

WHAT IS CLAIMED IS:

1. A method for genetic modification of a myeloid cell, the method comprising transfecting the myeloid cell with a gene editing reagent targeting a genetic site of interest, wherein the myeloid cell is not transduced with a viral vector.
2. The method of claim 1, wherein the myeloid cell is a primary myeloid cell.
3. The method of claim 1, wherein the myeloid cell is a monocyte, macrophage, neutrophil, basophil, eosinophil, erythrocyte, dendritic cell, or megakaryocyte.
4. The method of claim 1, wherein the cell is transfected via electroporation.
5. The method of claim 1, wherein the cell is transfected via nucleofection.
6. The method of claim 1, wherein the cell is transfected via lipid-based or polymer-based transfection.
7. The method of claim 6, wherein the cell is transfected via lipid-based transfection.
8. The method of claim 1, wherein the cell is not subjected to a selection step and/or an enrichment step after transfection.
9. The method of claim 1, wherein the gene editing reagent comprises an RNA-guided nuclease.
10. The method of claim 9, wherein the RNA-guided nuclease is a CRISPR-Cas system.
11. The method of claim 10, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.
12. The method of claim 10, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

13. The method of claim 1, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.
14. The method of claim 13, wherein the Cas protein and guide RNA are non-covalently associated.
15. The method of claim 13, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.
16. The method of claim 1, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.
17. The method of claim 1, wherein the cells are contacted with an electroporation enhancer during transfection.
18. The method of claim 17, wherein the electroporation enhancer is selected from a carrier DNA, a single stranded DNA, a combination of single stranded and double stranded DNA, a polymeric additive, and an oligonucleotide.
19. The method of claim 18, wherein the carrier DNA is a single-stranded DNA oligonucleotide.
20. The method of claim 18, wherein the carrier DNA is non-homologous to human, mouse, and/or rat genomes.
21. The method of claim 1, wherein the myeloid cell is differentiated prior to electroporation.
22. The method of claim 21, wherein the myeloid cell is differentiated into a dendritic cell.
23. The method of claim 21, wherein the myeloid cell is differentiated into a macrophage.

24. The method of claim 1, wherein the myeloid cell is not activated prior to or during genetic modification.
25. The method of claim 1, wherein two or more distinct crispr RNAs (crRNA) to the site of interest are introduced into the myeloid cell.
26. The method of claim 25, wherein introducing the crRNAs into the myeloid cell comprises transfecting the cell with the crRNAs.
27. The method of claim 25, wherein the crRNAs are contacted with tracrRNAs.
28. The method of claim 27, wherein the crRNAs are annealed to the tracrRNAs.
29. The method of claim 25, wherein the crRNAs are single guide RNAs (sgRNAs).
30. The method of claim 1, wherein multiple genetic sites of interest are targeted.
31. The method of claim 30, wherein two or more distinct crRNAs to each site of interest are introduced into the myeloid cell.
32. The method of claim 1, wherein the myeloid cell is a human cell.
33. A method for genetic modification of a plurality of myeloid cells, the method comprising transfecting the plurality of myeloid cells in the presence of a gene editing reagent targeting a site of interest, wherein the myeloid cells are not transduced with a viral vector.
34. The method of claim 33, wherein the myeloid cells are cultured myeloid cells.
35. The method of claim 33, wherein the cell is transfected via electroporation.

36. The method of claim 33, wherein the cell is transfected via lipid-based transfection.
37. The method of claim 33, wherein the site of interest is modified in at least 70% of the plurality of myeloid cells.
38. The method of claim 37, wherein the site of interest is modified in at least 80% of the plurality of myeloid cells.
39. The method of claim 37, wherein the site of interest is modified in at least 85% of the plurality of myeloid cells.
40. The method of claim 37, wherein the site of interest is modified in at least 90% of the plurality of myeloid cells.
41. The method of claim 33, wherein the plurality of myeloid cells comprises dendritic cells (DCs) and the site of interest is modified in at least 50% of the DCs.
42. The method of claim 33, wherein the viability of the plurality of myeloid cells after electroporation is at least 80%.
43. The method of claim 42, wherein the viability of the plurality of myeloid cells after electroporation is at least 90%.
44. The method of claim 33, wherein the gene editing reagent comprises an RNA-guided nuclease.
45. The method of claim 44, wherein the RNA-guided nuclease is a CRISPR-Cas system.
46. The method of claim 45, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.
47. The method of claim 45, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

48. The method of claim 33, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.

49. The method of claim 48, wherein the Cas protein and guide RNA are non-covalently associated.

50. The method of claim 48, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

51. The method of claim 33, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.

52. The method of claim 9 or 44, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to RNP is between 100:1 and 1:100.

53. The method of claim 52, wherein the ratio of guide RNA to RNP is less than or equal to about 3:1.

54. The method of claim 52, wherein the ratio of guide RNA to ribonucleoprotein (RNP) is less than or equal to about 2:1.

55. The method of claim 52, wherein the ratio of guide RNA to ribonucleoprotein (RNP) is about 3:1.

56. The method of claim 52, wherein the ratio of guide RNA to ribonucleoprotein (RNP) is about 2:1.

57. The method of claim 33, wherein at least 70% of the transfected cells are administered to a patient in need thereof, without selection or enrichment of the cells.

58. The method of claim 33, wherein at least 70% of the transfected cells are used in a subsequent reaction, without selection or enrichment of the cells.

59. A method of genetically modifying a plurality of myeloid cells, comprising transfecting the myeloid cells with a gene editing reagent, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not comprise a selection step or enrichment step following myeloid cell transfection.

60. The method of claim 59, wherein the cells are transfected via electroporation.

61. The method of claim 59, wherein the cells are transfected via lipid-based transfection.

62. The method of claim 59, wherein the site of interest is modified in at least 70% of the plurality of myeloid cells.

63. The method of claim 62, wherein the site of interest is modified in at least 80% of the plurality of myeloid cells.

64. The method of claim 62, wherein the site of interest is modified in at least 85% of the plurality of myeloid cells.

65. The method of claim 62, wherein the site of interest is modified in at least 90% of the plurality of myeloid cells.

66. The method of claim 59, wherein the plurality of myeloid cells comprises dendritic cells (DCs) and the site of interest is modified in at least 50% of the DCs.

67. The method of claim 59, wherein the viability of the plurality of myeloid cells after electroporation is at least 80%.

68. The method of claim 67, wherein the viability of the plurality of myeloid cells after electroporation is at least 90%.

69. The method of claim 59, wherein the gene editing reagent comprises an RNA-guided nuclease.

70. The method of claim 69, wherein the RNA-guided nuclease is a CRISPR-Cas system.

71. The method of claim 70, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

72. The method of claim 70, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

73. The method of claim 59, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.

74. The method of claim 73, wherein the Cas protein and guide RNA are non-covalently associated.

75. The method of claim 73, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

76. The method of claim 59, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.

77. A system for genetically modifying a myeloid cell in the absence of a viral vector, the system comprising a chamber compatible with a transfection system, multiple myeloid cells within the chamber in a media compatible with electroporation, and at least one gene editing system designed to target at least one site of interest in the genome of myeloid cells.

78. The system of claim 77, further comprising a transfection system.

79. The system of claim 77, wherein the transfection system comprises an electroporation apparatus.

80. The system of claim 77, wherein the myeloid cell is a primary myeloid cell.

81. The system of claim 77, wherein the myeloid cell is a cultured myeloid cell.
82. The system of claim 77, wherein the myeloid cell is a monocyte, a macrophage, or a dendritic cell.
83. The system of claim 77, wherein the cell is not subjected to a selection step and/or an enrichment step after transfection.
84. The system of claim 77, wherein the gene editing system comprises a gene editing reagent.
85. The system of claim 84, wherein the gene editing reagent comprises an RNA-guided nuclease.
86. The system of claim 85, wherein the RNA-guided nuclease is a CRISPR-Cas system.
87. The system of claim 85, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.
88. The system of claim 86, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.
89. The method of claim 84, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.
90. The method of claim 89, wherein the Cas protein and guide RNA are non-covalently associated.
91. The method of claim 89, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.
92. The system of claim 84, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.

93. The system of claim 77, wherein the cells are contacted with an electroporation enhancer during transfection.

94. The system of claim 93, wherein the electroporation enhancer is selected from a carrier DNA, a single stranded DNA, a combination of single stranded and double stranded DNA, a polymeric additive, and an oligonucleotide.

95. The system of claim 94, wherein the carrier DNA is a single-stranded DNA oligonucleotide.

96. The system of claim 77, wherein the myeloid cell is not activated prior to or during genetic modification.

97. A method of treating a disease treatable with a myeloid cell, comprising providing a genetically modified myeloid cell that has not been transduced with a virus, wherein the myeloid cell has been transfected with a gene editing reagent; and administering the myeloid cell to a patient in need thereof.

98. The method of claim 97, wherein the myeloid cells are primary myeloid cells.

99. The method of claim 97, wherein the myeloid cells are cultured myeloid cells.

100. The method of any one of claims 97-99, wherein the gene editing reagent comprises an RNA-guided nuclease.

101. The method of claim 100, wherein the RNA-guided nuclease is a CRISPR-Cas system.

102. The method of claim 101, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

103. The method of claim 101, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

104. The method of claim 97, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.

105. The method of claim 104, wherein the Cas protein and guide RNA are non-covalently associated.

106. The method of claim 104, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

107. The method of claim 97, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.

108. The method of claim 97, wherein administering comprises oral, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, or subcutaneous administration.

109. A composition comprising a plurality of myeloid cells, a gene editing reagent, a transfection buffer, and an electroporation enhancer, wherein the composition does not comprise a viral vector.

110. The composition of claim 109, wherein the myeloid cells are cultured myeloid cells.

111. The composition of claim 109, wherein the plurality of myeloid cells is a plurality of monocyte, macrophage, or dendritic cells.

112. The composition of claim 109, wherein the gene editing reagent comprises an RNA-guided nuclease.

113. The composition of claim 112, wherein the RNA-guided nuclease is a CRISPR-Cas system.

114. The composition of claim 113, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

115. The composition of claim 113, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

116. The composition of claim 109, wherein the gene editing reagent comprises a CRISPR-Cas system comprising a Cas protein, a guide RNA, and optionally a donor DNA.

117. The composition of claim 116, wherein the Cas protein and guide RNA are non-covalently associated.

118. The composition of claim 116, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

119. The composition of claim 109, wherein the gene editing reagent comprises transcription activator-like effector nuclease (TALEN), a zinc finger nuclease, or an Argonaut endonuclease.

120. The composition of claim 112, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to is between 100:1 and 1:100.

121. The composition of claim 112, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to is less than or equal to about 3:1.

122. The composition of claim 112, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to ribonucleoprotein (RNP) is less than or equal to about 2:1.

123. The composition of claim 112, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to ribonucleoprotein (RNP) is about 3:1.

124. The composition of claim 112, wherein the RNA-guided nuclease comprises a guide RNA and a ribonucleoprotein (RNP), wherein the ratio of guide RNA to ribonucleoprotein (RNP) is about 2:1.

125. The composition of claim 109, wherein the electroporation enhancer is selected from a carrier DNA, a single stranded DNA, a combination of single stranded and double stranded DNA, a polymeric additive, and an oligonucleotide.

126. The composition of claim 125, wherein the carrier DNA is a single-stranded DNA oligonucleotide.

127. A genetically modified myeloid cell made by the method of claim 1 or 33.

128. An assay for drug discovery comprising screening the effect of one or more compounds on the myeloid cell of claim 127.

129. A method for target validation of a compound, comprising contacting a myeloid cell of claim 127 with the compound and monitoring an effect on the cell.