

CLAIMS

1. A method of determining a presence of an adoptive cell therapy in a subject, comprising:
detecting an expression level of a 2A peptide in a sample from the subject that has
received the adoptive cell therapy, wherein the adoptive cell therapy comprises a cell
expressing the 2A peptide, or a fragment thereof.
2. The method of claim 1, wherein detection of the 2A peptide, or a fragment thereof, is
associated with the presence of the adoptive cell therapy.
3. The method of claim 1, wherein absence of the 2A peptide, or a fragment thereof, is
associated with inefficiency of the adoptive cell therapy.
4. The method of any one of claims 1-3, wherein detecting of the 2A peptide, or a fragment
thereof, occurs between 1 day and 30 days after the subject has received the adoptive cell
therapy.
5. The method of any one of claims 1-3, wherein detecting of the 2A peptide, or fragment
thereof, occurs between 1 month and 12 months after the subject has received the
adoptive cell therapy.
6. The method of any one of claims 1-3, wherein detecting of the 2A peptide or fragment
thereof occurs between 1 year and 5 years after the subject has received the adoptive cell
therapy.
7. A method of monitoring the persistence of an adoptive cell therapy in a subject that has
received the adoptive cell therapy, comprising: detecting an expression level of a 2A
peptide, or fragment thereof, in a sample from a first time point from the subject; and
detecting the expression level of the 2A peptide, or fragment thereof, in a sample from a
second time point from the subject; wherein the adoptive cell therapy comprises a cell
expressing the 2A peptide, and wherein detection of the 2A peptide, or fragment thereof,
in the sample of the second time point is associated with the persistence of the adoptive
cell therapy.
8. A method of monitoring the persistence of an adoptive cell therapy in a subject that has
received the adoptive cell therapy, comprising: detecting an expression level of a 2A
peptide, or fragment thereof, in a sample from a first time point from the subject; and
detecting the expression level of the 2A peptide, or fragment thereof, in a sample from a
second time point from the subject; wherein the adoptive cell therapy comprises a cell
expressing the 2A peptide, and wherein absence of the 2A peptide, or fragment thereof,
in the sample of the second time point is associated with the inefficiency of the adoptive
cell therapy.

9. The method of claim 7 or 8, further comprising administering a second adoptive cell therapy to the subject.
10. A method of monitoring the expansion of an adoptive cell therapy in a subject that has received the adoptive cell therapy, comprising: detecting an amount of cells expressing a 2A peptide, or fragment thereof, in a sample from a first time point from the subject; and detecting an amount of cells expressing a 2A peptide, or fragment thereof, in a sample from a second time point from the subject; wherein expansion of the adoptive cell therapy occurs when the amount of cells expressing a 2A peptide, or fragment thereof, in a sample of the second time point is increased compared to the amount of cells expressing a 2A peptide or fragment thereof in a sample of the first time point.
11. A method of monitoring the expansion of an adoptive cell therapy in a subject that has received the adoptive cell therapy, comprising: detecting an amount of cells expressing a 2A peptide or fragment thereof in a sample from a first time point from the subject; and detecting an amount of cells expressing a 2A peptide or fragment thereof in a sample from a second time point from the subject; wherein no expansion of the adoptive cell therapy occurs when the amount of cells expressing a 2A peptide or fragment thereof in a sample of the second time point is decreased or the same compared to the amount of cells expressing a 2A peptide or fragment thereof in a sample of the first time point.
12. The method of any one of claims 7-11, wherein the second time point occurs between 1 day and 30 days after the first time point.
13. The method of any one of claims 7-11, wherein the second time point occurs between 1 month and 12 months after the first time point.
14. The method of any one of claims 7-11, wherein the second time point occurs between 1 year and 5 years after the first time point.
15. The method of any one of claims 1-14, wherein the cell is a Cell Product.
16. The method of any one of claims 1-15, wherein the cell is a NeoTCR Product.
17. The method of any one of claims 1-16, wherein the sample is a blood sample.
18. The method of any one of claims 1-16, wherein the sample is a tumor sample.
19. The method of any one of claims 1-18, wherein the subject is human.
20. The method of any one of claims 1-19, wherein the subject has a cancer.
21. The method of any one of claims 1-20, wherein the 2A peptide, or fragment thereof, is detected by flow cytometry.
22. The method of any one of claims 1-20, wherein the 2A peptide, or fragment thereof, is detected by immunohistochemistry (IHC).

23. The method of claim 22, wherein the IHC is ChipCytometry.
24. A method for the prognosis of a subject treated with an adoptive cell therapy,
comprising: detecting the expression level of a 2A peptide, or fragment thereof, in a
sample from the subject wherein the adoptive cell therapy comprises a cell expressing
the 2A peptide, or fragment thereof, and determining the expression level of the 2A
peptide, or fragment thereof, in a sample from the subject, wherein detection of the 2A
peptide, or fragment thereof, is associated with efficacy of the adoptive cell therapy.
25. A method for the prognosis of a subject treated with an adoptive cell therapy,
comprising: detecting the expression level of a 2A peptide, or fragment thereof, in a
sample from the subject wherein the adoptive cell therapy comprises a cell expressing
the 2A peptide, or fragment thereof, and determining an expression level of a 2A peptide,
or fragment thereof, in a sample from the subject, and wherein no detection of the 2A
peptide, or fragment thereof, is associated with reduced efficacy of the adoptive cell
therapy.
26. The method of claim 24 or 25, further comprising administering a second adoptive cell
therapy to the subject.
27. The method of any one of claims 24-26, wherein detecting of the 2A peptide, or
fragment thereof, occurs between 1 day and 30 days after the subject has received the
adoptive cell therapy.
28. The method of any one of claims 24-26, wherein detecting of the 2A, or fragment
thereof, peptide occurs between 1 month and 12 months after the subject has received the
adoptive cell therapy.
29. The method of any one of claims 24-26, wherein detecting of the 2A, or fragment
thereof, peptide occurs between 1 year and 5 years after the subject has received the
adoptive cell therapy.
30. The method of any one of claims 24-29, wherein the cell is a Cell Product.
31. The method of any one of claims 24-29, wherein the cell is a NeoTCR Product.
32. The method of any one of claims 24-31, wherein the sample is a blood sample.
33. The method of any one of claims 24-31, wherein the sample is a tumor sample.
34. The method of any one of claims 24-33, wherein the subject is human.
35. The method of any one of claims 24-34, wherein the subject has a cancer.
36. The method of any one of claims 24-35, wherein the 2A peptide, or fragment thereof, is
detected by flow cytometry.

37. The method of any one of claims 24-35, wherein the 2A peptide, or fragment thereof, is detected by IHC.
38. The method of claim 37, wherein the IHC is ChipCytometry.
39. A method of treating a cancer in a subject that has received an adoptive cell therapy,
5 wherein the adoptive cell therapy comprises a cell expressing a 2A peptide, or fragment thereof, the method comprising:
- a) detecting the expression level of the 2A peptide, or fragment thereof, in a sample from the subject, wherein absence or decreased detection of of the 2A peptide, or fragment thereof, is associated with inefficiency of the adoptive cell therapy; and
 - 10 b) administering a second adoptive cell therapy, when the expression level of the 2A peptide is absent or decreased.
40. The method of claim 39, wherein detecting of the 2A peptide, or fragment thereof, occurs between 1 day and 30 days after the subject has received the adoptive cell therapy.
- 15 41. The method of claim 39, wherein detecting of the 2A peptide, or fragment thereof, occurs between 1 month and 12 months after the subject has received the adoptive cell therapy.
42. The method of claim 39, wherein detecting of the 2A peptide, or fragment thereof, occurs between 1 year and 5 years after the subject has received the adoptive cell
20 therapy.
43. The method of any one of claims 37-40, wherein the cell is a Cell Product.
44. The method of any one of claims 37-40, wherein the cell is a NeoTCR Product.
45. The method of any one of claims 37-42, wherein the sample is a blood sample.
46. The method of any one of claims 37-42, wherein the sample is a tumor sample.
- 25 47. The method of any one of claims 37-44, wherein the subject is human.
48. The method of any one of claims 37-45, wherein the 2A peptide, or fragment thereof, is detected by flow cytometry.
49. The method of any one of claims 37-45, wherein the 2A peptide or fragment thereof is detected by IHC.
- 30 50. The method of claim 49, wherein the IHC is ChipCytometry.
51. The method of any one of claims 1-50, wherein the 2A peptide, or fragment thereof, is a cleaved 2A peptide.
52. The method of any one of claims 1-51, wherein the 2A peptide is selected from the group comprising P2A, T2A, E2A, and F2A peptides.

53. The method of any one of claims 1-52, wherein the 2A peptide is a P2A or T2A peptide.
54. The method of any one of claims 1-53, wherein the 2A peptide is a P2A peptide.
55. The method of any one of claims 1-54, wherein the 2A peptide, or fragment thereof, is detected using an anti-2A antibody or antigen binding portion thereof.
- 5 56. The method of claim 55, wherein the anti-2A antibody cross-competes for binding to the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is a 3H4 antibody.
57. The method of claim 55, wherein the anti-2A antibody binds to the same epitope of the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the
10 reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is a 3H4 antibody.
58. The method of claim 55, wherein the anti-2A antibody cross-competes for binding to the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is an ABS31 antibody.
- 15 59. The method of claim 55, wherein the anti-2A antibody binds to the same epitope of the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is an ABS31 antibody.
60. The method of claim 55, wherein the anti-2A antibody or antigen-binding portion thereof
20 is a 3H4 antibody.
61. The method of claim 55, wherein the anti-2A antibody or antigen-binding portion thereof is an ABS31 antibody.
62. The method of any one of claims 55-59, wherein the anti-2A antibody binds to an epitope having an amino acid sequence set forth in SEQ ID NO: 3.
- 25 63. A method of determining whether a cell has been edited, comprising transducing or transfecting a cell with a vector encoding an antigen binding receptor and a 2A peptide; detecting an expression level of the 2A peptide, or fragment thereof, in the cell; wherein detection of the 2A peptide, or fragment thereof, is associated with efficiency of gene editing.
- 30 64. A method of determining whether a cell has been edited, comprising transducing or transfecting a cell with a vector encoding an antigen binding receptor and a 2A peptide; detecting an expression level of the 2A peptide, or fragment thereof, in the cell; wherein absence of the 2A peptide, or fragment thereof, is associated with inefficiency of gene editing.

65. The method of claim 63 or 64, wherein the antigen binding receptor is a T cell receptor (TCR).

66. The method of claim 65, wherein the vector comprises:

- 5 a) first and second homology arms homologous to first and second target nucleic acid sequences and oriented to facilitate homologous recombination;
- b) a nucleotide sequence encoding the TCR positioned between the first and second homology arms; and
- 10 c) a first nucleotide sequence encoding a 2A ribosome skipping element positioned upstream of the nucleotide sequence encoding the TCR polypeptide and a second nucleotide sequence encoding a 2A ribosome skipping element positioned downstream of the nucleotide sequence encoding the TCR polypeptide, wherein the first and second nucleotide sequences encoding the 2A ribosome skipping elements have the same amino acid sequences and are codon-diverged relative to each other.

15 67. A method of releasing a population of cells for adoptive cell therapy, comprising: transducing or transfecting the population of cells with a vector comprising an antigen binding receptor and a 2A peptide; detecting an amount of cells expressing the 2A peptide, or fragment thereof; releasing the population of cells for therapeutic use when the amount of cells expressing the 2A peptide is above a threshold.

20 68. The method of claim 67, wherein the antigen binding receptor is a T cell receptor (TCR).

69. The method of claim 68, wherein the vector comprises:

- 25 a) first and second homology arms homologous to first and second target nucleic acid sequences and oriented to facilitate homologous recombination;
- b) a nucleotide sequence encoding the TCR positioned between the first and second homology arms; and
- 30 c) a first nucleotide sequence encoding a 2A ribosome skipping element positioned upstream of the nucleotide sequence encoding the TCR polypeptide and a second nucleotide sequence encoding a 2A ribosome skipping element positioned downstream of the nucleotide sequence encoding the TCR polypeptide, wherein the first and second nucleotide sequences encoding the 2A ribosome skipping elements have the same amino acid sequences and are codon-diverged relative to each other.

70. The method of any one of claims 67-69, wherein the threshold is calculated by the formula:

$$\frac{\text{amount of cells expressing the 2A peptide}}{\text{amount of cells in the population of cell}} \times 100\%.$$

71. The method of claim 70, wherein the threshold is equal to or greater than 5%.
72. The method of any one of claims 67-71, wherein the population of cell comprises a Cell Product.
- 5 73. The method of any one of claims 67-71, wherein the population of cell comprises a NeoTCR Product.
74. The method of any one of claims 67-74, wherein the 2A peptide, or fragment thereof, is detected by flow cytometry.
75. The method of any one of claims 67-74, wherein the 2A peptide, or fragment thereof, is
10 detected by IHC.
76. The method of claim 75, wherein the IHC is ChipCytometry.
77. The method of any one of claims 67-76, wherein the 2A peptide is a cleaved 2A peptide.
78. The method of any one of claims 67-77 wherein the 2A peptide is selected from the group comprising P2A, T2A, E2A, and F2A peptides.
- 15 79. The method of claim 78, wherein the 2A peptide is P2A or T2A.
80. The method of claim 78, wherein the 2A peptide is P2A.
81. The method of any one of claims 67-80, wherein the 2A peptide, or fragment thereof, is detected using an anti-2A antibody or antigen binding portion thereof.
82. The method of claim 81, wherein the anti-2A antibody cross-competes for binding to the
20 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is a 3H4 antibody.
83. The method of claim 81, wherein the anti-2A antibody binds to the same epitope of the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion thereof, wherein the reference antibody or
25 antigen-binding portion is a 3H4 antibody.
84. The method of claim 81, wherein the anti-2A antibody cross-competes for binding to the 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is an ABS31 antibody.
85. The method of claim 81, wherein the anti-2A antibody binds to the same epitope of the
30 2A peptide with a reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion thereof, wherein the reference antibody or antigen-binding portion is an ABS31 antibody.

86. The method of claim 81, wherein the anti-2A antibody or antigen-binding portion thereof is a 3H4 antibody.
87. The method of claim 81, wherein the anti-2A antibody or antigen-binding portion thereof is an ABS31 antibody.
- 5 88. The method of any one of claims 67-85, wherein the anti-2A antibody binds to an epitope having an amino acid sequence set forth in SEQ ID NO: 3.
89. A method of treating a patient with a Cell Product, the method comprising:
- a) administering a patient in need thereof a Cell Product,
 - b) drawing blood or collecting a tumor sample from the patient at a future time
 - 10 point, and
 - c) analyzing the blood or tumor sample using FACS or IHC analysis to determine if the 2A peptide or antibody binding fragment thereof is present using an anti-2A antibody,
- wherein if the 2A peptide or antibody binding fragment thereof is detected then
- 15 the Cell Product is present and persisting in the patient and if the 2A peptide or antibody binding fragment thereof is not detected then the Cell Product is no longer in the patient,
- wherein if the Cell Product is present and persisting, the product is effective at implanting into the patient and no additional therapy is administered to the
- 20 patient, and
- wherein if the Cell Product is not present, the product is not effective at implanting into the patient and a new therapy is administered to the patient.
90. The method of claim 89, wherein the Cell Product is a NeoTCR product.
91. The method of claim 89 or 90, wherein the patient is a patient with cancer.
- 25 92. The method of any of claims 89-91, wherein the future time point is selected from a time point between 1-10 days, 11-30 days, 31-90 days, 91-180 days, and 181-365 days.
93. The method of any of claims 89-91, wherein the future time point is selected from a time point between 1-2 years, 2-5 years, 5-10 years, 10-20 years, and 20-50 years.
94. A method of detecting a cleaved 2A peptide or antibody binding fragment thereof in
- 30 cells of a Cell Product using FACS, comprising one of the following methods:
- a) Method 1, comprising:
 - i) staining the cells with a live/dead label
 - ii) fixing the cells with a fixative
 - iii) staining the cells with cell surface markers of interest

- iv) staining the cells with a permeabilization buffer with an anti-2A antibody
 - v) fixing the cells with a fixative
 - b) Method 2 comprising:
 - i) staining the cells with a live/dead label
 - 5 ii) staining the cells with cell surface markers of interest
 - iii) fixing the cells with a fixative
 - iv) staining the cells with a permeabilization buffer with an anti-2A antibody
 - v) fixing the cells with a fixative
 - c) Method 3 comprising:
 - 10 i) staining the cells with a live/dead label
 - ii) staining the cells with cell surface markers of interest
 - iii) staining the cells with a permeabilization buffer with an anti-2A antibody
 - iv) fixing the cells with a fixative
- wherein the stained cells from Methods 1, 2, or 3 are analyzed on a flow cytometer using
- 15 FACS analysis.
95. The method of claim 94, wherein the cells are gene edited using the methods described in Example 2 and/or 3.
96. A method of determining the prognosis of a patient treated with a Cell Product, the method comprising:
- 20 a) administering a patient in need thereof a Cell Product,
 - b) drawing blood or collecting a tumor sample from the patient at a future time point, and
 - c) analyzing the blood or tumor sample using FACS or IHC analysis to determine if the 2A peptide or antibody binding fragment thereof is present using an anti-2A
 - 25 antibody,
- wherein if the 2A peptide or antibody binding fragment thereof is detected then the Cell Product is present and persisting in the patient and if the 2A peptide or antibody binding fragment thereof is not detected then the Cell Product is no longer in the patient,
- 30 wherein if the Cell Product is present and persisting, the prognosis of the patient is good for responding to the Cell Product therapy, and
- wherein if the Cell Product is not present, the prognosis of the patient is bad for responding to the Cell Product therapy.
97. The method of claim 96, wherein the Cell Product is a NeoTCR product.

98. The method of claim 95 or 96, wherein the patient is a patient with cancer.
99. The method of any of claims 95-98, wherein a good prognosis is partial or complete response to the Cell Therapy.
100. The method of any of claims 95-99, wherein a bad prognosis is no response to the Cell Therapy and/or disease progression.
101. A method of determining the persistence and/or presence of a Cell Product in a patient after infusion of the Cell Product, the method comprising:
- a) drawing blood or collecting a tumor sample from the patient at a time point following administration of the Cell Product, and
 - b) analyzing the blood or tumor sample using FACS or IHC analysis to determine if the 2A peptide or antibody binding fragment thereof is present using an anti-2A antibody,
- wherein the Cell Product is present and has persisted in the patient if the 2A peptide or antibody binding fragment thereof is detected using the anti-2A antibody.
102. A method of determining the expansion of a Cell Product in a patient after infusion of the Cell Product, the method comprising:
- a) drawing blood or collecting a tumor sample from the patient at two or more time points following administration of the Cell Product,
 - b) analyzing the blood or tumor samples using FACS or IHC analysis to determine if the 2A peptide or antibody binding fragment thereof is present using an anti-2A antibody, and
 - c) determining the difference between the number of cells stained with the anti-2A antibody at the two or more timepoints,
- wherein, an increase in the cell numbers stained with the anti-2A antibody between a first time point and a second is evidence of cell expansion, and
- wherein a decrease or no increase in the cell numbers stained with the anti-2A antibody between a first time point and a second is evidence of no cell expansion.
103. A method for detecting the rate of gene editing of a gene editing reaction, the method comprising:
- a) permeabilizing and staining the cells from the gene editing reaction with an anti-2A peptide antibody,
 - b) sorting the cells using FACS or IHC to identify the number of unstained and stained cells, and

- c) calculating the rate of gene editing using the following calculation: (number of cells positive for anti-2A antibody staining) ÷ (total number of cells).
104. The method of 103 wherein step (a) of permeabilizing and staining the cells is selected from the group consisting of Method 1, Method 2, and Method 3 of claim 6.
- 5 105. A method for qualifying a Cell Product for release for the treatment of patients in need thereof, the method comprising:
- a) taking a sample of the Cell Product,
 - b) analyzing the sample using FACS or IHC analysis to determine if the 2A peptide or antibody binding fragment thereof is present using an anti-2A antibody, and
 - 10 c) determining the percent of edited cells,
- wherein, the Cell Product is released for the treatment of patients if the percent of edited cells meets the product specification for release.
106. The method of claim 105, wherein the percent of edited cells is determined using the following formula: (number of cells positive for anti-2A antibody staining) ÷ (total number of cells).
- 15 107. The method of claim 105 or 106, wherein the product specification for release is at least 5% gene edited cells.
108. The method of any of claims 89-107, wherein the future time point is selected from a time point between 1-10 days, 11-30 days, 31-90 days, 91-180 days, and 181-365 days.
- 20 109. The method of any of claims 89-107, wherein the future time point is selected from a time point between 1-2 years, 2-5 years, 5-10 years, 10-20 years, and 20-50 years.
110. The method of any of claims 89-109, wherein the anti-2A antibody binds to the epitope DVEENPG (SEQ IN NO: 3).
- 25 111. The method of any of claims 89-110, wherein the anti-2A antibody is the 3H4 or the ABS31 antibody.
112. The method of any of claims 89-111, wherein the anti-2A antibody competes for binding with the 3H4 and/or ABS31 antibody.
113. The method of any of claims 89-112, wherein the anti-2A antibody binds the same epitope as the 3H4 and/or ABS31 antibody.
- 30 114. The method of any of claims 89-93 or 96-113, wherein the IHC is ChipCytometry.