

What is claimed is:

1. A modified primary human T cell comprising: at least one nucleic acid sequence comprising at least one heterologous gene non-virally inserted into one or both of: an endogenous T cell receptor alpha subunit constant gene (TRAC); and an endogenous T cell receptor beta subunit constant gene (TRBC), wherein the at least one heterologous gene encodes at least one antigen-specific receptor that specifically binds the target antigen expressed by a cancer, and wherein the at least one nucleic acid sequence is at least 500 bp in size.
2. The T cell of claim 1, wherein the modified human T cell does not comprise a viral vector.
3. The T cell of claim 1 or 2, wherein the at least one nucleic acid sequence is at least 1.5 kb in size.
4. The T cell of any one of claims 1-3, wherein the at least one nucleic acid sequence is non-virally inserted into an exon of the T cell receptor alpha subunit constant gene (TRAC) or into an exon of the T cell receptor beta subunit constant gene (TRBC).
5. The T cell of any one of claims 1-4, wherein the at least one heterologous gene comprises at least one of: (a) a variable region of a heterologous T cell receptor alpha (TCR- $\alpha$ ) chain and (b) a variable region of a heterologous T cell receptor beta (TCR- $\beta$ ) chain.
6. The T cell of any one of claims 1-5, wherein the at least one heterologous gene comprises at least one of:
  - (1) a) a variable region of a heterologous T cell receptor alpha (TCR- $\alpha$ ) chain or b) a variable region and constant region of the heterologous TCR- $\alpha$  chain; and
  - (2) a) a variable region of a heterologous T cell receptor beta (TCR- $\beta$ ) chain or b) a variable region and constant region of the heterologous TCR- $\beta$  chain.
7. The T cell of claim 6, wherein the at least one heterologous gene comprises each of:
  - (1) the a) variable region of the heterologous TCR- $\alpha$  chain or b) variable region and constant region of the heterologous TCR- $\alpha$  chain; and

- (2) the a) variable region of the heterologous TCR- $\beta$  chain or b) variable region and constant region of the heterologous TCR- $\beta$  chain.
8. The T cell of claim 7, wherein one or more coding sequences for the heterologous TCR- $\alpha$  chain and the heterologous TCR- $\beta$  chain are linked by a linker sequence or a multicistronic element.
  9. The T cell of claim 8, wherein the linker sequence is a cleavable linker sequence that is cleaved to generate the heterologous TCR- $\alpha$  chain gene and the heterologous TCR- $\beta$  chain gene.
  10. The T cell of claim 8, wherein the heterologous gene encodes the TCR- $\alpha$  chain and the heterologous TCR- $\beta$  chain and wherein the heterologous gene is inserted into TRAC.
  11. The T cell of any one of claims 1-5, wherein the at least one heterologous gene is non-virally inserted into an endogenous TRAC, wherein the at least one heterologous gene comprises each of:
    - (1) a) a variable region of the heterologous TCR- $\alpha$  chain or b) a variable region and constant region of the heterologous TCR- $\alpha$  chain; and
    - (2) a) a variable region of the heterologous TCR- $\beta$  chain or b) a variable region and constant region of the heterologous TCR- $\beta$  chain,
 wherein the heterologous TCR- $\alpha$  chain and the heterologous TCR- $\beta$  chain are operably linked by a cleavable linker sequence that is cleaved to generate the heterologous TCR- $\alpha$  chain and the heterologous TCR- $\beta$  chain, and wherein the heterologous TCR- $\alpha$  chain and the heterologous TCR- $\beta$  form an antigen-specific T cell receptor (TCR) that recognizes the target antigen expressed by the cancer.
  12. The T cell of any one of claims 1-11, wherein expression of the at least one heterologous gene is under the control of an endogenous promoter.
  13. The T cell of any one of claims 1-12, wherein the modified human T cell is a CD8+ T cell or a CD4+ T cell.

14. The T cell of claim 13, wherein the CD8+ T cell or CD4+ T cell is an effector T cell or a naïve T cell.
15. The T cell of any one of claims 1-14, wherein the primary human T cell is obtained from a subject having cancer and modified to comprise the at least one nucleic acid sequence comprising at least one heterologous gene.
16. A population of cells comprising a plurality of the primary human T cell of any one of claims 1-15.
17. The T cell of any one of claims 1-15 or the population of cells of claim 16 for use in therapy.
18. Use of the T cell of any one of claims 1-15 or the population of cells of claim 16 in the manufacture of a medicament for treating a cancer that expresses the target antigen.