typically involving a secreted complex comprising Granzyme B, perforin and granulysin, inducing tumor cell apoptosis.

Chimeric antigen receptor (CAR) genes encode artificial T cell receptors comprising an extra-cellular tumor antigen binding domain, typically derived from the single-chain antibody variable fragment (scFv) domain of a monoclonal antibody, fused via hinge and transmembrane domains to a cytoplasmic effector domain. The effector domain is typically derived from the CD3-zeta chain of the T cell co-receptor complex, and can also include domains derived from CD28 and/or CD137 receptor proteins. The CAR extra-cellular domain binds the tumor antigen in an MHC-independent manner leading to T cell activation and proliferation, culminating in cytotoxic anti-tumor activity as described for TCR engineered T cells.

To date, at least 15 different tumor antigens have been targeted in clinical trials of engineered T cells. In several trials, anti-tumor activity has been reported. The greatest success has been achieved in hematologic malignancies. For example, adoptive transfer of CAR-T cells engineered to target the B cell antigen, CD19, led to multiple partial and complete responses in subjects with lymphoma, acute lymphoblastic leukemia, acute lymphocytic leukemia and B-cell acute lymphocytic leukemia. In contrast, trials targeting other tumor types, especially solid tumors, including renal cell carcinoma, neuroblastoma, colorectal cancer, breast cancer, ovarian cancer, melanoma, sarcoma and prostate cancer, have been less successful. In many of these trials, very few patients experienced objective responses. Thus, there is a need to improve the anti-tumor efficacy of adoptively transferred engineered T cells.

SUMMARY

In one aspect, provided herein is a modified cell comprising a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and a gRNA molecule that targets T cell receptor alpha (TRAC) or T cell receptor beta (TRBC), wherein the gRNA molecule comprises a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002.

In another aspect, provided herein is a modified cell comprising a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and a gRNA molecule, wherein the complex localizes to a site in the TRAC or TRBC locus, which site comprises a target domain and an NGG sequence, wherein the gRNA molecule comprises a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002.

In another aspect, provided herein is an *ex vivo* method of editing a cell, comprising contacting the cell with, or delivering to the cell, a composition comprising a non-viral RNP complex comprising:
(a) a gRNA molecule comprising a targeting domain that is complementary to a target sequence on a target nucleic acid comprising the TRAC or TRBC gene, wherein the targeting domain comprises a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002; and
(b) an *S. pyogenes* Cas9 polypeptide.

In another aspect, provided herein is an ex vivo method of editing a population of cells, comprising contacting the population of cells with a composition comprising a non-viral RNP complex comprising:
(a) a gRNA molecule comprising a targeting domain that is complementary to a target sequence on a target nucleic acid comprising the TRAC or TRBC gene, wherein the targeting domain comprises a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002; and
(b) an *S. pyogenes* Cas9 polypeptide or a nucleic acid encoding a Cas9 polypeptide, wherein at least 6% of the cells are edited.

In another aspect, provided herein is a population of modified cells, wherein the modified cells comprise a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and a gRNA molecule that targets TRAC or TRBC, wherein the gRNA molecule comprises a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002.

Methods and compositions discussed herein provide for the treatment of cancer using an immunotherapy approach comprising administration of genetically engineered T cells or T cell precursors to a subject. An approach to treat a subject suffering from cancer is to isolate T cells from the subject, genetically modify them to target an antigen expressed by the cancer cells, then re-introduce them into the subject; a process referred to as adoptive cell transfer. Methods to genetically modify T cells include introduction of T cell receptor (TCR) or chimeric antigen receptor (CAR) genes encoding trans-membrane TCR or CAR proteins, respectively, which specifically recognize particular cancer antigens. While not wishing to be bound by theory, it is believed that engagement of the tumor-expressed antigen with the antigen binding domain of the TCR or CAR protein initiates a signaling cascade leading to T cell activation, proliferation and, ultimately, destruction of the cancer cell via a cytotoxic immune response (Kershaw et al., 2013 NatRevCancer 13, 525-541).

Adoptive cell transfer utilizing genetically modified T cells has entered clinical testing as a therapeutic for solid and hematologic malignancies. Results to date have been mixed. In hematologic

CLAIMS

What is claimed is:

1. A modified cell comprising a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and a gRNA molecule that targets T cell receptor alpha (TRAC) or T cell receptor beta (TRBC), wherein the gRNA molecule comprises~~ing~~ a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, ~~which is complementary with~~ a target~~ing~~ domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002~~from the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene~~.
2. A modified cell comprising a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and ~~The~~a gRNA molecule, wherein the complex localizes to a site in the TRAC or TRBC locus, which site ~~of claim 1, wherein the~~comprises a target~~ing~~ domain and an NGG sequence, wherein the gRNA molecule comprises a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002 ~~is configured to provide a cleavage event selected from a double strand break and a single strand break, within 500, 400, 300, 200, 100, 50, 25, or 10 nucleotides of a T cell target knockout position~~.
3. The modified cell~~gRNA molecule~~ of claim 1, wherein the modification comprises a deletion or mutation of all or part of the TRAC or TRBC gene~~targeting domain is configured to target an enzymatically inactive Cas9 (eiCas9) or an eiCas9 fusion protein, sufficiently close to a T cell target knockdown position to reduce, decrease or repress expression of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene~~.
4. The modified cell~~gRNA molecule~~ of claim 1~~3~~, said modified cell further comprising a modification in a second T-cell expressed gene~~wherein the targeting domain is configured to target the promoter region of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene~~.
5. The modified cell~~gRNA molecule~~ of claim 1, wherein expression of TRAC or TRBC in the modified cell is modulated~~the targeting domain comprises a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32~~.
6. ~~The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 1A-F or Tables 13A-K.~~

7. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 2A-I or Tables 14A-K.
8. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 3A-H or Tables 15A-F.
9. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 4A-I or Tables 16A-K.
10. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 5A-I or Tables 17A-K.
11. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 6A-I or Tables 18A-K.
12. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 7A-H, Tables 19A-J, Table 31 or Table 32.
13. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 8A-H or Tables 20A-J.
14. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 9A-I or Tables 21A-K.
15. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 10A-I or Tables 22A-K.
16. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 11A-I or Tables 23A-J.
17. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 12A-I or Tables 24A-K.
18. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 25A-G or Table 29.
19. The gRNA molecule of claim 1, wherein the targeting domain is selected from those in Tables 26A-G or Table 27.
20. The gRNA molecule of any of claims 1-19, wherein the gRNA is a modular gRNA molecule.
21. The gRNA molecule of any of claims 1-19, wherein the gRNA is a chimeric gRNA molecule.
22. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 16 or 17 nucleotides or more in length.
23. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 16 or 17 nucleotides in length.
24. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 18 nucleotides in length.
25. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 19 nucleotides in length.
26. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 20 nucleotides in length.
27. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 21 nucleotides in length.

28. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 22 nucleotides in length.

29. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 23 nucleotides in length.

30. The gRNA molecule of any of claims 1-19, wherein the targeting domain is 24, 25, or 26 nucleotides in length.

31. The gRNA molecule of any of claims 1-30, comprising from 5' to 3':

a targeting domain;

a first complementarity domain;

a linking domain;

a second complementarity domain;

a proximal domain; and

a tail domain.

32. The gRNA molecule of any of claims 1-30, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

33. The gRNA molecule of any of claims 1-30, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

34. The gRNA molecule of any of claims 1-30, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 35 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

35. The gRNA molecule of any of claims 1-30, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

36. A nucleic acid that comprises: (a) sequence that encodes a gRNA molecule comprising a targeting domain that is complementary with a T cell target domain in the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

37. The nucleic acid of claim 36, wherein the gRNA molecule is a gRNA molecule of any of claims 1-35.

38. The nucleic acid of claim 36, wherein the targeting domain is configured to provide a cleavage event selected from a double strand break and a single strand break, within 500, 400, 300, 200, 100, 50, 25, or 10 nucleotides of the T cell target knockout position.

39. The nucleic acid of claim 36, wherein the targeting domain is configured to target an enzymatically inactive Cas9 (eiCas9) or an eiCas9 fusion protein, sufficiently close to a T cell target knockdown

position to reduce, decrease or repress expression of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene.

40. The nucleic acid of claim 36, wherein the targeting domain is configured to target the promoter region of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene.

41. The nucleic acid of claim 36, wherein the targeting domain comprises a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.

42. The nucleic acid of claim 36, wherein the targeting domain is selected from those in Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.

43. The nucleic acid of any of claims 36-42, wherein the gRNA is a modular gRNA molecule.

44. The nucleic acid of any of claims 36-42, wherein the gRNA is a chimeric gRNA molecule.

45. The nucleic acid of any of claims 36-42, wherein the targeting domain is 16 or 17 nucleotides or more in length.

46. The nucleic acid of any of claims 36-42, wherein the targeting domain is 16 or 17 nucleotides in length.

47. The nucleic acid of any of claims 36-42, wherein the targeting domain is 18 nucleotides in length.

48. The nucleic acid of any of claims 36-42, wherein the targeting domain is 19 nucleotides in length.

49. The nucleic acid of any of claims 36-42, wherein the targeting domain is 20 nucleotides in length.

50. The nucleic acid of any of claims 36-42, wherein the targeting domain is 21 nucleotides in length.

51. The nucleic acid of any of claims 36-42, wherein the targeting domain is 22 nucleotides in length.

52. The nucleic acid of any of claims 36-42, wherein the targeting domain is 23 nucleotides in length.

53. The nucleic acid of any of claims 36-42, wherein the targeting domain is 24, 25, or 26 nucleotides in length.

54. The nucleic acid of any of claims 36-54, comprising from 5’ to 3’:

a targeting domain;

a first complementarity domain;

a linking domain;

a second complementarity domain;

a proximal domain; and

a tail domain.

55. The nucleic acid of any of claims 36-54, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

56. The nucleic acid of any of claims 36-54, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

57. The nucleic acid of any of claims 36-54, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 35 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

58. The nucleic acid of any of claims 36-54, comprising:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

59. The nucleic acid of any of claims 36-58, further comprising: (b) sequence that encodes a Cas9 molecule.

60. The nucleic acid of claim 59, wherein the Cas9 molecule is an eaCas9 molecule.

61. The nucleic acid of claim 60, wherein the eaCas9 molecule comprises a nickase molecule.

62. The nucleic acid of claim 60, wherein the eaCas9 molecule forms a double strand break in a target nucleic acid.

63. The nucleic acid of claim 60, wherein the eaCas9 molecule forms a single strand break in a target nucleic acid.

64. The nucleic acid of claim 63, wherein the single strand break is formed in the strand of the target nucleic acid to which the targeting domain of the gRNA molecule is complementary.

65. The nucleic acid of claim 63, wherein the single strand break is formed in the strand of the target nucleic acid other than the strand to which to which the targeting domain of the gRNA is complementary.

66. The nucleic acid of claim 60, wherein the eaCas9 molecule comprises HNH-like domain cleavage activity but has no, or no significant, N-terminal RuvC-like domain cleavage activity.

67. The nucleic acid of claim 60, wherein the eaCas9 molecule is an HNH-like domain nickase.

68. The nucleic acid of claim 60, wherein the eaCas9 molecule comprises a mutation at D10.

69. The nucleic acid of claim 60, wherein the eaCas9 molecule comprises N-terminal RuvC-like domain cleavage activity but has no, or no significant, HNH-like domain cleavage activity.
70. The nucleic acid of claim 60, wherein the eaCas9 molecule is an N-terminal RuvC-like domain nickase.
71. The nucleic acid of claim 60, wherein the eaCas9 molecule comprises a mutation at H840.
72. The nucleic acid of claim 59, wherein the Cas9 molecule is an eiCas9 molecule.
73. The nucleic acid of claim 72, wherein the Cas9 molecule is an eiCas9 fusion protein molecule (e.g., an eiCas9 transcription repressor domain fusion, e.g., an eiCas9 KRAB domain fusion).
74. The nucleic acid of any of claims 36-73, further comprising: (c) sequence that encodes a second gRNA molecule described herein having a targeting domain that is complementary to a second target domain of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.
75. The nucleic acid of claim 74, wherein the second gRNA molecule is a gRNA molecule of any of claims 1-35.
76. The nucleic acid of claim 74, wherein the targeting domain of the second gRNA is configured to provide a cleavage event selected from a double strand break and a single strand break, within 500, 400, 300, 200, 100, 50, 25, or 10 nucleotides of the T cell target knockout position.
77. The nucleic acid of claim 74, wherein the targeting domain of the second gRNA is configured to target an enzymatically inactive Cas9 (eiCas9) or an eiCas9 fusion protein, sufficiently close to a T cell target knockdown position to reduce, decrease or repress expression of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene.
78. The nucleic acid of claim 74, wherein the targeting domain of the second gRNA is configured to target the promoter region of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, or *PTPN6* gene.
79. The nucleic acid of claim 74, wherein the targeting domain of the second gRNA comprises a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.
80. The nucleic acid of claim 74, wherein the targeting domain of the second gRNA is selected from those in Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.

81. The nucleic acid of any of claims 74-80, wherein the second gRNA molecule is a modular gRNA molecule.

82. The nucleic acid of any of claims 74-80, wherein the second gRNA molecule is a chimeric gRNA molecule.

83. The nucleic acid of any of claims 74-80, wherein the targeting domain is 16 or 17 nucleotides or more in length.

84. The nucleic acid of any of claims 74-80, wherein the targeting domain is 16 or 17 nucleotides in length.

85. The nucleic acid of any of claims 74-80, wherein the targeting domain is 18 nucleotides in length.

86. The nucleic acid of any of claims 74-80, wherein the targeting domain is 19 nucleotides in length.

87. The nucleic acid of any of claims 74-80, wherein the targeting domain is 20 nucleotides in length.

88. The nucleic acid of any of claims 74-80, wherein the targeting domain is 21 nucleotides in length.

89. The nucleic acid of any of claims 74-80, wherein the targeting domain is 22 nucleotides in length.

90. The nucleic acid of any of claims 74-80, wherein the targeting domain is 23 nucleotides in length.

91. The nucleic acid of any of claims 74-80, wherein the targeting domain is 24, 25, or 26 nucleotides in length.

92. The nucleic acid of any of claims 74-91, wherein the second gRNA molecule comprises from 5' to 3':
a targeting domain;
a first complementarity domain;
a linking domain;
a second complementarity domain;
a proximal domain; and
a tail domain.

93. The nucleic acid of any of claims 74-92, wherein the second gRNA molecule comprises:
a linking domain of no more than 25 nucleotides in length;
a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and
a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

94. The nucleic acid of any of claims 74-92, wherein the second molecule gRNA molecule comprises:
a linking domain of no more than 25 nucleotides in length;
a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and
a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

95. The nucleic acid of any of claims 74-92, wherein the second gRNA molecule comprises:
a linking domain of no more than 25 nucleotides in length;
a proximal and tail domain, that taken together, are at least 35 nucleotides in length; and
a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

96. The nucleic acid of any of claims 74-92, wherein the second gRNA molecule comprises:

a linking domain of no more than 25 nucleotides in length;

a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and

a targeting domain of 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length.

97. The nucleic acid of any of claims 74-96, further comprising a third gRNA molecule.

98. The nucleic acid of claim 97, further comprising a fourth gRNA molecule.

99. The nucleic acid of claim 36, further comprising: (b) sequence that encodes a Cas9 molecule of any of claims 59-73.

100. The nucleic acid of claim 99, wherein the nucleic acid does not comprise (c) a sequence that encodes a second gRNA molecule.

101. The nucleic acid of claim 100, wherein each of (a) and (b) is present on the same nucleic acid molecule.

102. The nucleic acid of claim 100, wherein the nucleic acid molecule is an AAV vector.

103. The nucleic acid of claims 101, wherein: (a) is present on a first nucleic acid molecule; and (b) is present on a second nucleic acid molecule.

104. The nucleic acid of claim 103, wherein the first and second nucleic acid molecules are AAV vectors.

105. The nucleic acid of claim 36, further comprising:

(b) sequence that encodes a Cas9 molecule of any of claims 59-73; and

(c) sequence that encode a second gRNA molecule of clams 74-96.

106. The nucleic acid of claim 105, wherein each of (a), (b), and (c) are present on the same nucleic acid molecule.

107. The nucleic acid of claim 106, wherein the nucleic acid molecule is an AAV vector.

108. The nucleic acid of claim 105, wherein:

one of (a), (b), and (c) is encoded on a first nucleic acid molecule; and

a second and third of (a), (b), and (c) is encoded on a second nucleic acid molecule.

109. The nucleic acid of claim 108, wherein the first and second nucleic acid molecules are AAV vectors.

110. The nucleic acid of claim 108, wherein: (a) is present on a first nucleic acid molecule; and (b) and (c) are present on a second nucleic acid molecule.

111. The nucleic acid of claim 110, wherein the first and second nucleic acid molecules are AAV vectors.

112. The nucleic acid of claim 108, wherein: (b) is present on a first nucleic acid molecule; and (a) and (c) are present on a second nucleic acid molecule.

113. The nucleic acid of claim 112, wherein the first and second nucleic acid molecules are AAV vectors.

114. The nucleic acid of claim 108, wherein: (c) is present on a first nucleic acid molecule; and (b) and (a) are present on a second nucleic acid molecule.

115. The nucleic acid of claim 114, wherein the first and second nucleic acid molecules are AAV vectors.

116. The nucleic acid of any of claims 103, 108, 110, 112, or 114, wherein the first nucleic acid molecule is other than an AAV vector and the second nucleic acid molecule is an AAV vector.

117. The nucleic acid of any of claims 36-58, wherein the nucleic acid comprises a promoter operably linked to the sequence that encodes the gRNA molecule of (a).

118. The nucleic acid of any of claims 74-96, wherein the nucleic acid comprises a second promoter operably linked to the sequence that encodes the second gRNA molecule of (c).

119. The nucleic acid of any of claims 117 or 118, wherein the promoter and second promoter differ from one another.

120. The nucleic acid of any of claims 117 or 118, wherein the promoter and second promoter are the same.

121. The nucleic acid of any of claims 59-73, wherein the nucleic acid comprises a promoter operably linked to the sequence that encodes the Cas9 molecule of (b).

122. The nucleic acid of any of claims 36-96, further comprising a gRNA molecule targeting a second gene selected from the group consisting of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

123. The nucleic acid of any of claims 36-96, further comprising a gRNA molecule targeting at least one additional gene selected from the group consisting of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

124. A composition comprising the (a) gRNA molecule of any of claims 1-35.

125. The composition of claim 124, further comprising (b) a Cas9 molecule of any of claims 59-73.

126. The composition of any of claims 124 or 125, further comprising (c) a second gRNA molecule of any of claims 74-96.

127. The composition of claim 126, further comprising a third gRNA molecule.

128. The composition of claim 127, further comprising a fourth gRNA molecule.

129. A composition comprising at least two gRNA molecules to target two or more of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

130. The composition of claim 129, wherein the at least two gRNA molecules to two or more of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes comprise a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables

~~15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.~~

~~131. The composition of claim 129, wherein the at least two gRNA molecules to two or more of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes are selected from those in Tables 1A-I, Tables 2A-I, Tables 3A-H, Tables 4A-I, Tables 5A-I, Tables 6A-I, Tables 7A-H, Tables 8A-H, Tables 9A-I, Tables 10A-I, Tables 11A-I, Tables 12A-I, Tables 13A-K, Tables 14A-K, Tables 15A-F, Tables 16A-K, Tables 17A-K, Tables 18A-K, Tables 19A-J, Tables 20A-J, Tables 21A-K, Tables 22A-K, Tables 23A-J, Tables 24A-K, Tables 25A-G, Tables 26A-G, Table 27, Table 29, Table 31, or Table 32.~~

~~132. The composition of any of claims 127-129, further comprising (b) a Cas9 molecule of any of claims 59-73.~~

~~133. The composition of any of claims 129-132, further comprising (c) a second gRNA molecule of any of claims 74-96 targeting two or more of *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.~~

~~134. The composition of claim 133, further comprising a third gRNA molecule.~~

~~135. The composition of claim 134, further comprising a fourth gRNA molecule.~~

~~136. A method of altering a cell comprising contacting the cell with:~~

~~(a) a gRNA of any of claims 1-35;~~

~~(b) a Cas9 molecule of any of claims 59-73; and~~

~~optionally, (c) a second gRNA molecule of any of claims 74-96.~~

~~137. The method of claim 136, further comprising a third gRNA molecule.~~

~~138. The method of claim 137, further comprising a fourth gRNA molecule.~~

~~139. The method of any of claims 136-138, comprising contacting the cell with (a), (b), and optionally (c).~~

~~140. The method of any of claims 136-139, wherein the gRNA molecule of (a) is selected from any of claims 1-35.~~

~~141. The method of any of claims 136-140, wherein the cell is from a subject suffering from cancer.~~

~~142. The method of any of claims 136-141, wherein the cell is from a subject having cancer or which could otherwise benefit from a mutation at a T cell target position of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.~~

~~143.~~6. The modified cell~~method~~ of ~~any of~~ claim~~s~~ 1~~36-142~~, wherein the cell is a T-~~-~~cell.

~~144.~~7. The modified cell~~method~~ of claim 1~~43~~, wherein the cell is a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a regulatory T cell, a lymphoid progenitor cell, a natural killer cell, or a dendritic cell ~~is an engineered T cell~~.

~~145.~~8. The modified cell~~method~~ of claim 7~~144~~, wherein the ~~engineered~~ T~~-~~cell is a~~n engineered chimeric antigen receptor (CAR)~~ CD8+ T cell.

~~146.~~9. The modified cell~~method~~ of claim 8~~144~~, wherein the CD8+~~engineered~~ T cell is a~~n~~ CD8+ naïve T cell, a central memory T cell, or an effector memory ~~engineered TCR (T-cell receptor)~~ T cell.

~~147.~~10. The modified cell~~method~~ of claim 1~~43~~, wherein the modification is within the first 500 bp of the coding sequence downstream from the start codon~~T cell is engineered to express a TCR or a CAR prior to introducing a T cell target position mutation in one or more of the *FAS, BID, CTLA4, PDCD1, CBLB, PTPN6, TRAC* or *TRBC* gene~~.

~~148.~~11. An~~The~~ *ex vivo* method of editing a cell~~claim 143~~, comprising contacting ~~wherein~~ the ~~T~~ cell with, or delivering to the cell, a composition comprising a non-viral RNP complex comprising:

(a) a gRNA molecule comprising a targeting domain that is complementary to a target sequence on a target nucleic acid comprising the TRAC or TRBC gene, wherein the targeting domain comprises a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002; and

(b) an *S. pyogenes* Cas9 polypeptide ~~is engineered to express a TCR or a CAR after introducing a T cell target position mutation in one or more of the *FAS, BID, CTLA4, PDCD1, CBLB, PTPN6, TRAC* or *TRBC* gene~~.

~~149.~~12. The method of claim 1~~143~~, wherein said contacting results in modification of the target sequence~~the T cell is engineered to express a TCR or a CAR at the same time as introducing a T cell target position mutation in one or more of the *FAS, BID, CTLA4, PDCD1, CBLB, PTPN6, TRAC* or *TRBC* gene~~.

~~150.~~13. The method of claim 11~~136-149~~, wherein the target nucleic acid further comprises an NGG sequence~~contacting is performed *ex vivo*~~.

~~151.~~14. The method of claim ~~136-150~~11, wherein the ~~contacted~~ cell is a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a regulatory T cell, a lymphoid progenitor cell, a natural killer cell, or a dendritic cell ~~is returned to the subject's body~~.

~~152.~~15. An~~The~~ *ex vivo* method of editing a population of cells, comprising contacting the population of cells with a composition comprising a non-viral RNP complex comprising:~~any of claims 136-151, wherein the cell is from a subject that has cancer~~

(a) a gRNA molecule comprising a targeting domain that is complementary to a target sequence on a target nucleic acid comprising the TRAC or TRBC gene, wherein the targeting domain comprises a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002; and

(b) an *S. pyogenes* Cas9 polypeptide or a nucleic acid encoding a Cas9 polypeptide, wherein at least 6% of the cells are edited.

153.16. The method of ~~any of~~ claim~~s~~ 15~~136-152~~, wherein the target nucleic acid further comprise~~s~~~~ing~~ an NGG sequence~~acquiring knowledge of the sequence of the T cell target position in the cell~~.

154.17. The method of claim 15~~153~~, wherein the cells are T cells~~comprising acquiring knowledge of the sequence of the T cell target position in the cell by sequencing a portion of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene~~.

155.18. The method of ~~any of~~ claim~~s~~ 15~~136-154~~, wherein the cell is a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a regulatory T cell, a lymphoid progenitor cell, a natural killer cell, or a dendritic cell~~comprising inducing a T cell target position mutation~~.

156.19. A population of modified cells~~The method of any of claims 136-155~~, wherein the modified cells comprise a modification in the TRAC or TRBC gene made by delivering a non-viral RNP complex comprising an *S. pyogenes* Cas9 polypeptide and a gRNA molecule that targets TRAC or TRBC, wherein the gRNA molecule comprises a targeting domain comprising a sequence that is the same, or differs by no more than 3 nucleotides from, a targeting domain sequence selected from any one of SEQ ID NOs: 49274-49291 or 49951-50002~~contacting comprises contacting the cell with a nucleic acid that encodes at least one of (a), (b), and (c)~~.

~~157. The method of any of claims 136-156, wherein contacting comprises contacting the cell with a nucleic acid of any of claims 36-123.~~

~~158. The method of any of claims 136-157, wherein contacting comprises delivering to the cell the Cas9 molecule of (b) and a nucleic acid which encodes (a) and optionally (c).~~

~~159. The method of any of claims 136-158, wherein contacting comprises delivering to the cell the Cas9 molecule of (b), the gRNA molecule of (a) and optionally the second gRNA molecule of (c).~~

~~160. The method of any of claims 136-159, wherein contacting comprises delivering to the cell the gRNA molecule of (a), optionally the second gRNA molecule of (c) and a nucleic acid that encodes the Cas9 molecule of (b).~~

~~161. A method of treating a subject, comprising contacting a subject (or a cell from the subject) with:~~

~~(a) a gRNA of any of claims 1-35;~~

~~(b) a Cas9 molecule of any of claims 59-73; and~~

~~optionally, (c) a second gRNA of any of claims 74-96.~~

~~162. The method of claim 161, further comprising a third gRNA molecule.~~

~~163. The method of claim 162, further comprising a fourth gRNA molecule.~~

~~164. The method of any of claims 161-163, further comprising contacting the subject with (a), (b), and optionally (c).~~

~~165. The method of any of claims 161-164, wherein the subject is suffering from cancer.~~

~~166. The method of claim 165, wherein the cancer is selected from the group consisting of:~~

~~lymphoma, chronic lymphocytic leukemia (CLL), B cell acute lymphocytic leukemia (B-ALL), acute~~

lymphoblastic leukemia, acute myeloid leukemia, non-Hodgkin's lymphoma (NHL), diffuse large cell lymphoma (DLCL), multiple myeloma, renal cell carcinoma (RCC), neuroblastoma, colorectal cancer, breast cancer, ovarian cancer, melanoma, sarcoma, prostate cancer, lung cancer, esophageal cancer, hepatocellular carcinoma, pancreatic cancer, astrocytoma, mesothelioma, head and neck cancer, and medulloblastoma.

167. The method of any of claims 161-166, wherein the subject would benefit from a mutation at the T cell target position of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

168. The method of any of claims 161-167, wherein the subject would benefit from a mutation in one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

169. The method of any of claims 161-167, wherein the subject would benefit from a mutation in two or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

170. The method of any of claims 161-167, wherein the subject would benefit from a mutation in three or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

171. The method of any of claims 161-167, wherein the subject would benefit from a mutation in four or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

172. The method of any of claims 161-167, wherein the subject would benefit from a mutation in five or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

173. The method of any of claims 161-167, wherein the subject would benefit from a mutation in six or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

174. The method of any of claims 161-167, wherein the subject would benefit from a mutation in seven or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* and *TRBC* genes.

175. The method of any of claims 161-167, wherein the subject would benefit from a mutation in each of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB* , *PTPN6*, *TRAC* and *TRBC* genes.

176. The method of any of claims 161-175, comprising acquiring knowledge of the sequence of the T cell target position in the subject.

177. The method of claim 176, comprising acquiring knowledge of the sequence of the T cell target position in the subject by sequencing one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene or a portion thereof.

178. The method of any of claims 161-177, comprising inducing a T cell target position mutation in the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

179. The method of any of claims 161-178, wherein a cell of the subject is contacted *ex vivo* with (a), (b), and optionally (c).

180. The method of claim 179, wherein the cell of the subject is a T cell.

181. The method of claim 180, wherein the T cell is engineered to express a TCR or a CAR.

182. The method of claim 180, wherein the T cell is engineered to express a TCR or a CAR prior to inducing a T cell target position mutation in one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

183. The method of claim 180, wherein the T cell is engineered to express a TCR or a CAR after to inducing a T cell target position mutation in one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

184. The method of claim 180, wherein the T cell is engineered to express a TCR or a CAR at the same time as inducing a T cell target position mutation in one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* gene.

185. The method of any of claims 179-184, wherein the cell is returned to the subject's body.

186. The method of any of claims 179-185, wherein treatment comprises introducing a cell into the subject's body, wherein the cell subject is contacted *ex vivo* with (a), (b), and optionally (c).

187. The method of any of claims 161-186, wherein contacting comprises contacting the subject with a nucleic acid that encodes at least one of (a), (b), and (c).

188. The method of any of claims 161-187, wherein contacting comprises contacting the subject with a nucleic acid of any of any of claims 36-123.

189. The method of any of claims 161-188, wherein contacting comprises delivering to the subject the Cas9 molecule of (b) and a nucleic acid which encodes and (a) and optionally (c).

190. The method of any of claims 161-188, wherein contacting comprises delivering to the subject the Cas9 molecule of (b), and the gRNA of (a) and optionally the second gRNA of (c).

191. The method of any of claims 161-188, wherein contacting comprises delivering to the subject the gRNA of (a), optionally the second gRNA of (c) and a nucleic acid that encodes the Cas9 molecule of (b).

192. A reaction mixture comprising a gRNA, a nucleic acid, or a composition described herein, and a cell from a subject having cancer, or a subject which would benefit from a mutation at a T cell target position in one or more of the *FAS*, *BID*, *CTLA4*, *PDCD1*, *CBLB*, *PTPN6*, *TRAC* or *TRBC* genes.

193. A kit comprising, (a) gRNA molecule of any of claims 1-35, or nucleic acid that encodes the gRNA, and one or more of the following:
(b) a Cas9 molecule of any of claims 59-73;
(c) a second gRNA molecule of any of claims 74-96; and
(d) nucleic acid that encodes one or more of (b) and (c).

194. The kit of claim 193, comprising nucleic acid that encodes one or more of (a), (b) and (c).

195. The kit of claim 194, further comprising a third gRNA molecule targeting a T cell target position.

196. The kit of claim 195, further comprising a fourth gRNA molecule targeting a T cell target position.

197. A gRNA of any of claims 1-35, wherein the gRNA comprises a modification at or near its 5' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of its 5' end).

198. A gRNA of any of claims 1-35, wherein the gRNA comprises a modification at or near its 3' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of its 3' end).

199. A gRNA of any of claims 1-35, wherein the gRNA comprises a modification at or near its 5' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of its 5' end) and a modification at or near its 3' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of its 3' end).

200. A gRNA of any of claims 197-199, wherein the modification causes the gRNA to exhibit increase stability towards nucleases when introduced into a T cell.

201. A gRNA of any of claims 197-199, wherein the modification causes the gRNA to exhibit a reduced innate immune response when introduced into a T cell.

202. A gRNA of claim 201, wherein the innate immune response involves the induction of cytokine expression.