

SUMMARY

[0006] Provided herein are reagents and processes that can improve the viability and health of cells for use in cell-based therapies and minimize wastage in the manufacturing and drug product process.

5 [0007] Preserving cell viability during the formulation and filling process is critical and challenging. Optimizing formulation compositions and drug product processes are important for maintaining overall cell health and functionality. The instant disclosure provides improved formulations and/or processes that maintain better cell health and viability and allow sufficient time to complete the drug product process. It is especially
10 advantageous for processing engineered cells, such as engineered immune cells, that can be produced in large batches in a drug product process to minimize wastage, while ensuring overall cell health and viability and uniformity of dosage content.

[0008] Thus, in one aspectexample, the present disclosure provides a drug product process that maintains cell health and/or homogeneity of cells. In certain

15 embodimentexamples, the present disclosure provides a drug product process of preparing a drug product comprising engineered immune cells, said method comprising the steps of formulating engineered immune cells in a composition containing a cryopreservative to form a drug product, and mixing and filling the drug product into one or more containers, wherein the drug product maintains homogeneity. In certain embodimentexamples, the step
20 of mixing and filling takes at least about two hours. In certain embodimentexamples, the step of mixing and filling takes about two hours, about three hours, about four hours, about five hours or about 6 hours. In certain embodimentexamples, the mixing comprises intermittent mixing. In some embodimentexamples, the mixing comprises continuous mixing. In certain embodimentexamples, the intermittent mixing is in a shaker flask. In
25 certain embodimentexamples, the intermittent mixing is in a spinner flask. In certain embodimentexamples, the intermittent mixing comprises mixing for about 1 to about 3 minutes, about every 10 to about every 40 minutes. In certain embodimentexamples, the mixing comprises continuous mixing in a spinner flask. In certain embodimentexamples, the mixing comprises continuous mixing in a shaker flask for about two hours. In certain
30 embodimentexamples, the mixing comprises continuous mixing in a shaker flask for no more than two hours. In certain embodimentexamples, the mixing comprises continuous mixing in a shaker flask for less than two hours.

[0008a] In one aspect, the present disclosure refers to a process of preparing a drug product comprising engineered immune cells, said method comprising the steps of formulating engineered immune cells in a composition containing a cryopreservative to form a drug product, and mixing and filling the drug product into one or more containers, wherein the drug product maintains homogeneity.

[0009] In certain embodimentexamples, the mixing and filling step is at about 4 °C. In certain embodimentexamples, the mixing and filling step is at room temperature. In certain embodimentexamples, the mixing is at a speed of about 30 to about 85 rpm. In certain embodimentexamples, the cryopreservative comprises DMSO or glycerol. In certain embodimentexamples, the cryopreservative comprises about 3% DMSO to about 10% DMSO. In certain embodimentexamples, the cryopreservative comprises about 5% DMSO. In certain embodimentexamples, the composition further comprises an excipient that enhances viscosity. In certain embodimentexamples, the engineered immune cells are T cells, inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes, helper T-lymphocytes, effector T-lymphocytes, tumor infiltrating lymphocytes (TILs), NK cells, NK-T-cells, TCR-expressing cells, TCR knockout T cells, dendritic cells, macrophages, killer dendritic cells, mast cells, or B-cells. In certain embodimentexamples, the engineered immune cells are CAR T cells. In certain embodimentexamples, the engineered immune cells are allogeneic CAR T cells. In certain embodimentexamples, the engineered immune cells are autologous CAR T cells. In some embodimentexamples, the engineered immune cells are human cells.

[0010] In another aspectexample, the instant disclosure provides a drug product comprising engineered immune cells prepared by the drug product process disclosed herein.

[0010a] In another aspect, the present disclosure refers to a drug product comprising engineered immune cells prepared by the process as disclosed herein.

[0011] In yet another aspectexample, the instant disclosure provides methods of treating a subject in need of a treatment comprising administering to the subject the drug product disclosed herein. In certain embodimentexamples, the subject is a human.

[0011a] In another aspect, the present disclosure refers to use of the drug product as disclosed herein in the manufacture of a medicament for treating a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** shows an exemplary workflow of final steps of manufacturing of engineered cells for a drug product.

[0013] **FIGs. 2A-2B** show impact of orbital shaker mixing of CAR T cell fomulation on viable cell density (VCD) and cell viability.

[0014] **FIGs. 3A-3B** show impact of orbital shaker mixing and formulaton hold temperature on VCD and viability.

[0015] **FIGs. 4A-4B** show results of time course analysis of cell settling in a cell formulation containing 20E6/ml or 100E6/ml of CAR T cells.

[0016] The data in **FIGs. 5A-5B** compare the impact of continuous shaker flask mixing, intermittent shaker flask mixing with continueous spinner flask mixing on the VCD and

What is claimed is:

1. A process of preparing a drug product comprising engineered immune cells, said method comprising the steps of formulating engineered immune cells in a composition containing a cryopreservative to form a drug product, and mixing and filling the drug product into one or more containers, wherein the drug product maintains homogeneity.
2. The process of claim 1, wherein the step of mixing and filling takes at least about two hours.
3. The process of claim 1 or 2, wherein the step of mixing and filling takes about two hours, about three hours, about four hours, about five hours or about 6 hours.
4. The process of any one of claims 1-3, wherein the mixing comprises intermittent mixing or continuous mixing.
5. The process of claim 4, wherein the intermittent mixing is in a shaker flask.
6. The process of claim 4, wherein the intermittent mixing is in a spinner flask.
7. The process of any one of claims 4-6, wherein the intermittent mixing comprises mixing for about 1 to about 3 minutes, about every 10 to about every 40 minutes.
8. The process of any one of claims 1-4, wherein the mixing comprises continuous mixing in a spinner flask.
9. The process of any one of the preceding claims, wherein the mixing and filling step is at about 4 °C.
10. The process of any one of claims 1-8, wherein the mixing and filling step is at room temperature.
11. The process of any one of the preceding claims, wherein the mixing is at a speed of about 30 to about 85 rpm.
12. The process of any one of the preceding claims wherein the cryopreservative comprises DMSO or glycerol.
13. The process of any one of the preceding claims wherein the cryopreservative comprises about 3% DMSO to about 10% DMSO.
14. The process of claim 13, wherein the cryopreservative comprises about 5% DMSO.
15. The process of any one of the preceding claims wherein the composition further comprises an excipient that enhances viscosity.
16. The process of any one of the preceding claims wherein the engineered immune cells are T cells, inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory

T-lymphocytes, helper T-lymphocytes, effector T lymphocytes, tumor infiltrating lymphocytes (TILs), NK cells, NK-T-cells, TCR-expressing cells, TCR knockout T cells, dendritic cells, macrophages, killer dendritic cells, mast cells, or B-cells.

17. The process of any one of the preceding claims wherein the engineered immune cells are CAR T cells.

18. The process of claim 17 wherein the engineered immune cells are allogeneic CAR T cells.

19. The process of claim 17 wherein the engineered immune cells are autologous CAR T cells.

20. The process of any one of the preceding claims, wherein the engineered immune cells are human cells.

21. A drug product comprising engineered immune cells prepared by the process of any one of claims 1-20.

22. Use of the drug product of claim 21 in the manufacture of a medicament for
~~method of~~ treating a subject in need thereof ~~comprising administering to the subject~~
~~the drug product of claim 21.~~