CLAIMS

1. A method of generating a composition comprising cytotoxic T cells (CTLs) that comprise (a) a T cell receptor (TCR) that binds to a first antigen, and (b) a chimeric antigen receptor (CAR) that binds to a second antigen, said method comprising:

(i) preparing a culture of cells comprising human peripheral blood mononuclear cells (PBMCs);

(ii) co-culturing said culture comprising PBMCs with human B cell lymphoblastoid cells (BLCLs) that have been engineered to express said first antigen;

(iii) maintaining said culture comprising PBMCs and BLCLs for a time sufficient to induce the selective proliferation of CTLs comprising a TCR that binds to said first antigen;

(iv) transducing the CTLs of said culture with a viral vector comprising a nucleic acid sequence encoding a CAR that binds to said second antigen, thereby providing CTLs that comprise (a) a TCR that binds to said first antigen and (b) a CAR that binds to said second antigen;

(v) culturing the CAR-expressing first-antigen-specific CTLs to allow proliferation of such CTLs;

(vi) optionally re-culturing said CAR-expressing, first-antigen-specific CTLs one or more times with BLCLs that have been engineered to express said first antigen, thereby further sensitizing said CAR-expressing first-antigen-specific CTLs to said first antigen; and

(vii) harvesting a composition comprising CTLs that comprise (a) a TCR that binds to said first antigen, and (b) a CAR that binds to said second antigen.

2. A method of preparing an adoptive immunotherapy composition comprising cytotoxic T cells (CTLs) that comprise (a) a T cell receptor (TCR) that binds to a first antigen and, (b) a chimeric antigen receptor (CAR) that binds to a second antigen, said method comprising:

(i) preparing a culture of cells comprising human peripheral blood mononuclear cells (PBMCs);

(ii) co-culturing said culture comprising PBMCs with human B cell lymphoblastoid cells (BLCLs) that have been engineered to express said first antigen;

(iii) maintaining said culture comprising PBMCs and BLCLs for a time sufficient to induce the selective proliferation of CTLs comprising a TCR that binds to said first antigen;

(iv) transducing the CTLs of said culture with a viral vector comprising a nucleic acid sequence encoding a CAR that binds to said second antigen, thereby providing CTLs that comprise (a) a TCR that binds to said first antigen and (b) a CAR that binds to said second antigen;

(v) culturing the CAR-expressing first-antigen-specific CTLs to allow proliferation of such cells;

(vi) optionally re-culturing said CAR-expressing, first-antigen-specific CTLs one or more times with BLCLs that have been engineered to express said first antigen, thereby further sensitizing said CAR-expressing first-antigen-specific CTLs to said first antigen; and

(vii) harvesting a composition comprising CTLs that comprise (a) a TCR that binds to said first antigen, and (b) a CAR that binds to said second antigen, thereby providing said adoptive immunotherapy composition.

3. The method of claim 1 or 2, further comprising incubating the culture of any of steps (i), (ii), (iii), (v), or (vi), or any combination thereof, with one or more cytokines.

4. The method of claim 3, wherein the one or more cytokines comprises IL-2.

5. The method of any one of claims 1-4, wherein said co-culturing of step (ii) is performed at a ratio of PBMCs to BLCLs of approximately 20:1.

6. The method of any one of claims 1-5, wherein the transducing of step (iv) occurs approximately three (3) days after the co-culturing of step (ii).

7. The method of any one of claims 1-6, comprising re-culturing step (vi).

8. The method of any one of claims 1-7, wherein step (vi) comprises re-culturing said CAR-expressing first-antigen-specific CTLs with BLCLs that have been engineered to express said first antigen approximately eight (8) days after said transducing step (iv).

9. The method of claim 7 or 8 wherein said re-culturing step (vi) is performed at a ratio of CTLs to BLCLs of approximately 0.2 to 1.

10. The method of any one of claims 7 to 9, wherein immediately prior to the re-culturing step (vi), CD56+ cells are depleted from the cell culture.

11. The method of any one of claims 1 to 10, wherein step (vi) comprises

(a) re-culturing said CAR-expressing first-antigen-specific CTLs with BLCLs that have been engineered to express said first antigen approximately eight (8) days after said transduction step (iv), and

(b) again re-culturing said CAR-expressing first-antigen-specific CTLs with BLCLs that have been engineered to express said first antigen at least fourteen (14) days after said transduction step (iv).

12. The method of claim 11, wherein said re-culturing step (a) is performed at a ratio of CTLs to BLCLs of approximately 0.2 to 1 and said re-culturing step (b) is performed at a ratio of CTLs to BLCLs of approximately 4:1.

13. The method of claim 11 or 12, wherein immediately prior to the re-culturing step (a), CD56+ cells are depleted from the cell culture.

14. The method of any one of claims 1 to 13, wherein the PBMCs and BLCLs are derived from the same donor.

15. The method of any one of claims 1 to 14, wherein the BLCLs are gamma irradiated prior to co-culturing with said PBMCs.

16. The method of any one of claims 1 to 15, wherein the BLCLs are infected with a virus comprising at least one immunogenic peptide antigen, and wherein said at least one immunogenic peptide antigen comprises said first antigen.

17. The method of claim 16, wherein said first antigen is an Epstein-Bar virus (EBV), cytomegalovirus (CMV), human papilloma virus (HPV), BK virus (BKV), John-Cunningham virus (JCV), Merkel cell virus (MCV), human T-lymphotropic virus (HTLV) or human immunodeficiency virus (HIV) antigen.

18. The method of ~~any one of~~ claim~~s~~ 16 ~~t~~o<u>r</u> 17, wherein said first antigen comprises an EBV LMP1 peptide or fragment thereof, an EBV LMP2A peptide or fragment thereof, or an EBV EBNA1 peptide or fragment thereof.

19. The method of any one of claims 1 to 18, comprising the step of cryopreserving said CAR-expressing first-antigen-specific CTLs for administration to a patient in need at a future date.

20. The method of any one of claims 1 to 19, wherein the viral vector comprising the nucleic acid sequence encoding the CAR is a lentiviral or retroviral vector, such as a replication-incompetent lentiviral or retroviral vector.

21. The method of any one of claims 1 to 20, wherein the CAR comprises one or more signal transduction domains, optionally wherein the one or more signal transduction domains comprise a CD28 signaling domain, a 4-1BB signaling domain, a CD3 signaling domain, or a variant or fragment thereof.

22. The method of claim 21, wherein the CAR comprises a variant CD3ζ domain lacking at least one functional ITAM region.

23. The method of claim 21, wherein the CAR comprises a Mut06 domain.

24. The method of any one of claims 1 to 23, wherein the viral vector comprising the nucleic acid sequence encoding the CAR further comprises a nucleic acid sequence encoding a selectable marker, such as a selectable marker that confers resistance to the antibiotic blasticidin.

25. A method for improving the proliferative capacity of CAR-expressing antigen-specific CTLs, comprising performing the method of any one of the preceding claims.

26. A method for improving the transduction efficiency of antigen-specific CTLs, comprising performing the method of any one of the preceding claims.

27. A method for improving the viability of CAR-expressing antigen-specific CTLs, comprising performing the method of any one of the preceding claims.

~~28. Use of the CAR-expressing, first-antigen-specific CTLs obtained by the method of any one of the preceding claims in the manufacture of a medicament for the treatment of a disorder associated with expression of a peptide antigen in a patient in need thereof.~~

28~~29~~. A composition comprising CAR-expressing antigen-specific CTLs prepared by the method of any one of the preceding claims.