

tMUC1 CAR T cells to express more IFN $\gamma$  and thus release IFN $\gamma$  in the absence of the antigen that tMUC1 CAR binds (tMUC1).

[00346] FIG. 39 provides a histogram showing GZMB release by tMUC1 CAR T cells and CD19 CAR T cells. Various cells were cultured on Day 7, and flow cytometry assays were performed on Day 8. Flow cytometry assays showed GZMB release by the activated CD19 CAR T cells and MUC1 CAR T cells. The statistical analysis of the convective graphs (proportional comparison MFI) indicates that the activation of CD19 CAR T cells can cause MUC1 CAR T cells to release GZMB, and such release was enhanced in the presence of PBMC. The mCAR-group is not all CD19 CAR positive cells, and the statistical value is relative. These results show that activation of CD19 CAR T cells induced MUC1 CAR T cells to release intracellular GZMB.

[00347] FIG. 40 and 41 show proliferation of MUC1 CAR T cells in various embodiments. CFSE reactions were performed and used to indicate levels of cell proliferation. Various cells were cultured on Day 7, and flow cytometry assays were performed on Day 8. As shown on FIG. 40, the first row is the experimental group of coupled CAR T cells co-cultured with two substrate cells, and the second row is the control group of MUC1 CAR T cells co-cultured with two substrate cells. As shown in the third and fourth columns of the first and second rows, activation of CD19 CAR T cells with K19 induces the proliferation of MUC1 CAR T cells. The fifth and sixth columns show that MCF-7 activates and includes the proliferation of MUC1CAR T cells. FIG. 41 shows counting results from the flow cytometry shown in FIG. 40. The volume calibration was performed, tMUC1 CAR cell population was gated, and the number of cells of each group of tMUC1 CAR was statistically analyzed. As shown in FIG. 41, the number of cells in the group including CD19 CAR T cells and tMUC1CAR T cells was higher than that in the control group, and the proliferation of the group including CD19 CAR T cells and tMUC1 CAR T cells in the presence of PBMC was the highest. The results show that activation of CD19 CAR T cells can enhance the proliferation of MUC1 CAR T cells, which can be enhanced and/or mediated through PBMC.

[00348] FIG. 42 shows the proliferation CD19 CART cells in various embodiments. CFSE reactions were performed and used to indicate levels of cell proliferation. Various cells were cultured on Day 7, and flow cytometry assays were performed on Day 8. The groups of cells comprising CD19 CAR T cells, tMUC1 CAR T cells, MCF-7 in the presence or absence of PBMC showed the proliferation of CD19 CAR T cells. These results show that activation of tMUC1 CAR T cells can enhance the proliferation of CD19 CAR T cells, which may be enhanced and/or mediated through PBMC. The combination of the results shown in FIGS. 40-42 indicates that the mixture of CD19 CAR T cells and tMUC1 CAR T cells may form a positive circle through PBMC such that activation of CD19 CAR T cells or tMUC1 CAR T cells may further activate each other to enhance the proliferation of CD19 CAR T cells and tMUC1 CAR T cells and/or the release of cytokines by CD19 CAR T cells and tMUC1 CAR T cells, which may be mediated and/or enhanced by PBMC (See FIG. 62). These results can also explain the reason that tMUC1 CAR T cells expanded more in subjects infused with a population of cells comprising coupled CAR T

CAR T cells co-cultured with two substrate cells, and the second row comprising the control group CD19 CAR T cells co-cultured with two substrate cells. FIG. 57 shows that activation of CLDN18.2 CAR T cells with KATO3+ cells can induce the proliferation of CD19 CAR T cells. The fifth and sixth columns show that PBMC can further enhance proliferation of CD19 CAR T cells. The results demonstrate that CLDN18.2 CAR T cells were activated by KATO3+ cells in the coupled CAR group and activated CLDN18.2 CAR T cells can activate CD19 CAR T cells to promote the proliferation of CD19 CAR T cells, which can be further enhanced by PBMC.

[00362] FIGS. 58-60 show cytokine release in various cell cultures. Various cells were cultured on Day 7, and flow cytometry assays were performed on Day 8. As shown, limited amounts of IL12, IFN $\gamma$  and GZMB were released in the control group. The coupled CAR T cell group and single CAR T cell group were labeled with solid line and dotted line, respectively. The amount of IL12, IFN $\gamma$  and GZMB released is similar in the absence of PBMC. When PBMC was added, the amount of IL12, IFN $\gamma$  and GZMB released increased.

Table 20: CAR T cells and substrate cells used in Group 4

| Coupled CAR T cells | CD19 CAR T cells & ACPP CAR cells |                |                      |
|---------------------|-----------------------------------|----------------|----------------------|
| CAR T Cell ID       | CAR                               | Substrate Cell | Notes                |
| 6404                | BCMA CAR                          | 8226           | BCMA positive Cell   |
| 6701                | GUCY2C CAR                        | T84            | GUCY2C positive cell |

[00363] FIG. 84 shows other histograms of CD137 expression in various cell cultures. Peripheral blood of healthy volunteers was collected on Day 0. CD3+ T cells were sorted and collected using Pan T kits, and CD3/CD28 Dynabeads were added at a 1:1 ratio to the collected CD3+ T cells. On Day 1, CD3+ T cells were transfected with lentivirus encoding BCMA CAR and GUCY2C CAR, respectively. The binding domains of BMCA CAR and GUCY2C CAR include SEQ ID NOs: 60 and 488, respectively. On Day 2, the lentivirus and the Dynabeads were removed, and fresh media were added. On Day 7, CAR T cells and target cells (e.g., 8226) were co-cultured for 24 hours and various assays were performed on Day 8. Flow cytometry assays were performed, and the results show expression of BMCA CAR and GUCY2C CAR T cells. As shown in FIG. 84, the activation of the GUCY2C CAR T cells was higher, and the activation was increased in the presence of PBMC. These results show activation of BCMA CAR T cells by 8226 can activate GUCY2C CAR T cells, and this effect is enhanced by PBMC. Since PBMC includes B cells and plasma cells that include BCMA, PBMC can activate BCMA CAR T cells. The activation of BCMA CAR T cells by PMBC is enhanced by GUCY2C CAR T cells.

[00364] FIG. 85 shows the proliferation of GUCY2C CAR T cells. Various cells were cultured on Day 7, and flow cytometry assays were performed on Day 8. Further, CFSE reaction was measured to evaluate levels of proliferation. PMBC includes B cells and plasma cells, which include BCMA. As shown in FIG. 85, the activation of BCMA CAR T cells with PMBC can induce the proliferation of GUCY2C CAR T cells.

## CLAIMS

1. Use of a composition in the manufacture of a medicament for enhancing expansion of cells in a subject having colorectal cancer, wherein the composition comprises a first population of T cells comprising a first chimeric antigen receptor (CAR) binding CD19 or BCMA, and a second population of T cells comprising a second CAR binding GUCY2C, the first and second CAR each comprising an extracellular antigen-binding domain, a transmembrane domain, a co-stimulatory domain, and a CD3 zeta signaling domain,

wherein the medicament is to be administered to the subject to allow the first and second population of T cells to expand, and

wherein expansion of the second population of T cells in the subject is enhanced as compared to a subject to be administered a composition comprising the second population of T cells without the first population of T cells.

2. The use of claim 1, wherein the first CAR binds CD19.

3. The use of claim 1 or 2, wherein the extracellular antigen-binding domain of the first CAR comprises SEQ ID NO: 5.

4. The use of any one of claims 1-3, wherein the extracellular antigen-binding domain of the second CAR comprises SEQ ID NO: 11.

5. The use of any one of claims 1-4, wherein the co-stimulatory domain comprises an intracellular domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a combination thereof.

6. The use of any one of claims 1-5, wherein the co-stimulatory domain comprises an intracellular domain of 4-1BB.

7. The use of claim 6, wherein the co-stimulatory domain comprises SEQ ID NO: 127.

8. The use of any one of claims 1-7, wherein the transmembrane domain comprises a transmembrane domain of CD8, CD4, CD28, CD27, CD25, or 4-1BB.

9. The use of any one of claims 1-8, wherein the transmembrane domain comprises a transmembrane domain of CD8.

10. The use of claim 9, wherein the transmembrane domain comprises SEQ ID NO: 37, SEQ ID NO: 40, or SEQ ID NO: 126.

11. The use of claim 10, wherein the transmembrane domain comprises SEQ ID NO: 37.

12. The use of any one of claims 1-11, wherein each of the first CAR and the second CAR further comprises a hinge domain, which comprises SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 125.

13. The use of claim 12, wherein the hinge domain comprises SEQ ID NO: 35.

14. The use of any one of claims 1-13, wherein each of the first CAR and the second CAR comprises SEQ ID NO: 30.

15. The use of any one of claims 1-4, 6, 7, 9, 11, and 13, wherein the first CAR comprises an anti-CD19 scFv comprising SEQ ID NO: 5, a hinge domain comprising SEQ ID NO: 35, a transmembrane domain comprising SEQ ID NO: 37, a co-stimulatory domain comprising SEQ ID NO: 127, and a CD3 zeta signaling domain.

16. The use of any one of claims 1-4, 6, 7, 9, 11, and 13, wherein the second CAR comprises an anti-GUCY2C scFv comprising SEQ ID NO: 11, a hinge domain comprising SEQ ID NO: 35, a transmembrane domain comprising SEQ ID NO: 37, a co-stimulatory domain comprising SEQ ID NO: 127, and a CD3 zeta signaling domain.

17. The use of any one of claims 1-16, wherein the CD3 zeta signaling domain comprises an amino acid sequence encoded by SEQ ID NO: 41.

18. The use of any one of claims 1-17, wherein the first population of T cells comprises a vector encoding the first CAR and a cytokine.

19. The use of claim 18, wherein the cytokine is selected from IL-6, IL-12, IFN- $\gamma$ , and combinations thereof.

20. The use of claim 19, wherein the IL-6 comprises SEQ ID NO: 482.