ transposon vector.

50. The method of claim 49, wherein the PIGGYBAC™ transposon vector comprises PIGGYBAC™ inverted terminal repeat sequences flanking at least one nucleotide sequence encoding at least one of the transcription factors.

51. The method of claim 49 or 50 further comprising introducing a PIGGYBAC™ transposase or an engineered nucleic acid encoding a PIGGYBAC™ transposase into the PSCs.

52. The method of any one of claims 31-51, further comprising culturing the OPCs to produce oligodendrocytes.

53. A population of cells produced by the method of any one of claims 31-52.

54. A pharmaceutical composition comprising OPCs produced by the method of any one of claims 31-51.

55. A pharmaceutical composition comprising oligodendrocytes produced by the method of claim 52.

56. A method comprising administering to a subject having a demyelinating disorder the pharmaceutical composition of claim 54 or 55.

57. The method of claim 56, wherein the demyelinating disorder is selected from multiple sclerosis, transverse myelitis, metachromatic leukodystropy (MLD), and adrenoleukodystropy (ALD).

58. The method of any one of claim 31-57, wherein the differentiation agent consists of SOX9, or one or more copies of a nucleic acid encoding SOX9.

What is claimed is:

CLAIMS

1. A method of generating a population of oligodendrocyte progenitor cells (OPCs) comprising:
    a. providing a pluripotent stem cell (PSC);
    b. overexpressing SOX9 in said PSC; and
    c. generating said population of OPCs from said PSC.

2. The method of claim 1, further comprising contacting SOX9 or a nucleic acid molecule encoding SOX9 with said PSC.

3. The method of claim 2, wherein said nucleic acid molecule encoding SOX9 is expressed in said PSC via a vector.

4. The method of claim 1, further comprising contacting a protein encoding SOX9 with said PSC.

5. The method of claim 2, wherein said nucleic acid molecule encoding SOX9 is engineered.

6. The method of claim 2, wherein said PSC is transfected with said nucleic acid molecule encoding SOX9.

7. The method of claim 2, wherein said PSC comprises less than 20 copies of said nucleic acid molecule encoding SOX9 per cell.

8. The method of claim 1, wherein at least 30% of said population of OPCs expresses at least one OPC-specific marker.

9. The method of claim 1, wherein at least one of the following pluripotency genes is down-regulated in a cell of said population of OPCs: POU Class 5 Homeobox 1 (POU5F1), Nanog Homeobox (NANOG), and SRY-Box Transcription Factor 2 (SOX2).

10. The method of claim 1, wherein said PSC is an induced PSC (iPSC).

11. The method of claim 1, wherein (b) further comprises the step of contacting said PSC with an engineered nucleic acid encoding IL- 10 or IFNβ.

12. The method of claim 11, wherein said population of OPCs express or secrete IL- 10 or IFNβ.

13. The method of claim 1, further comprising culturing said population of OPCs to produce an oligodendrocyte.

14. The method of claim 13, wherein said population of OPCs or said oligodendrocyte express at least one biomarker selected from the group consisting of O4, NG2, Myelin Basic Protein (MBP), NK2 Homeobox 1 (NKX2-1), Myelin Oligodendrocyte Glycoprotein (MOG), Myelin Associated Oligodendrocyte Basic Protein (MOBP), OLIG2 (Oligodendrocyte Transcription Factor 2), Chondroitin Sulfate Proteoglycan 4 (CSPG4), Oligodendrocyte Transcription Factor 1 (OLIG1), SRY-Box Transcription Factor 8 (SOX8), SRY-Box Transcription Factor 10 (SOX10), Myelin Regulatory Factor (MYRF), Platelet Derived Growth Factor Receptor Alpha (PDGFRA), Matrix Metallopeptidase 15 (MMP15), Proteolipid Protein 1 (PLP1), Transmembrane Protein 88B (TMEM88B), Ectonucleotide Pyrophosphatase/Phosphodiesterase 6 (ENPP6), A2B5, 2',3'-Cyclic Nucleotide 3' Phosphodiesterase (CNPase), Galactosylceramidase (GALC), Myelin Associated Glycoprotein (MAG), and Neurofascin (NFASC).

15. The method of claim 14, further comprising culturing a first population of said PSCs with at least a second population of PSCs.

16. The method of claim 15, wherein said second population of PSCs comprises a nucleic acid encoding a lineage- specifying gene.

17. The method of claim 15, wherein said second population of PSCs comprises unmodified PSCs.

18. The method of claim 17, wherein said nucleic acid encoding SOX9 is operably linked to an inducible promoter.

19. The method of claim 18, wherein culturing said first population of PSCs with said second population of PSCs induces expression of said nucleic acid molecule encoding SOX9.

20. The method of claim 1, wherein said population of OPCs is to be administered to a patient suffering from demyelination.