expression pattern that do not provoke a strong response from the immune system appear on a cancer cell as neoantigens. Tumors formed of cancer cells evolve to produce a tumor microenvironment with immunosuppressive attributes and continue to proliferate, resulting in severely dysfunctional immune systems (Spranger 2015; Blank 2016; Poch 2007; Ohm & Carbone 2002).

[0005] As such, there is a need for new cancer therapies which are designed to treat each patient's unique cancer by targeting their specific neoantigens.

SUMMARY

[0006] In one aspect, the present disclosure refers to a method of generating a population of T cells expressing one or more T cell receptors (TCRs) that specifically bind one or more antigens, comprising: (i). obtaining a blood sample from a subject with cancer or a viral infection; (ii). identifying one or more antigens associated with the cancer or the viral infection; (iii). preparing one or more mRNA molecules encoding the one or more antigens associated with the cancer or the viral infection; (iv). isolating monocytes from peripheral blood mononuclear cells (PBMCs) of the blood sample and preserving a remainder of cells from the sample, the remainder of cells comprising T cells; (v). differentiating the isolated monocytes into dendritic cells; (vi). transfecting the dendritic cells with the one or more mRNA molecules; and (vii). stimulating the T cells from the remainder of cells by contacting them with the transfected dendritic cells, thereby generating a population of T cells that express one or more TCRs that specifically bind the one or more antigens associated with the cancer or the viral infection. In some examples, populations of T cells derived from methods according to various embodiments disclosed herein are disclosed.

[0007] In some examples, there are disclosed methods of generating a population of T cells expressing one or more T cell receptors (TCRs) that specifically bind an antigen, comprising: (i). transfecting a population of dendritic cells with one or more mRNA molecules encoding one or more antigens; and (ii). stimulating a population of naïve T cells by contacting them with the transfected dendritic cells of step (i), thereby generating a population of T cells that express one or more T cells receptors that specifically bind the one or more antigens encoded by the one or more mRNA molecules.

[0008] In some examples, isolated engineered T cells comprising T cell receptors (TCRs) targeting a plurality of cancer neoantigens selected from the neoantigens set forth in Tables 1-9 and 11 are disclosed.

[0009] In some examples, populations of engineered T cells comprising T cell receptors (TCRs) targeting one or more antigens are disclosed, the population comprising less than 5% regulatory T cells, less than 5% exhausted T cells, and more memory T cells than effector T cells.

[0010] In some examples, methods of treating cancer in a subject in need thereof are disclosed, comprising: (i). obtaining a blood sample from the subject; (ii). identifying one or more neoantigens associated with the subject's cancer; (iii). preparing one or more mRNA molecules encoding the one or more neoantigens; (iv). isolating monocytes from peripheral blood mononuclear cells (PBMCs) of the blood sample and preserving a remainder of cells from the sample, the remainder of cells comprising T cells; (v). differentiating the isolated monocytes into dendritic cells; (vi). transfecting the dendritic cells with the one or more mRNA molecules; (vii). stimulating the T cells from the remainder of cells by contacting them with the transfected dendritic cells, thereby generating a population of T cells that express one or more T cells receptors (TCRs) that specifically bind the one or more neoantigens associated with the cancer; and (viii). administering all or a portion of the resultant population of T cells to the subject.

[0011] In some examples, methods of treating cancer in a subject in need thereof are disclosed, comprising: (i). identifying two or more neoantigens associated with the subject's cancer; and (ii). administering to the subject a population of T cells, the population of T cells comprising a plurality of T cells that each express two or more T cell receptors (TCRs) that specifically bind at least two of the two or more neoantigens and further comprise a deletion or disruption in an endogenous β2-microglobulin (B2M) gene.

[0012] In some examples, methods of treating a viral infection in a subject in need thereof are disclosed, comprising: (i). identifying two or more viral antigens associated with the subject's viral infection; and (ii). administering to the subject a plurality of T cells expressing two or more T cell receptors (TCRs) that specifically bind the two or more viral antigens.

[0013] In some examples, methods of transiently expressing one or more pro-inflammatory proteins and/or one or more exogenous enzymes that alter an extracellular matrix in a T cell are disclosed, comprising transfecting the T cell with one or more mRNA molecules encoding the one or more pro-inflammatory proteins and/or the one or more exogenous enzymes that alter an extracellular matrix.

[0014] In some examples, methods of altering a tumor microenvironment in a subject are disclosed, comprising administering to the subject a population of T cells transiently expressing one or more pro-inflammatory proteins and/or one or more exogenous enzymes that alter an extracellular matrix.

[0015] In some examples, methods of preparing a composition comprising dendritic cells encoding and/or expressing one or more neoantigens associated with a subject's cancer are disclosed, comprising: (i). obtaining a blood sample from the subject; (ii). sequencing cell free deoxyribonucleic acid (cfDNA) derived from the blood sample to identify one or more neoantigens associated with the subject's cancer; (iii). preparing an mRNA encoding the one or more neoantigens associated with the subject's cancer or a peptide corresponding to the one or more neoantigens associated with the subject's cancer; (iv). isolating monocytes from peripheral blood mononuclear cells (PBMCs) of the blood sample; (v). differentiating the isolated monocytes into dendritic cells; and (vi). combining the dendritic cells with the mRNA or peptide from step (iii) to obtain dendritic cells encoding and/or expressing the one or more neoantigens associated with the subject's cancer.

[0016] In some examples, compositions comprising one or more T cells encoding and/or expressing a T cell receptor (TCR) that binds to a neoantigen associated with a subject's cancer are disclosed, wherein the one or more T cells comprise one or more CD4+ T cell, one or more CD8+ T cell, one or more CD3+ T cell, and wherein the CD4+ T cells and CD8+ T cells are present in the composition in a ratio of about 1:1, about 1:2, or about 1:4.

[0017] In some examples, compositions comprising one or more T cells encoding and/or expressing a TCR that binds to a neoantigen associated with a subject's cancer are disclosed.

[0018] In some examples, compositions comprising one or more T cells encoding and/or expressing a TCR that binds to one or more

neoantigens associated with a cancer are disclosed, wherein (a) the one or more neoantigens are associated with a specific type of cancer, or (b) the one or more neoantigens are associated with a cancer specific to a subject, and wherein the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, acute myeloid leukemia (AML), melanoma, bladder cancer, hematologic cancer, pancreatic cancer, and glioblastoma.

[0019] In some examples, compositions comprising one or more T cells encoding and/or expressing a TCR that binds to a neoantigen associated with a subject's cancer are disclosed, wherein the one or more T cells comprise $CD4^+$ T cells, a $CD8^+$ T cells, a $CD3^+$ T cells, and wherein the $CD4^+$ T cells and $CD8^+$ T cells are present in the composition in a ratio ranging from about 1:4 to about 1:1, e.g., in a ratio of about 1:1, about 1:2, or about 1:4.

[0020] In some examples, the composition comprises about 80%, by weight, of a total weight of the composition, the one or more T cells encoding and/or expressing the TCR.

[0021] In some examples, the composition comprises less than 20%, by weight, of any cell other than the one or more T cells encoding and/or expressing the TCR.

[0022] In some examples, the one or more T cells is a $CD4^+$ T cell, a $CD8^+$ T cell, a $CD3^+$ T cell, or combination thereof.

[0023] In some examples, the one or more T cells is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, an NK cell, or any combination thereof.

[0024] In some examples, the composition comprises greater than about 70%, by weight, of a total weight of the composition, $CD3^+$ and $CD8^+$ T cells or $CD3^+$ and $CD4^+$ T cells.

[0025] In some examples, the composition comprises greater than about 70%, by weight, of the total weight of the composition, central memory T cells.

[0026] In some examples, the composition comprises greater than about 70%, by weight, of the total weight of the composition, effector memory T cells.

[0027] In some examples, the composition comprises greater than about 70%, by weight, of a total weight of the composition, $CD4^+$ T cells.

[0028] In some examples, the composition comprises greater than about 70%, by weight, of a total weight of the composition, $CD8^+$ T cells.

[0029] In some examples, the composition comprises greater than about 70%, by weight, of a total weight of the composition, $CD3^+$ T cells.

[0030] In some examples, the composition comprises T cells, wherein the T cells display minimal exhaustion markers including PD-1, LAG3, TIM-3, CTLA4, BTLA, TIGIT.

[0031] In some examples, the compositions further comprise a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, and/or pharmaceutically acceptable diluent.

[0032] In some examples, the cells are infused into the patient for treatment or prophylaxis.

[0033] In other examples, the RNA used to make the T cell product can be administered to the same patient before or after the T cell product as a prime boost.

[0034] In some examples, the RNA or T cells can be a neoantigen vaccine.

[0035] In some examples, the neoantigen is one or more of KRAS G12A, KRAS G12C, KRAS G12D, KRAS G12R, KRAS G12S, KRAS G12V, KRAS G13D, KRAS G13C, KRAS Q61K, TP53 E285K, TP53 G245S, TP53 R158L, TP53 R175H, TP53 R248Q, TP53 R248W, TP53 R273C, TP53 273H, TP53 R282W, and TP53 V157F.

[0036] In some examples, methods of treating cancer in a subject in need thereof are disclosed, comprising administering to the subject the composition according to any of the embodiments of the present technology.

[0037] In some examples, methods of preparing a composition comprising T cells encoding and/or expressing a TCR that binds to a neoantigen associated with a subject's cancer are disclosed, the method comprising: (a) obtaining a blood sample from the subject; (b) sequencing cell free deoxyribonucleic acid (cfDNA) derived from the blood sample to identify one or more neoantigens associated with the subject's cancer; (c) preparing a messenger ribonucleic acid (mRNA) encoding the one or more neoantigens associated with the subject's cancer or a peptide corresponding to the one or more neoantigens associated with the subject's cancer; (d) isolating monocytes from the blood sample and preserving a remainder of cells in the blood

sample, wherein the remainder of cells comprise T cells; (e) differentiating the isolated monocytes into dendritic cells ("DCs"); (f) combining the DCs with the mRNA or peptide from (c) to obtain DCs encoding and/or expressing one or more neoantigens associated with the subject's cancer; (g) stimulating the T cells from (d) by contacting the T cells from (d) with the DCs from (f); and (h) obtaining a composition comprising T cells encoding and/or expressing a TCR that binds to the neoantigen associated with the subject's cancer.

[0038] In some examples, the mRNA encodes all neoantigens associated with the subject's cancer.

[0039] In some examples, the mRNA encodes a plurality of neoantigens associated with the subject's cancer.

[0040] In some examples, the peptide further comprises a plurality of peptides that includes all neoantigens associated with the subject's cancer.

[0041] In some examples, the mRNA encodes all common neoantigens associated with the subject's cancer.

[0042] In some examples, the peptide further comprises a plurality of peptides that includes all common neoantigens associated with the subject's cancer.

[0043] In some examples, the neoantigen is a KRAS gene, a TP53 gene, or both.

[0044] In some examples, the neoantigen is one or more of KRAS G12A, KRAS G12C, KRAS G12D, KRAS G12R, KRAS G12S, KRAS G12V, KRAS G13D, KRAS G13C, KRAS Q61K, TP53 E285K, TP53 G245S, TP53 R158L, TP53 R175H, TP53 R248Q, TP53 R248W, TP53 R273C, TP53 273H, TP53 R282W, and TP53 V157F.

[0045] In some examples, the neoantigen is one of listed in **Tables 1-10 and 11**.

[0046] In some examples, the antigens are tumor associated antigens.

[0047] In some examples, the mRNA and/or peptide are at least about 80% pure and/or mRNA has a cap1 5' structure and substitution of chemically modified uracil nucleotides such as 5-methoxy-uracil.

[0048] In some examples, the mRNA and/or peptide comprises less than 20% of any other material.

[0049] In some examples, step (g) is repeated at least once.

[0050] In some examples, differentiating the monocytes into DCs includes contacting the monocytes with a plurality of cytokines.

[0051] In some examples, all or substantially all of the monocytes are differentiated into DCs.

[0052] In some examples, differentiating the monocytes into DCs further comprises maturing the DCs by contacting the DCs with a maturation composition.

[0053] In some examples, the DC's and T cells are cultured in a single or multiple closed system bioreactors.

[0054] In some examples, the RNA can be introduced by nucleofection, preferably 4D nucleofection.

[0055] In some examples, the RNA can be introduced by lipid nanoparticles.

[0056] In other examples, the DC's can be seeded and released from the cartridge in a closed system.

[0057] In some examples, stimulating T cells further comprises introducing cytokines to the cells.

[0058] In some examples, stimulating T cells promotes expansion of $CD4^+$, $CD3^+$, and/or $CD8^+$ T cells.

[0059] In some examples, the full genetic diversity of MHC and TCR present within the patient are used to target multiple neoantigens in a single bioreactor.

[0060] In other examples, the capacity of T cells to kill tumor cells is measured through the killing of cells expressing tumor antigens in a Real time Cell Adhesion assay (RTCA).

[0061] In some examples, the capacity of T cells to kill tumor cells is assayed by killing cells transfected with RNA.

[0062] In other examples, the capacity of T cells to be activated to antigens can be assayed by ELISpot where RNA expresses the antigen targets.

[0063] In some examples, methods of preparing a composition comprising DCs encoding and/or expressing one or more neoantigens associated with a subject's cancer are disclosed, the method comprising: (a) obtaining a blood sample from the subject; (b) sequencing cfDNA derived from the blood sample to identify one or more neoantigens associated with the subject's cancer; (c) preparing an mRNA encoding the one or more neoantigens associated with the subject's cancer or a peptide corresponding to the one or more neoantigens associated with the subject's cancer; (d) isolating monocytes from the blood sample; (e) differentiating the isolated monocytes into DCs; (f) combining the DCs with the mRNA or peptide from (c) to obtain DCs encoding and/or expressing one or more neoantigens associated with the subject's cancer.

[0064] In some examples, methods of treating and/or preventing cancer in a subject in need thereof are disclosed, the method comprising administering to the subject the composition comprising T cells encoding and/or expressing a TCR that binds to a neoantigen associated with a subject's cancer, wherein the T cells are derived from the subject.

[0065] In some examples, the cancer treatment comprises inhibiting cancer cell growth in the subject, reducing a number of cancer cells in the subject, slowing the progression of cancer in the subject, decreasing the likelihood of recurrence of cancer in the subject, or reducing one or more symptoms associated with the cancer in the subject.

[0066] In some examples, the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, AML, melanoma, bladder cancer, hematologic cancer, and glioblastoma.

[0067] In some examples, the cancer comprises a solid tumor.

[0068] In some examples, the subject is administered a dose of T cells between about 1×10^5 to about 5×10^5 cells/kg of the subject's body weight.

[0069] In some examples, the subject's cancer is treated after a first administration of the composition.

[0070] In some examples, the methods further comprise transferring one or more genes into the T cells.

[0071] In some examples, transferring one or more genes into the T cells includes transferring one or more vectors comprising nucleic acids that correspond to the

one or more genes into the T cells, where in the one or more vectors is a lentivirus vector, a plasmid vector, and/or an adenovirus vector.

[0072] In some examples, the methods further comprise treating the T cells with an apoptosis inhibitor such as a Rho kinase (ROCK) inhibitor in step (g) or vaccinia virus B18R recombinant protein.

[0073] In some examples, the ROCK inhibitor is ROCK1 inhibitor, ROCK2 inhibitor, or both.

[0074] In some examples, the methods further comprise stimulating the T cells by seeding the T cells with additional T cells.

[0075] In some examples, the methods further comprise transfecting the T cells with RNA.

[0076] In some examples, the transfection increases the longevity and activity of the T cells through transient expression of molecules.

[0077] In some examples, the methods further comprise using CRISPR, Talen, Zinc Finger, Meganucleases, sleeping beauty or other gene editing technologies to knockout the β2-microglobulin (B2M) gene in T cells.

[0078] In some examples, allogeneic cell products with the β2-microglobulin knockout administered to patients without the need for (or only needing only low levels) conditioning by chemotherapy, radiation or immunosuppressive agents.

[0079] In other examples, they can be administered to manage a patient before administration of an autologous T cell product.

[0080] In some examples, allogeneic cell products with the β2-microglobulin knockout are administered to patients without the need for (or only needing only low levels) conditioning by chemotherapy, radiation or immunosuppressive agents having a longer half-life in the blood than those cells with wild type β2-microglobulin.

[0081] In some examples, allogeneic cell products with the β2-microglobulin knockout demonstrate longer survival in the presence of partial MHC matched or fully mismatched T cells than those cells with wild type β2-microglobulin.

[0082] In still other examples, such allogeneic cell products with the β2-microglobulin knockout demonstrate a longer half-life in a patient's blood.

[0083] In other examples, the T cells can be modified by nucleofection, transfection with lipid nanoparticles or by other means of RNA to enhance the T cell's ability to suppress, overcome or modify a tumor microenvironment. In some embodiments, the introduced nucleic acids results in proinflammatory changes in the tumor microenvironment.

[0084] In some examples, the introduced nucleic acids consist of circularized RNA, self-replicating RNA or chemically synthesized mRNAs, all with or without substituted or modified nucleosides in order to extend the half-life of the introduced RNA for prolonged expression.

[0085] In some examples, this half-life can be 3 to 5 days. In other embodiments, this half-life can be 1 to 3 weeks. In other embodiments, the half-life can be a month, 2 months, 3 months, 6 months, 12 months or anything in between.

[0086] In other examples, T cells nucleofected with such RNA have survival advantages in the tumor microenvironment.

[0087] In other examples, T cells nucleofected with such RNA act as delivery vehicles for such microenvironment modifying molecules across the tumor.

[0088] In other preferred examples, T cells reactive to multiple cancer antigens nucleofected with such RNA act as delivery vehicles for such microenvironment modifying molecules across the heterogenous tumor.

[0089] In other examples, the T cells are stimulated against multiple neoantigens, their T cells single cell sequenced for TCR, and the repertoire of TCR's transfected into fresh T cells.

[0090] In some examples, transferring one or more genes into the T cells includes inserting one or more nucleic acids that correspond to the one or more genes into the genome of the T cells via clustered regularly interspaced short palindromic repeat (CRISPR)-mediated insertion.

[0091] In some examples, the insertion is by a knock out of the endogenous TCR with sequential or simultaneous knock in of the transfected TCR.

[0092] In other examples, the RNA encoding the viral, neoantigens or antigens is transfected directly into the PBMC's using lipid nanoparticle formulations or

nucleofection and T cells are then exposed to cytokines and anti-CD3, anti-CD28 antibody to expand the T cell product.

[0093] In some examples, methods of treating a viral infection in a subject in need thereof are disclosed, the method comprising administering to the subject the composition comprising T cells encoding and/or expressing a TCR that binds to a viral antigen associated with a virus, wherein the T cells are derived from the subject.

[0094] In some examples, the viral antigen is a protein expressed by one or more of cytomegalovirus, Epstein-Barr virus, hepatitis B virus, human papillomavirus, adenovirus, herpes virus, human immunodeficiency virus, influenza virus, human respiratory syncytial virus, vaccinia virus, varicella-zoster virus, yellow fever virus, Ebola virus, coronavirus (e.g., SARS-CoV, MERS-CoV, SARS-CoV-2), Eastern equine encephalitis virus, Polyomavirus hominis1 (BKV), SV40 and Zika virus.

[0095] In some examples, the subject's viral infection is treated after a first administration of the composition.

[0096] In other examples, a vaccine containing antigens from multiple viral proteins.

[0097] In preferred examples, this vaccine is RNA or DNA.

[0098] In other preferred examples, this vaccine targets antigens from multiple viral proteins of SARS-COV-2.

[0099] In preferred examples, the antigens selected reflect the effective clearance response in natural immunity to a virus.

[0100] In other preferred examples, this vaccine targets antigens from multiple viral proteins of SARS-COV-2 including two or more of the following: Cov-2 S, M, N, 3a, 7a, 8.

[0101] In some examples, T cells reactive to viral and neoantigens are both present in the T cell product to treat or prevent cancer.

[0102] In other examples, the T cell products and/or DCs can be manufactured in a closed system.

CLAIMS

I/We claim:

1. A method of generating a population of T cells expressing one or more T cell receptors (TCRs) that specifically bind one or more antigens, comprising:

   (i). obtaining a blood sample from a subject with cancer or a viral infection;

   (ii). identifying one or more antigens associated with the cancer or the viral infection;

   (iii). preparing one or more mRNA molecules encoding the one or more antigens associated with the cancer or the viral infection;

   (iv). isolating monocytes from peripheral blood mononuclear cells (PBMCs) of the blood sample and preserving a remainder of cells from the sample, the remainder of cells comprising T cells;

   (v). differentiating the isolated monocytes into dendritic cells;

   (vi). transfecting the dendritic cells with the one or more mRNA molecules; and

   (vii). stimulating the T cells from the remainder of cells by contacting them with the transfected dendritic cells,

   thereby generating a population of T cells that express one or more TCRs that specifically bind the one or more antigens associated with the cancer or the viral infection.

2. The method of claim 1, wherein the one or more antigens are viral antigens or cancer neoantigens selected from the neoantigens set forth in Table 1-9 and 11.

3. The method of claim 1 or 2, wherein the one or more antigens are identified by sequencing cell free deoxyribonucleic acid (cfDNA) associated with the cancer or the viral infection.

4. The method of claim 3, wherein the sequencing comprises next generation sequencing.

5. The method of any one of claims 1-4, wherein the one or more antigens are about 15 to about 50 amino acids in length.

6. The method of any one of claims 1-5, wherein the mRNA is at least about 80% pure.

7. The method of any one of claims 1-6, wherein the one or more mRNA molecules comprise coding sequences for a plurality of the antigens each separated by a polylinker comprises an amino acid sequence of GGSGGGSS.

8. The method of any one of claims 1-7, wherein the one or more mRNA molecules each comprise a signal peptide, a 5' untranslated region (UTR), a 3' untranslated region (UTR), and/or a polyadenine (poly (A)) tail.

9. The method of any one of claims 1-8, wherein the differentiating the isolated monocytes into dendritic cells of step (v) occurs in media containing one or more cytokines selected from the group consisting of GM-CSF, IL-4, IL-1β, IL-6, TNF-a, and PGE2.

10. The method of any one of claims 1-9, wherein all or substantially all of the monocytes are differentiated into dendritic cells in step (v).

11. The method of any one of claims 1-10, wherein the method further comprises incubating the dendritic cells of step (v) with one or more antigen peptides associated with the cancer or the viral infection prior to step (vii).

12. The method of any one of claims 1-11 wherein the transfecting the dendritic cells with the one or more mRNA molecules of step (vi) is by cation lipid transfection, lipofection, or nucleofection.

13. The method of any one of claims 1-12, wherein the ratio of the dendritic cells to the T cells in step (vii) is about 1:2 to about 1:4.

14. The method of any one of claims 1-17, wherein the stimulating the T cells of step (vii) occurs in media comprising IL-7 and IL-15.

15. The method of any one of claims 1-14, wherein the stimulating the T cells of step (vii) is repeated for 2, 3, 4, or more times.

16. The method of any one of claims 1-15, wherein the method further comprises stimulating the T cells of step (vii) with tetrameric antibodies that bind CD3, CD28, and CD2.

17. The method of any one of claims 1-16, wherein the T cells have a deletion or disruption in an endogenous β2-microglobulin (B2M) gene.

18. The method of any one of claims 1-17, wherein the T cells are further exposed to one or more apoptosis inhibitors selected from the group consisting of 10058-F4, 4'-methoxyflavone, AZD5438, BAG1 (72- end) protein, BAX Inhibiting peptide, BEPP monohydroxychloride, BI-6C9, BTZO, Bongkrekic acid, CTP inhibitor, CTX1 , Calpeptin, Clofarabine, Clusterin nuclear form protein, Combretastatin A4, Cyclic Pifithrin-a hydroxybromide, EM20-25, Fasentin, Ferrostatin-1, GNF-2, IM-54, Ischemin-Calbiochem A cell permeable azobenezene, Liproxstatin-1, MDL28170, Mdivi-1 , Mitochondrial Fusion Promoter, N-Ethylmaleimide, N-Ethylmaleimide, NS3694, NSCI, Necrostatin-1 , Oridonin, PD151746, PDI inhibitor 16F16, Pentostatin, Pifithrin-a, Pifithrin-a p-Nitro Cyclic, Pifithrin-u, S-15176 difumarate, UCF-101 , p53-Snail binding inhibitor GFI25, TW-37, and Z-VAD-FMK, during step (vii).

19. The method of any one of claims 1-18, wherein the T cells are further exposed to one or more Rho-associated protein kinase (ROCK) inhibitors selected from the group consisting of Y-276322HCI, Thiazovivin, Fasudil (FIA-1077) HCI, GSK429286A, RKI-1447, Azaindole 1 (TC-S 7001), GSK269962A HCI, Netarsudil (AR-13324), Y-39983 HCI, ZINC00881524, KD025 (SLx-2119), Ripasudil (K-115), Hydroxyfasudil (HA-1100) AT13148, AMA-0076, AR-1286, ATS907, DE-104, INS-115644, INS-117548, PG324, Y-39983;RKI-983, SNJ-1656, Wf-563,

Azabenzimidazole-aminofurazans, H-1152P, XD-4000, HMN-1152, Rhostatin, 4-(1-aminoakyl)-N-(4-pyridl)cyclohexane-carboamides, BA-207, BA-215, BA-285, BA-1037, Ki-23095, VAS-012, quinazoline, Netarsudil, and ITRI-E-212, at the initiation of step (vii).

20. The method of any one of claims 1-19, wherein the dendritic cells and the T cells are cultured in a single closed system bioreactor.