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 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.
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 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.
3. The modified cell of claim 1, wherein the modification comprises a deletion or mutation of all or part of the TRAC or TRBC gene.
4. The modified cell of claim 1, said modified cell further comprising a modification in a second T-cell expressed gene.
5. The modified cell of claim 1, wherein expression of TRAC or TRBC in the modified cell is modulated.
6. The modified cell of claim 1, wherein the cell is a T-cell.
7. The modified cell of claim 1, 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.
8. The modified cell of claim 7, wherein the T-cell is a CD8+ T cell.
9. The modified cell of claim 8, wherein the CD8+ T cell is a CD8+ naïve T cell, a central memory T cell, or an effector memory T cell.
10. The modified cell of claim 1, wherein the modification is within the first 500 bp of the coding sequence downstream from the start codon.
11. 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.

12. The method of claim 11, wherein said contacting results in modification of the target sequence.
13. The method of claim 11, wherein the target nucleic acid further comprises an NGG sequence.
14. The method of claim 11, 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.
15. 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.
16. The method of claim 15, wherein the target nucleic acid further comprises an NGG sequence.
17. The method of claim 15, wherein the cells are T cells.
18. The method of claim 15, 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.
19. 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.