

WHAT IS CLAIMED IS:

1. A system for modifying an endogenous beta-2 microglobulin (B2M) gene in a cell, comprising:
 - a nuclease capable of cleaving a targeted locus in an endogenous B2M gene in a genome of the cell, or a nucleic acid encoding the nuclease; and optionally:
 - a guide RNA (gRNA) comprising a sequence complementary to the B2M gene, and/or
 - a repair template comprising a first homology arm, a second homology arm, and a nucleic acid encoding a payload therebetween, wherein the first homology arm and/or the second homology arm has homology to a sequence in the B2M gene.
2. The system of claim 1, wherein the gRNA comprises: a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity with the nucleotide sequence of any one of SEQ ID NOs: 01-04; a nucleotide sequence of any one of SEQ ID NOs: 01-04; or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 01-04.
3. The system of claim 1 or 2, wherein the gRNA is adapted to inactivate the endogenous B2M gene.
4. The system of any one of claims 1-3, wherein the targeted locus is within a first coding exon of the B2M gene.
5. The system of any one of claims 1-4, wherein the nuclease comprises a Cas nuclease.
6. The system of claim 5, wherein the Cas nuclease comprises a Cas9 nuclease.
7. The system of claim 1, wherein the nuclease is selected from the group consisting of a zinc finger nuclease, a transcription activator-like effector nuclease (TALEN), a homing endonuclease (HEs), and a combined TALEN-HE protein (megaTALs).
8. The system of any one of claims 1-7, wherein the repair template lacks a sequence targeted by the nuclease, and/or lacks a sequence capable of hybridizing to a sequence of the gRNA or complement thereof.

9. The system of claim 8, wherein the payload lacks the sequence targeted by the nuclease, and/or lacks a sequence capable of hybridizing to a sequence of the gRNA or complement thereof.

10. The system of claim 8, wherein the first arm of homology and/or the second arm of homology lack the sequence targeted by the nuclease, and/or lack a sequence capable of hybridizing to a sequence of the gRNA or complement thereof.

11. The system of any one of claims 1-10, wherein the payload comprises a first nucleic acid encoding a first polypeptide.

12. The system of claim 11, wherein the payload comprises a second nucleic acid encoding a second polypeptide.

13. The system of claim 12, wherein a nucleic acid encoding a ribosome skip sequence is located between the first nucleic acid and the second nucleic acid.

14. The system of any one of claims 11-13, wherein the first polypeptide or second polypeptide encodes a B2M cDNA.

15. The system of any one of claims 11-14, wherein the first polypeptide or second polypeptide encodes a non-polymeric HLA polypeptide, or a therapeutic polypeptide.

16. The system of claim 15, wherein the non-polymeric HLA polypeptide is selected from HLA-E, HLA-F, or HLA-G.

17. The system of claim 15 or 16, further comprising a third polypeptide encoding a self-peptide to be presented by the non-polymeric HLA polypeptide.

18. The system of claim 15, wherein the therapeutic polypeptide comprises an enzyme, an antibody or antigen binding fragment thereof, a receptor, a chimeric antigen receptor, or a cytokine.

19. The system of claim 15, wherein the therapeutic polypeptide comprises factor IX, angiotensin-converting enzyme 2 (Ace2), beta-glucocerebrosidase (GBA), alpha-galactosidase A (GLA), or acid alpha-glucosidase (GAA).

20. The system of any one of claims 11-19, wherein the first nucleic acid is operably linked to a promoter of the endogenous B2M gene.

21. The system of any one of claims 11-20, wherein the first polypeptide is in-frame with a polypeptide encoded by the endogenous B2M gene.

22. The system of any one of claims 11-21, wherein the payload comprises a heterologous promoter, and the first nucleic acid is operably linked to the heterologous promoter.
23. The system of claim 22, wherein the heterologous promoter is a constitutive promoter.
24. The system of claim 22 or 23, wherein the promoter selected from an MND promoter, and an EF1 alpha promoter, or a PGK promoter.
25. The system of any one of claims 1-24, wherein a vector comprises the repair template.
26. The system of claim 25, the vector comprises a viral vector.
27. The system of claim 26, wherein the viral vector comprises an adeno-associated virus (AAV) vector, or a lentiviral vector.
28. The system of any one of claims 1-27, further comprising a cell, wherein the cell comprises the nuclease; and optionally, the gRNA, and/or repair template.
29. The system of claim 28, wherein the cell comprises the nuclease
30. The system of claim 28 or 29; wherein the cell comprises the gRNA, and/or repair template.
31. The system of any one of claims 28-30, wherein the cell is selected from the group consisting of a B cell, a hematopoietic stem cell, an early pro-B cell, a late pro-B cells, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal zone B cell, a mature B cell, a naïve B cell, a plasmablast (short lived) cell, a GC B cell, a memory B cell, and a long lived plasma cell.
32. The system of any one of claims 28-31, wherein the cell is a B cell.
33. The system of any one of claims 28-32, wherein the cell is human.
34. The system of any one of claims 28-33, wherein the cell is autologous to a subject.
35. The system of any one of claims 28-33, wherein the cell is allogeneic to a subject.
36. The system of any one of claims 28-35, wherein the cell is *ex vivo*.
37. The system of any one of claims 28-35, wherein the cell is *in vivo*.

38. A pharmaceutical composition comprising the system of any one of claims 1-37 and a pharmaceutically acceptable excipient.
39. A method for preparing a modified cell, comprising:
obtaining the system of any one of claims 1-37;
introducing the nuclease into a first cell; and optionally:
introducing the gRNA into the cell, and/or introducing the repair template into the cell, thereby preparing a modified cell comprising a modified B2M locus in the genome of the modified cell.
40. The method of claim 39, wherein the modified B2M locus comprises an inactivated B2M endogenous gene.
41. The method of claim 39 or 40, wherein the gRNA is adapted to inactivate the endogenous B2M gene.
42. The method of any one of claims 39-41, further comprising selecting for the modified cell.
43. The method of claim 42, wherein the selecting comprising contacting the modified cell with an immune cell.
44. The method of claim 43, wherein the immune cell is allogeneic to the first cell.
45. The method of claim 43 or 44, wherein the immune cell comprises an MHC-I different from an MHC-I of the first cell.
46. The method of any one of claims 43-45, wherein the immune cell is selected from a T cell, such as a cytotoxic CD8⁺ T cell, or a natural killer cell.
47. The method of any one of claims 43-46, wherein the immune cell is *in vivo*.
48. The method of any one of claims 43-46, wherein the immune cell is *ex vivo*.
49. The method of claim 39, wherein the modified B2M locus expresses an active B2M gene or B2M cDNA.
50. The method of claim 49, wherein the repair template lacks a sequence targeted by the nuclease, and/or lacks a sequence capable of hybridizing to a sequence of the gRNA or complement thereof, such that the modified B2M locus lacks the sequence targeted by the nuclease, and/or lacks the sequence capable of hybridizing to a sequence of the gRNA or complement thereof.

51. The method of claim 49 or 50, wherein the repair template comprises a B2M cDNA.
52. The method of any one of claims 49-51, further comprising selecting for the modified cell.
53. The method of claim 52, wherein the selecting comprising contacting the modified cell with an immune cell.
54. The method of claim 53, wherein the immune cell is autologous to the first cell.
55. The method of claim 53 or 54, wherein the immune cell is selected from a T cell, such as a cytotoxic CD8+ T cell, or a natural killer cell.
56. The method of any one of claims 53-55, wherein the immune cell is *in vivo*.
57. The method of any one of claims 53-55, wherein the immune cell is *ex vivo*.
58. The method of claim 39, wherein the modified B2M locus expresses a replacement MHC-I.
59. The method of claim 58, wherein the modified B2M locus comprises an inactivated B2M endogenous gene.
60. The method of claim 58 or 59, wherein the repair template comprises a payload encoding a B2M cDNA, a non-polymeric HLA polypeptide, and/or a self-peptide to be presented by the non-polymeric HLA polypeptide.
61. The method of any one of claims 58-60, further comprising selecting for the modified cell.
62. The method of claim 61, wherein the selecting comprising contacting the modified cell with an immune cell.
63. The method of claim 62, wherein the immune cell is allogeneic to the first cell.
64. The method of claim 62 or 63, wherein the immune cell is selected from a T cell, such as a cytotoxic CD8+ T cell, or a natural killer cell.
65. The method of any one of claims 62-64, wherein the immune cell is *in vivo*.
66. The method of any one of claims 62-64, wherein the immune cell is *ex vivo*.
67. The method of any one of claims 39-66, wherein the first cell is a B cell.
68. A cell prepared by the method of any one of claims 39-67.
69. A method for enriching for a modified B cell having increased resistance to killing by a T cell or natural killer cell, comprising:

preparing a modified cell by the method of claim 39, wherein the first cell is a B cell, and wherein the modified B2M locus is inactivated; and

contacting the modified cell with a T cell or natural killer cell, wherein the T cell or natural killer cell is allogeneic to the first cell, and wherein the modified B cell has an increased resistance to killing by the T cell or natural killer cell compared to a B cell expressing a B2M gene.

70. A method for enriching for a modified B cell, comprising:

preparing a modified cell by the method of claim 39, wherein the modified B2M locus expresses an active B2M gene or B2M cDNA; and

contacting the modified cell with a T cell or natural killer cell, wherein the T cell or natural killer cell is autologous to the first cell, and wherein a cell not expressing an active B2M gene or B2M cDNA is killed by the T cell or natural killer cell.

71. A method for enriching for a modified B cell, comprising:

preparing a modified cell by the method of claim 39, wherein the modified B2M locus expresses a replacement MHC-I, wherein the repair template comprises a payload encoding a B2M cDNA, a non-polymeric HLA polypeptide, and a self-peptide to be presented by the non-polymeric HLA polypeptide; and

contacting the modified cell with a T cell or natural killer cell, wherein the T cell or natural killer cell is allogenic to the first cell.

72. A method for preparing a modified B cell for an allogeneic infusion, comprising:

preparing a modified cell by the method of claim 39, wherein the cell is a B cell and the repair template comprises a payload encoding a B2M cDNA, a non-polymeric HLA polypeptide, and a self-peptide to be presented by the non-polymeric HLA polypeptide.

73. The method of claim 72, further comprising administering the modified B cell to a subject, wherein the B cell is allogeneic to the subject.

74. The method of claim 72 or 73, wherein the non-polymeric HLA polypeptide is selected from HLA-E, HLA-F, or HLA-G.

75. The method of any one of claims 72-74, wherein the subject is mammalian.

76. The method of any one of claims 72-75, wherein the subject is human.

77. An isolated nucleic acid comprising a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the nucleotide sequence of any one of SEQ ID NOs: 01-04; a nucleotide sequence of any one of SEQ ID NOs: 01-04; or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 01-04.