

CLAIMS

1. An immune cell expressing a chimeric antigen receptor (CAR),

wherein the CAR comprises an intracellular signaling domain comprising a signaling domain or fragment of a CD28 protein that is functional in the absence of lymphocyte-specific protein tyrosine kinase (LCK), and

wherein the immune cell ~~has been modified~~ comprises or has been contacted with a nucleic acid sequence capable of downregulating or eliminating gene expression of LCK, such that the expression and/or function of LCK has been reduced or eliminated.

- ~~2. The immune cell of claim 1, wherein the intracellular signaling domain comprises a signaling domain or fragment of a CD28 protein that is functional in the absence of LCK.~~

- ~~3.2.~~ The immune cell of claim 1, wherein the immune cell is a T cell or an NK cell.

- ~~4. The immune cell of claim 1, wherein the immune cell comprises or has been contacted with an inhibitor of LCK.~~

- ~~5. The immune cell of claim 4, wherein the inhibitor of LCK is a nucleic acid sequence capable of downregulating or eliminating gene expression or modifying the function of LCK.~~

- ~~6.3.~~ The immune cell of claim ~~5~~1, wherein the nucleic acid sequence capable of downregulating gene expression of LCK is selected from the group consisting of an antisense RNA, antagomir RNA, siRNA, shRNA, a CRISPR system, a zinc finger nuclease system and a transcription activator-like effector based nuclease (TALEN) system.

- ~~7. The immune cell of claim 4, wherein the inhibitor of LCK is an inhibitor of LCK protein.~~

- ~~8. The immune cell of claim 7, wherein the inhibitor of LCK is selected from the group consisting of Aminoquinazolin, A 420983, A770041, Dasatinib, Saractinib and Masatinib.~~
- ~~9.4.~~ The immune cell of claim 1, wherein the immune cell comprises a vector comprising a nucleic acid encoding the CAR.
- ~~10.5.~~ The immune cell of claim 1, wherein the immune cell comprises a vector comprising a nucleic acid encoding an inhibitor of LCK capable of downregulating or eliminating gene expression or modifying the function of LCK.
- ~~11.6.~~ The immune cell of claim 1, wherein the immune cell comprises a vector comprising a nucleic acid encoding the CAR and a nucleic acid encoding an inhibitor of LCK capable of downregulating or eliminating gene expression of LCK.
- ~~12.7.~~ The immune cell of claim 1, wherein the CAR comprises an extracellular antigen-binding domain.
- ~~13.8.~~ The immune cell of claim 1, wherein the intracellular signaling domain further comprises a primary-signaling domain comprising a functional signaling domain of a protein selected from CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, FcR gamma, Fc epsilon RI beta, CD79a, CD79b, Fc gamma RIIa, DAP10, or DAP12.
- ~~14. The immune cell of claim 1, wherein the intracellular signaling domain further comprises a costimulatory domain comprising a functional signaling domain of a protein selected from the group consisting of DAP10, CD28, CARD11, SLAMF1, LCK1, LCK3, LAT, OX40, CD27, CDS, ICAM 1, LFA 1 (CD11a/CD18), ICOS (CD278), and 4 1BB (CD137).~~
- ~~15. The immune cell of claim 1, wherein the immune cell has reduced PD 1 expression as compared to a cell that has not been modified such that the expression and/or function of LCK has been reduced or eliminated.~~
- ~~16.9.~~ An immune cell expressing a CAR, wherein the immune cell has been modified such that expression ~~or function~~ of the LCK gene has been disrupted.

~~17.10.~~ 10. A method of manufacturing ~~the~~^{an} immune cell of claim 1, the method comprising contacting an immune cell with an inhibitor of LCK for a sufficient time and under conditions to ~~reduce~~^{downregulate} or eliminate the gene expression and/or function of LCK, wherein the inhibitor of LCK is a nucleic acid sequence.

~~18.11.~~ 11. The method of claim ~~17~~10, wherein the inhibitor of LCK is a nucleic acid sequence capable of downregulating gene expression of LCK.

~~19.12.~~ 12. The method of claim ~~18~~11, wherein the inhibitor of LCK is a CRISPR system.

~~20.13.~~ 13. The method of claim ~~17~~10, wherein the method further comprises introducing a nucleic acid encoding a CAR into the immune cell.

~~21.14.~~ 14. A vector system comprising 1) a vector comprising a nucleic acid sequence encoding an inhibitor of LCK and 2) a vector comprising a nucleic acid sequence encoding a CAR.

~~22.15.~~ 15. A vector comprising a nucleic acid sequence encoding an inhibitor of LCK and a nucleic acid sequence encoding a CAR.

~~23.~~ 16. ~~The vector of claim 22, wherein the nucleic acid sequence encoding a CAR is an inhibitor of LCK.~~

~~24.16.~~ 16. An *ex vivo* method of improving the efficacy of a CAR-expressing immune cell in a cell therapy, the method comprising contacting the CAR-expressing immune cell with an inhibitor of LCK for a sufficient time and under conditions to reduce or eliminate the gene expression and/or function of LCK, wherein the inhibitor of LCK is a nucleic acid sequence.

~~25.17.~~ 17. The method of claim ~~24~~16, wherein the CAR-expressing immune cell is a Car-expressing T (CART) cell.

~~26.~~ 18. ~~The method of claim 24, wherein the off-target effects of the CAR-expressing immune cell are reduced.~~

~~27. The method of claim 24, wherein the exhaustion phenotype in the CAR-expressing cell is reduced.~~

~~28. The method of claim 24, wherein the memory of the CAR-expressing immune cell is improved.~~

~~29. A method of treating a subject in need thereof, the method comprising administering an immune cell according to any one of claims 1-16 for a sufficient time and under conditions to treat the subject.~~

~~30. The method of claim 29, wherein the subject has a disease associated with expression of a tumor antigen (e.g., a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of the tumor antigen).~~

~~31.~~18. An immune cell according to any one of claims 1-~~16~~9 for use in treating a subject in need thereof.

~~32.~~19. Use of an immune cell according to any one of claims 1-~~16~~9 in the manufacture of a medicament for treating a subject in need, wherein the subject has a disease associated with expression of an antigen, in particular a proliferative disease, a precancerous condition, a cancer, or a non-cancer related indication associated with expression of the tumor antigen, an infectious disease or an autoimmune disease.