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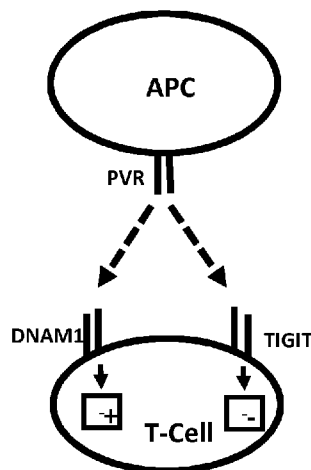
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Figure 2



(57) Abstract: Described herein are methods for activating V δ 2⁺ gamma-delta T cells ($\gamma\delta$ T cells) in a mammalian subject, comprising antagonizing TIGIT activity of V δ 2⁺ $\gamma\delta$ T cells. The invention also relates to methods of treating cancers with tissue-infiltrated $\gamma\delta$ T cells and methods of treating cancers comprising administration of pharmaceutical compositions comprising V δ 2⁺ $\gamma\delta$ T cells.



METHODS OF ACTIVATING V DELTA 2 NEGATIVE GAMMA DELTA T CELLS

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No 62/519,714 filed June 14, 2017, which is hereby incorporated by reference in its entirety.

2. FIELD OF THE INVENTION

[0002] The invention relates to methods for activating tissue-infiltrating gamma delta T cells, particularly V δ 2⁻ gamma-delta T cells ($\gamma\delta$ T cells), in a mammalian subject by antagonizing TIGIT activity of V δ 2⁻ $\gamma\delta$ T cells.

3. BACKGROUND

[0003] The growing interest in T cell immunotherapy for cancer has focused on the evident capacity of subsets of CD8⁺ and CD4⁺ $\alpha\beta$ T cells to recognize cancer cells and to mediate host-protective functions, particularly when de-repressed by clinically mediated antagonism of inhibitory pathways exerted by PD-1, CTLA-4, and other receptors. Nonetheless, problems remain. For example, there are clinical scenarios in which the efficacy of such treatments is poor. Moreover, there are often profound adverse events, the capacity to predict either efficacy or adverse events is extremely limited, and there is very little explanation of the interactions that allow the host to sense tumor cells (“immunogenicity”) that precede the activation of conventional, antigen-specific CD8⁺ and CD4⁺ $\alpha\beta$ T cell responses.

[0004] Upon global analysis of leukocyte populations and prognostic patterns of many cancer types (where higher levels of estimated T cell fractions were found to generally correlate with superior survival), intra-tumoral gamma delta T cells ($\gamma\delta$ T cells) emerged as one of the most significant favorable prognostic leukocyte population (Gentles *et al.*, 2015). $\gamma\delta$ T cells represent a subset of T cells that express on their surface a distinct, defining $\gamma\delta$ T-cell receptor (TCR). The $\gamma\delta$ TCR is made up of one gamma (γ) and one delta (δ) chain. Human $\gamma\delta$ T cells can be broadly classified into two types: peripheral blood-resident $\gamma\delta$ T cells and non-hematopoietic, tissue-resident, $\gamma\delta$ T cells. Most blood-resident $\gamma\delta$ T cells express a V δ 2 TCR; however, a smaller population of V δ 2⁻ $\gamma\delta$ T cells can be detected among $\gamma\delta$ T cells in blood. Conversely, tissue-resident $\gamma\delta$ T cells more frequently use V δ 1 and/or other V δ chains (V δ 2⁻

$\gamma\delta$ T cells); however, a smaller population of $V\delta 2^+ \gamma\delta$ T cells can be detected among $\gamma\delta$ T cells in tissue. Therefore, there is a need for novel methods for the specific activation and/or de-repression of $\gamma\delta$ T cells, and in particular, the $V\delta 2^- \gamma\delta$ T cells.

4. SUMMARY OF THE INVENTION

[0005] In certain embodiments, the application herein discloses methods of activating and/or de-repressing $V\delta 2^-$ gamma-delta T cells ($\gamma\delta$ T cells) in a mammalian subject, comprising antagonizing TIGIT activity of $V\delta 2^- \gamma\delta$ T cells present in the subject to an extent sufficient to activate the $V\delta 2^- \gamma\delta$ T cells *in vivo*. In certain aspects, the $V\delta 2^- \gamma\delta$ T cells are $V\delta 1^+$ cells. In certain aspects, the $V\delta 2^- \gamma\delta$ T cells are $V\delta 1^- V\delta 2^-$ double negative (DN) cells. In certain aspects, antagonizing TIGIT activity comprises administering at least one TIGIT inhibitor to the subject. In an aspect, the at least one TIGIT inhibitor is administered adjunctively with $V\delta 2^- \gamma\delta$ T cells that have been cultured *ex vivo*. In certain aspects, the at least one TIGIT inhibitor is added to the $V\delta 2^- \gamma\delta$ T cells during culturing *ex vivo*. In certain aspects, the cultured $V\delta 2^- \gamma\delta$ T cells have been expanded *ex vivo*. In aspect, the at least one TIGIT inhibitor is administered in a composition comprising the cultured $\gamma\delta$ T cells. In certain aspects, the at least one TIGIT inhibitor is administered separately from the *ex vivo* expanded $\gamma\delta$ T cells. In certain aspects, the methods further comprise administering to the subject at least one additional agent in an amount sufficient to activate $V\delta 2^- \gamma\delta$ T cells. In an aspect, the at least one additional agent is an agonist of DNAM-1. In certain aspects, the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with Poliovirus receptor (PVR), an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL3 binding to TIGIT, and combinations thereof. In an aspect, the TIGIT inhibitor is an antibody. In an aspect, the antibody binds specifically to TIGIT. In an aspect, the antibody binds to PVR to specifically inhibit PVR interaction with TIGIT. In an aspect, the antibody is a full length IgG antibody. In an aspect, the antibody is an antigen-binding antibody fragment. In an aspect, the antibody is a single domain antibody. In an aspect, the

antibody is fully human. In an aspect, the antibody is humanized. In an aspect, the TIGIT inhibitor is a small molecule inhibitor. In certain aspects, the *ex vivo* expanded V δ 2⁻ γ δ T cells were obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture. In an aspect, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2. In an aspect, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of interleukin-15 (IL-15). In an aspect, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2 and IL-15. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of TCR activation or co-stimulation signals. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of a T cell receptor pathway agonist. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of stromal or epithelial cells. In an aspect, stromal or epithelial cells are removed prior to culture. In an aspect, the lymphocytes are cultured in the absence of fibroblasts. In an aspect, the lymphocytes have been obtained from skin, the gastrointestinal tract (*e.g.* colon), mammary gland tissue, lung, liver, pancreas or prostate. In an aspect, the *ex vivo* expanded V δ 2⁻ γ δ T cells were obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of IL-2, IL-15, and a factor selected from the group consisting of IL-4, IL-21, IL-6, IL-7, IL-8, IL-9, IL-12, IL-18, IL-33, IGF-1, IL-1 β , human platelet lysate (HPL), and stromal cell-derived factor-1 (SDF-1) for at least 5 days to produce an expanded population of γ δ T cells. In an aspect, within 14 days of culture, the expanded population of γ δ T cells comprises at least 20-fold the number of γ δ T cells as the γ δ T cells obtained from a non-hematopoietic tissue. In an aspect, within 7 days of culture, the expanded population of γ δ T cells comprises at least 2-fold the number of γ δ T cells as the γ δ T cells obtained from a non-hematopoietic tissue. In an aspect, the expanded population of γ δ T cells is at least 50% V δ 1⁺ cells. In an aspect, the expanded population of γ δ T cells is at least 70% V δ 1⁺ cells. In an aspect, the expanded population of γ δ T cells is at least 90% V δ 1⁺ cells. In an aspect, the subject has cancer. In an aspect, the cancer has high infiltration of γ δ T cells. The application further discloses a TIGIT inhibitor as disclosed in any of the aspects and embodiments described herein for use in any of the methods described herein of activating and/or de-repressing V δ 2⁻ gamma-delta T cells (γ δ T cells) in a mammalian subject.

[0006] In certain embodiments, the application discloses herein a method of treating cancers having high infiltration of $\gamma\delta$ T cells, comprising administering at least one TIGIT inhibitor to a patient with a cancer having high infiltration of $\gamma\delta$ T cells, in an amount sufficient to activate tissue-infiltrating $\gamma\delta$ T cells in the patient. In an aspect, the cancer-infiltrating $\gamma\delta$ T cells are $V\delta 2^-$ cells. In an aspect, the $V\delta 2^-$ cells are $V\delta 1^+$ or DN cells. In an aspect, the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL3 binding to TIGIT, and combinations thereof. In an aspect, the TIGIT inhibitor is an antibody. In an aspect, the antibody binds specifically to TIGIT. In an aspect, the antibody binds to PVR to specifically inhibit PVR interaction with TIGIT. In an aspect, the antibody is a full length IgG antibody. In an aspect, the antibody is an antigen-binding antibody fragment. In an aspect, the antibody is a single domain antibody. In an aspect, the antibody is fully human. In an aspect, the antibody is humanized. In an aspect, the TIGIT inhibitor is a small molecule inhibitor. In an aspect, the methods further comprise the antecedent step of selecting for treatment a patient whose cancer shows high levels of $\gamma\delta$ T cell infiltration. In an aspect, the $\gamma\delta$ T cell infiltration is detected by analysis of a tumor biopsy by a method comprising an assay selected from the group consisting of immunohistochemistry, polymerase chain reaction, in situ hybridization, and combinations thereof. In an aspect, the $\gamma\delta$ T cell infiltration comprises $V\delta 1^+$ or double negative (DN) cells. In an aspect, at least one additional therapeutic agent is administered. In an aspect, the at least one or more additional agents comprises an agonist of DNAM-1. In an aspect, the at least one or more additional agents comprises an anti-cancer agent. In an aspect, the anti-cancer agent is selected from the group consisting of radiation, a chemotherapeutic or growth inhibitory agent, a targeted therapeutic agent, a small molecule inhibitor, a T cell expressing a chimeric antigen receptor, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate, an angiogenesis inhibitor, an antineoplastic agent, a cancer vaccine, an adjuvant, and combinations thereof. In an aspect, the anti-cancer agent is a chemotherapeutic. In an aspect, the anti-cancer agent is a small molecule inhibitor.

In an aspect, the anti-cancer agent is an antibody. In an aspect, the TIGIT inhibitor is administered continuously. In an aspect, the TIGIT inhibitor is administered intermittently. In an aspect, the anti-cancer agent is administered continuously. In an aspect, the anti-cancer agent is administered intermittently. In an aspect, wherein the TIGIT inhibitor is administered before the anti-cancer agent. In an aspect, the TIGIT inhibitor is administered simultaneous with the anti-cancer agent. In an aspect, the TIGIT inhibitor is administered after the anti-cancer agent. The application further discloses a TIGIT inhibitor as disclosed in any of the aspects and embodiments described herein for use in any of the methods described herein of treating cancers having high infiltration of $\gamma\delta$ T cells.

[0007] In certain embodiments, the application discloses herein a method of increasing the number of tissue infiltrating $\gamma\delta$ T cells in a mammalian subject recipient, comprising obtaining $\gamma\delta$ T cells from tissue comprising $\gamma\delta$ T cells from a donor; expanding the $\gamma\delta$ T cells *ex vivo*; transplanting the expanded $\gamma\delta$ T cells into a recipient, and administering at least one TIGIT inhibitor to the recipient. In an aspect, the obtained and/or expanded $\gamma\delta$ T cells are $V\delta 2^-$. In an aspect, the $V\delta 2^-$ cells are $V\delta 1^+$ or DN. In an aspect, the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling mediated by PVRL3 binding to TIGIT, or combinations thereof. In an aspect, the at least one TIGIT inhibitor is an antibody. In an aspect, the antibody binds specifically to TIGIT. In an aspect, the antibody binds to PVR. In an aspect, the antibody is a full length IgG antibody. In an aspect, the antibody is an antigen-binding antibody fragment. In an aspect, the antibody is a single domain antibody. In an aspect, the antibody is fully human. In an aspect, the antibody is humanized. In an aspect, the at least one TIGIT inhibitor comprises a small molecule inhibitor. In an aspect, the *ex vivo* expanded $V\delta 2^-$ $\gamma\delta$ T cells were obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture. In an aspect, the culturing step comprises culturing the lymphocytes obtained from

human or non-human animal non-hematopoietic tissue in the presence of IL-2. In an aspect, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of interleukin-15 (IL-15). In an aspect, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2 and IL-15. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of TCR activation or co-stimulation signals. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of a T cell receptor pathway agonist. In an aspect, the culturing step comprises culturing the lymphocytes in the absence of stromal or epithelial cells. In an aspect, stromal or epithelial cells are removed prior to culture. In an aspect, the lymphocytes are cultured in the absence of fibroblasts. In an aspect, the lymphocytes have been obtained from skin, the gastrointestinal tract (e.g. colon), mammary gland tissue, lung, liver, pancreas or prostate. In an aspect, the *ex vivo* expanded V δ 2⁻ $\gamma\delta$ T cells were obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of IL-2, IL-15, and a factor selected from the group consisting of IL-4, IL-21, IL-6, IL-7, IL-8, IL-9, IL-12, IL-18, IL-33, IGF-1, IL-1 β , human platelet lysate (HPL), and stromal cell-derived factor-1 (SDF-1) for at least 5 days to produce an expanded population of $\gamma\delta$ T cells. In an aspect, within 14 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 20-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue. In an aspect, within 7 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 2-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue. In an aspect, the expanded population of $\gamma\delta$ T cells is at least 50% V δ 1⁺ cells. In an aspect, the expanded population of $\gamma\delta$ T cells is at least 70% V δ 1⁺ cells. In an aspect, the expanded population of $\gamma\delta$ T cells is at least 90% V δ 1⁺ cells. In an aspect, the administration of the at least one TIGIT inhibitor is performed prior to, during, after the transplantation, or combinations thereof. In an aspect, at least one additional agent is administered to the recipient. In an aspect, the at least one additional agent is an anti-cancer agent. In an aspect, the anti-cancer agent is selected from the group consisting of radiation, a chemotherapeutic or growth inhibitory agent, a targeted therapeutic agent, a small molecule inhibitor, a T cell expressing a chimeric antigen receptor, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate, an angiogenesis inhibitor, an antineoplastic agent, a cancer vaccine, an adjuvant, and combinations thereof. In an aspect, the recipient has cancer. In an aspect, the $\gamma\delta$ T cells are obtained from an autologous donor. In an aspect, the non-hematopoietic tissue is tumor

tissue. In an aspect, the non-hematopoietic tissue is skin tissue. In an aspect, the method further comprises separating $V\delta 1^{+}\gamma\delta$ T cells or DN cells from the $\gamma\delta$ T cells prior to transplantation. In an aspect, at least one additional checkpoint inhibitor is administered. In an aspect, the additional checkpoint inhibitor does not inhibit PD1. In an aspect, the additional checkpoint inhibitor inhibits TIM-3. In an aspect, the additional checkpoint inhibitor inhibits LAG-3. In an aspect of the methods, the administration of the TIGIT inhibitor results in elevated release of cytokines from the $\gamma\delta$ T cells, selected from the group consisting of IFN- γ , TNF- α , interleukins, and combinations thereof. The application further discloses expanded $\gamma\delta$ T cells obtained from tissue comprising $\gamma\delta$ T cells from a donor as disclosed in any of the aspects and embodiments described herein and at least one TIGIT inhibitor as disclosed in any of the aspects and embodiments described herein for use in any of the methods described herein of increasing the number of tissue infiltrating $\gamma\delta$ T cells in a mammalian subject recipient.

[0008] In certain embodiments, described herein are methods of treating cancer, comprising administering a therapeutically effective amount of a TIGIT inhibitor to a cancer patient who has been determined to have an elevated representation of $V\delta 1^{+}$ cells in a sample of peripheral blood. In certain aspects, the methods further comprise the prior step of determining the representation of $V\delta 1^{+}$ cells in the peripheral blood sample. In an aspect, the methods further comprise the step, after determining the representation of $V\delta 1^{+}$ cells and before administering the TIGIT inhibitor, of selecting the patient for TIGIT inhibitor treatment if the patient's sample has been determined to have an elevated representation of $V\delta 1^{+}$ cells. In certain aspects, the elevated representation is an increase in $V\delta 1^{+}$ cells as a percentage of total lymphocytes in the sample. In certain aspects, greater than 0.5% of total lymphocytes in the sample are determined to be $V\delta 1^{+}$ cells. In certain aspects, greater than 1.0% of total lymphocytes are determined to be $V\delta 1^{+}$ cells. In certain aspects, greater than 2.0% of total lymphocytes are determined to be $V\delta 1^{+}$ cells. In certain aspects, wherein the elevated representation is an increase in $V\delta 1^{+}$ cells as a percentage of total $\gamma\delta$ T cells in the sample. In certain aspects, greater than 10% of total $\gamma\delta$ T cells in the sample are $V\delta 1^{+}$ cells. In certain aspects, greater than 15% of total $\gamma\delta$ T cells in the sample are $V\delta 1^{+}$ cells. In certain aspects, the elevated representation is an increase in the ratio of $V\delta 1^{+}$ cells to $V\delta 2^{+}$ cells in the sample. In certain aspects, the ratio of $V\delta 1^{+}$: $V\delta 2^{+}$ cells is greater than 1:9. In certain aspects, the ratio of $V\delta 1^{+}$: $V\delta 2^{+}$ cells is greater than 1:8. In certain aspects, the ratio of $V\delta 1^{+}$: $V\delta 2^{+}$ cells is

greater than 1:7. In certain aspects, the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:6. In certain aspects, the TIGIT inhibitor is an antibody or antigen-binding antibody fragment that binds specifically to TIGIT. In certain aspects, the methods further comprise administration of at least one additional anti-cancer therapy. In certain aspects, at least one of the at least one additional anti-cancer therapy is administration of a non-TIGIT checkpoint inhibitor. In certain aspects, the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PD-1. In certain aspects, the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PDL1. In certain aspects, the cancer is a solid, non-hematopoietic, cancer. In certain aspects, the cancer is a breast cancer. In certain aspects, the cancer is a hematological cancer. The application further discloses a TIGIT inhibitor as disclosed in any of the aspects and embodiments described herein for use in any of the methods described herein of treating cancer in a patient who has been determined to have an elevated representation of $V\delta 1^+$ cells in a sample of peripheral blood.

5. BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0009] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0010] **Figure 1A** provides a conceptual schema of sub-populations of T cells.

[0011] **Figure 1B** shows results of flow cytometry performed on human peripheral blood mononuclear cells (“human blood”) and human skin-derived lymphocytes (“human tissue”) stained with antibodies specific for CD3, $V\delta 1$, and $V\delta 2$.

[0012] **Figure 2** illustrates PVR expression on Antigen Presenting Cells (APC) and antagonistic action on T Cells through DNAM1 and TIGIT.

[0013] **Figure 3** shows results of flow cytometry (“FACS”) analysis for DNAM 1 expression of $\gamma\delta$ T cells derived from human peripheral blood mononuclear cells (“PBMCs”) and human skin lymphocytes. The shaded histogram is the signal of the Fluorescence minus one (FMO) control. “Percent of Max” refers to the percentage of maximum count.

[0014] **Figure 4** shows results of FACS analysis for TIGIT expression of $\gamma\delta$ T cells derived from human PBMCs, both unstimulated (left panel) and stimulated for 72 hours with PHA.

[0015] **Figure 5** shows results of FACS analysis for TIGIT expression on $\gamma\delta$ T cells derived from human skin.

[0016] **Figure 6** shows results of FACS analysis for DNAM 1 and TIGIT expression on $V\delta 1^+$, $V\delta 2^+$, and DN $\gamma\delta$ T cells derived from a representative donor of human PBMCs from peripheral blood (PB).

[0017] **Figure 7** shows results of FACS analysis for DNAM 1 and TIGIT expression on $V\delta 1^+$, $V\delta 2^+$, and DN $\gamma\delta$ T cells derived from multiple donors of human PBMCs from PB.

[0018] **Figure 8** shows results of FACS analysis for DNAM 1 and TIGIT expression on $V\delta 1^+$ and DN $\gamma\delta$ T cells derived from a representative donor of human skin leukocytes.

[0019] **Figure 9** shows results of FACS analysis for DNAM 1 and TIGIT expression on $V\delta 1^+$ and DN $\gamma\delta$ T cells derived from multiple donors of human skin leukocytes.

[0020] **Figure 10** shows interferon gamma ($IFN\gamma$) production in anti-CD3 stimulated $V\delta 1^+$, $V\delta 2^+$, and DN $\gamma\delta$ T cells derived from human PBMCs upon exposure to a recombinant human PVR Fc Chimera Protein (PVR) or an isotype control antibody (iso), demonstrating that PVR inhibits TCR stimulation in DN/ $V\delta 1^+$ T cells from human PBMCs.

[0021] **Figure 11** shows results from FACS analysis of Interferon gamma ($IFN\gamma$) and Tumor Necrosis Factor alpha ($TNF\alpha$) production in anti-CD3 stimulated $V\delta 1^+$, $V\delta 2^+$, and DN $\gamma\delta$ T cells derived from human PBMCs and human skin leukocytes upon exposure to PVR or an isotype control antibody (iso), demonstrating that PVR inhibits TCR stimulation in DN/ $V\delta 1^+$ T cells. For the graphs showing $V\delta 1^+$ and DN $\gamma\delta$ T cells, $n = 6$ peripheral blood-derived $\gamma\delta$ cell samples from different donors and 8 skin-derived $\gamma\delta$ cell samples (2 out of 8 are sorted $\gamma\delta$ cells). For the graphs showing $V\delta 2^+$ $\gamma\delta$ T cells, $n = 3$ peripheral blood-derived $\gamma\delta$ cell samples and 1 skin-derived $\gamma\delta$ cell samples. The graphs for $V\delta 1^+$ and DN cell samples are generated from exactly all the same donors, while the graphs for $V\delta 2^+$ are generated with a different set of donors. The donors used for the $V\delta 2^+$ graphs are all included among the donors for $V\delta 1^+$ and DN samples.

[0022] **Figure 12** shows results of FACS analysis for proliferation indicator/dye retention of anti-CD3 stimulated $V\delta 1^+$ and DN $\gamma\delta$ T cells derived from human skin leukocytes upon exposure to PVR or an isotype control antibody, demonstrating the PVR inhibits TCR-induced proliferation in DN/ $V\delta 1^+$ T cells from human skin.

[0023] **Figure 13** shows quantitation of results from FACS analysis for proliferation indicator/dye retention of anti-CD3 stimulated $V\delta 1^+$ and DN $\gamma\delta$ T cells derived from human skin leukocytes upon exposure to PVR or an isotype control antibody, demonstrating that PVR inhibits TCR-induced proliferation in DN/ $V\delta 1^+$ T cells from human skin.

[0024] **Figure 14** shows quantification of results from FACS analysis of Interferon gamma ($\text{IFN}\gamma$) and Tumor Necrosis Factor alpha ($\text{TNF}\alpha$) production in anti-CD3 stimulated $\text{V}\delta 1^+$ and DN $\gamma\delta$ T cells derived from human skin leukocytes upon exposure to an IgG control (mIgG), anti-CD3 (αCD3), and anti-CD3 plus PVR, demonstrating that PVR specifically inhibits TCR signaling.

[0025] **Figure 15** shows quantification of results from FACS analysis of Interferon gamma ($\text{IFN}\gamma$) production in anti-CD3 stimulated $\text{V}\delta 1^+$ and DN $\gamma\delta$ T cells derived from human skin leukocytes upon exposure to PVR and/or MICA, anti-DNAM1 or an IgG control.

[0026] **Figure 16** shows quantification of results from FACS analysis of Tumor Necrosis Factor alpha ($\text{TNF}\alpha$) production in anti-CD3 stimulated $\text{V}\delta 1^+$ and DN $\gamma\delta$ T cells derived from human skin leukocytes upon exposure to PVR and/or MICA, anti-DNAM1 or an IgG control, demonstrating that PVR specifically inhibits TCR signaling.

[0027] **Figure 17** shows quantification of results from FACS analysis of granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma ($\text{IFN}\gamma$) and Tumor Necrosis Factor alpha ($\text{TNF}\alpha$) production of $\gamma\delta$ T cells from human skin lymphocytes of 5 different donors sorted with a pan $\gamma\delta$ T cell antibody upon exposure to an IgG control (mIgG), anti-CD3 (αCD3), and anti-CD3 plus PVR, demonstrating that PVR is a general inhibitor of TCR stimulation.

[0028] **Figure 18** shows quantification of results from FACS analysis for TIGIT expression of tissue-derived $\text{V}\delta 1^+$ and $\text{V}\delta 3^+$ cells cultured in the presence of IL-2 and IL-15 (culture method 1), or in the presence of IL-2, IL-4, IL-15 and IL-21 (culture method 2), demonstrating that cells grown using culture method 2 express low levels of TIGIT.

[0029] **Figure 19** are graphs showing quantification of results from FACS analysis of tissue-derived $\text{V}\delta 1^+$ and $\text{V}\delta 3^+$ cells from an individual donor cultured in the presence of IL-2, IL-4, IL-15 and IL-21 upon exposure to an IgG control (mIgG), anti-CD3 (αCD3), and anti-CD3 plus PVR, demonstrating that the PVR inhibition of $\text{IFN}\gamma$ and $\text{TNF}\alpha$ was lost in $\text{V}\delta 1^+$ and $\text{V}\delta 3^+$ cells expressing low levels of TIGIT.

[0030] **Figure 20** are graphs showing quantification of results from FACS analysis of tissue-derived $\text{V}\delta 1^+$ and $\text{V}\delta 3^+$ cells from an individual donor cultured in the presence of IL-2, IL-4, IL-15 and IL-21 upon exposure to an IgG control (mIgG), anti-CD3 (αCD3), and anti-CD3 plus PVR, demonstrating that the PVR inhibition of $\text{IFN}\gamma$ and $\text{TNF}\alpha$ was lost in $\text{V}\delta 1^+$ and $\text{V}\delta 3^+$ cells expressing low levels of TIGIT.

6. DETAILED DESCRIPTION

6.1. Advantages and utility

[0031] Briefly, and as described in more detail below, described herein are methods for activating tissue-infiltrating $\gamma\delta$ T cells, particularly $V\delta 2^-$ $\gamma\delta$ T cells, in mammalian subjects, by antagonizing TIGIT activity of the $V\delta 2^-$ $\gamma\delta$ T cells, and methods for the treatment of cancer comprising administration of at least one TIGIT inhibitor.

6.2. Definitions

[0032] Terms used herein have the meanings ascribed to them by those of skill in the art unless explicitly defined as set forth below.

[0033] “ **$V\delta 2^-$ $\gamma\delta$ T cells**” refers to any $\gamma\delta$ T cell that does not express $V\delta 2$.

[0034] “**Double negative (DN)**” refers to $\gamma\delta$ T cells that do not express either $V\delta 1$ or $V\delta 2$. DN cells can express, but are not limited to those that express, $V\delta 3$, $V\delta 4$, $V\delta 5$, $V\delta 6$, $V\delta 7$, and $V\delta 8$.

[0035] The phrase “**activate a T cell**” includes activation, de-repression, and both activation and de-repression of the T cell.

[0036] “**TIGIT inhibitor**” refers to any agent or compound capable of disrupting TIGIT activity or disrupting TIGIT interaction with PVR. A “TIGIT inhibitor” can increase PVR interaction with DNAM1. For example, a “TIGIT inhibitor” may block the interaction of PVR with TIGIT, leading to sequestration of TIGIT and increased binding of PVR to DNAM1. A “TIGIT inhibitor” can be an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVRL3 binding to TIGIT, and combinations thereof.

[0037] As used herein, an “**expanded population of $\gamma\delta$ cells**” refers to a population of cells that has been cultured under conditions and for a duration sufficient to cause proliferation of $\gamma\delta$ cells within that population. Likewise, an “**expanded population of $V\delta 1^+$ T cells**,” as used herein, refers to a population of cells that has been cultured under conditions and for a duration sufficient to cause proliferation of $V\delta 1^+$ T cells within that population.

[0038] “**Tissue-resident**”, “**tissue-infiltrated**” and “**non-hematopoietic**” refers to cells that are located at non-vascular anatomical sites.

[0039] “**Antigen binding fragment**” of an antibody or “**antigen binding antibody fragment**” refers to any single chain or multiple chain portion of an antibody that is capable of binding specifically to an epitope of an antigen. Antigen binding fragment includes, without limitation, Fab, F(ab)₂, and single-chain Fv (scFv).

[0040] “**Human antibody**” unless otherwise indicated is one whose sequences correspond to (*i.e.*, are identical in sequence to) an antibody that could be produced by a human and/or consists entirely of amino acid sequences that are encoded in the human genome. A “human antibody” as used herein can be produced using various techniques known in the art, including phage-display libraries and by administering the antigen (*e.g.*, TIGIT) to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized Xenomice.

[0041] “**Humanized antibodies**” refer to non-human (*e.g.*, murine) antibodies that are chimeric antibodies and contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin variable domain are replaced by corresponding non-human residues. These modifications may be made to further refine antibody performance. Furthermore, in a specific embodiment, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. In an embodiment, the humanized antibodies do not comprise residues that are not found in the recipient antibody or in the donor antibody. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are

those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In an embodiment where the humanized antibodies do comprise residues that are not found in the recipient antibody or in the donor antibody, the Fc regions of the antibodies are modified.

[0042] “**Binds specifically**”, “**capable of specifically binding**”, “**specifically binds**”, or “**preferentially binds**” refers to the property of an antibody or fragment of binding to the (specified) antigen with a dissociation constant that is $<1 \mu\text{M}$, preferably $<1 \text{ nM}$ and most preferably $<10 \text{ pM}$. In an embodiment, the K_d of the antibody that binds specifically to TIGIT is 250-500 pM. An epitope that “binds specifically” or “binds preferentially” (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecular entity is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a TIGIT conformational epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other TIGIT epitopes or non-TIGIT epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding.

[0043] In certain embodiments, cancers or tissues with “**high $\gamma\delta$ T cell infiltration**” refers to tissues (*e.g.*, tumor from tumor biopsies) wherein the fraction of $\gamma\delta$ T cells to total leukocytes, either measured or estimated, is greater than the fraction of $\gamma\delta$ T cells in a given reference tissue, such as non-tumor tissue of a similar origin. In certain embodiments, tissues with “high $\gamma\delta$ T cell infiltration” refers to tissues wherein the fraction of $\gamma\delta$ T cells to total leukocytes is greater than 0.0001, greater than 0.001, greater than 0.01, greater than 0.1, greater than 0.2, greater than 0.3, greater than 0.4, or greater than 0.5. In certain embodiments, tissues with “high $\gamma\delta$ T cell infiltration” refers to tissues in which the fraction of $\gamma\delta$ T RNA to total

leukocyte RNA is greater than 0.0001, greater than 0.001, greater than 0.01, or greater than 0.1.

[0044] “**T-cell checkpoints**” or “**checkpoints**” refers to any protein or gene that functions to inhibit or suppress the activation of a T-cell. T-cell checkpoints include, but are not limited to: A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3 and VISTA.

[0045] “**T-cell checkpoint inhibitor**” or “**checkpoint inhibitor**” refers to any molecule that inhibits the activity of a T-cell checkpoint.

[0046] “**Sufficient amount**” means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to modulate $\gamma\delta$ T cell activity.

[0047] “**Therapeutically effective amount**” is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a “prophylactically effective amount” as prophylaxis can be considered therapy.

[0048] The phrases “**adjunctive administration**” or “**administered to the subject adjunctively**” are used interchangeably herein to mean administering a second therapeutic agent in therapeutically effective temporal proximity to a first therapeutic agent. In typical embodiments, adjunctive administration improves the effectiveness of the second therapeutic agent, the first therapeutic agent, or both the first and the second therapeutic agent as compared to administration of the agent alone.

[0049] Abbreviations used in this application include, but are not limited to, the following: “ $\gamma\delta$ T cells” refers to gamma delta T cells; “TIGIT” refers to T-Cell-IG and ITIM domain; TCR refers to the T-cell Receptor, “PVR” refers to Poliovirus receptor, “IL-2” refers to native or recombinant interleukin 2; and “IL-15” refers to native or recombinant interleukin 15, *etc.*, “PB” refers to peripheral blood and “PBMC” refers to peripheral blood mononuclear cells.

[0050] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

6.3. Methods for activating $V\delta 2^-$ gamma-delta T cells

[0051] In a first aspect, methods are presented for activating $V\delta 2^-$ gamma-delta T cells ($\gamma\delta$ T cells) in a mammalian subject. The methods comprise antagonizing TIGIT activity of $V\delta 2^-$ $\gamma\delta$ T cells present in the subject to an extent sufficient to activate the $V\delta 2^-$ $\gamma\delta$ T cells *in vivo*. In some embodiments, the $V\delta 2^-$ $\gamma\delta$ T cells are $V\delta 1^+$ cells. In some embodiments, the $V\delta 2^-$ $\gamma\delta$ T cells are $V\delta 1^-$ $V\delta 2^-$ double negative (DN) cells.

[0052] In typical embodiments, antagonizing TIGIT activity comprises administering at least one TIGIT inhibitor to the subject. TIGIT inhibitors suitable for use in the methods are described below in Section 6.6.

6.3.1. Adjunctive administration with *ex vivo* cultured V δ 2⁻ $\gamma\delta$ T cells

[0053] In a variety of embodiments, the at least one TIGIT inhibitor is administered to the subject adjunctively with V δ 2⁻ $\gamma\delta$ T cells that have been cultured *ex vivo*.

[0054] In some embodiments, the at least one TIGIT inhibitor is administered in a composition that also comprises the cultured $\gamma\delta$ T cells. In certain of these embodiments, the at least one TIGIT inhibitor is added to the V δ 2⁻ $\gamma\delta$ T cells during culturing *ex vivo*. In certain embodiments, the at least one TIGIT inhibitor is added to the V δ 2⁻ $\gamma\delta$ T cells after culturing *ex vivo*.

[0055] In other embodiments, the at least one TIGIT inhibitor is administered to the subject separately from, but adjunctively with, the *ex vivo* expanded $\gamma\delta$ T cells.

6.3.1.1. $\gamma\delta$ T cells from hematopoietic tissue

[0056] Although V δ 2⁺ T cells predominate among the $\gamma\delta$ cells in hematopoietic tissues, V δ 2⁻ cells are also present, albeit in lower numbers. In some embodiments, the $\gamma\delta$ T cells are obtained for culture from hematopoietic tissues, such as peripheral blood. In certain embodiments, V δ 2⁻ $\gamma\delta$ T cells are obtained for culture from the hematopoietic tissues. In certain embodiments, $\gamma\delta$ T cells from hematopoietic tissues are stimulated to reduce V δ 2 expression and/or induce V δ 1 expression and/or selectively increase proliferation of V δ 2⁻ cells. In various embodiments, the $\gamma\delta$ T cells are stimulated according to a protocol described in WO 2012/156,958, the contents of which are incorporated herein by reference in their entirety. In select embodiments, $\gamma\delta$ T cells obtained from hematopoietic tissues are stimulated with a $\gamma\delta$ TCR antibody to expand V δ 2⁻ $\gamma\delta$ T cells and/or V δ 1⁺ T cells *ex-vivo*. In certain embodiments, $\gamma\delta$ T cells obtained from hematopoietic tissues are stimulated with a $\gamma\delta$ TCR antibody to increase TIGIT expression of the $\gamma\delta$ T cells *ex-vivo*.

6.3.1.2. $\gamma\delta$ T cells from non-hematopoietic tissue

[0057] In various embodiments, $\gamma\delta$ T cells are obtained for culture from non-hematopoietic tissues. Detailed methods for the isolation, culturing and expansion of $\gamma\delta$ T cells from non-

hematopoietic tissues are disclosed in WO 2017/072367, which is incorporated herein by reference in its entirety.

[0058] In a variety of embodiments, $V\delta 2^- \gamma\delta$ T cells for culture are obtained from any human or non-human mammal non-hematopoietic tissue that can be removed from a subject.

[0059] In some embodiments, the non-hematopoietic tissue from which the $V\delta 2^- \gamma\delta$ T cells are obtained for culture is skin (*e.g.*, human skin), which can be obtained by methods known in the art. In some embodiments, the skin is obtained by punch biopsy. In other embodiments, the $V\delta 2^- \gamma\delta$ T cells are obtained for culture from the gastrointestinal tract (*e.g.*, colon), mammary gland, lung, prostate, liver, spleen, and pancreas. In some embodiments, the $V\delta 2^- \gamma\delta$ T cells are obtained for culture from non-neoplastic tissues. In some embodiments, the $V\delta 2^- \gamma\delta$ T cells are obtained for culture from human cancer tissues, *e.g.*, tumors of the breast, the prostate, or other solid tumors.

[0060] The $\gamma\delta$ T cells that are dominant in the non-hematopoietic tissues are primarily $V\delta 1^+$ T cells, such that $V\delta 1^+$ T cells comprise about 70-80% of the non-hematopoietic tissue-resident $\gamma\delta$ T cell population. In various embodiments, the $V\delta 2^- \gamma\delta$ T cells that have been cultured *ex vivo* are $V\delta 1^+$.

[0061] Some $\gamma\delta$ T cells that are resident in non-hematopoietic tissues express neither $V\delta 1$ nor $V\delta 2$ TCR and we have named them double negative (DN) $\gamma\delta$ T cells. In some embodiments, the $V\delta 2^- \gamma\delta$ T cells that have been cultured *ex vivo* are $V\delta 1^- V\delta 2^-$ double negative (DN) cells. These DN $\gamma\delta$ T cells are likely to be mostly $V\delta 3$ -expressing with a minority of $V\delta 5$ -expressing T cells.

6.3.1.2.1. Expansion of non-hematopoietic tissue-resident $\gamma\delta$ T cells

[0062] In some embodiments, the cultured $V\delta 2^- \gamma\delta$ T cells have been expanded *ex vivo*.

[0063] In certain embodiments, the *ex vivo* expanded $V\delta 2^- \gamma\delta$ T cells are obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture.

[0064] In certain embodiments, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2. In certain embodiments, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of interleukin-15 (IL-

15). In particular embodiments, the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2 and IL-15.

[0065] In typical embodiments, the culturing step comprises culturing the lymphocytes in the absence of TCR activation or co-stimulation signals. In some embodiments, the culturing step comprises culturing the lymphocytes in the absence of a T cell receptor pathway agonist. In some embodiments, the culturing step comprises culturing the lymphocytes in the absence of stromal or epithelial cells. In some embodiments, stromal or epithelial cells are removed prior to culture. In some embodiments, the lymphocytes are cultured in the absence of fibroblasts. In various embodiments, the lymphocytes have been obtained from skin, the gastrointestinal tract (e.g., colon), mammary gland tissue, lung, liver, pancreas or prostate.

[0066] In some embodiments, the *ex vivo* expanded V δ 2⁻ $\gamma\delta$ T cells are obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of IL-2, IL-15, and a factor selected from the group consisting of IL-4, IL-21, IL-6, IL-7, IL-8, IL-9, IL-12, IL-18, IL-33, IGF-1, IL-1 β , human platelet lysate (HPL), and stromal cell-derived factor-1 (SDF-1) for at least 5 days to produce an expanded population of $\gamma\delta$ T cells.

[0067] In select embodiments, the *ex vivo* expanded V δ 2⁻ $\gamma\delta$ T cells are obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals under conditions in which, within 14 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 20-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue. In some embodiments, the *ex vivo* expanded V δ 2⁻ $\gamma\delta$ T cells were obtained by culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals under conditions in which, within 7 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 2-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue.

[0068] In particular embodiments, the expanded population of $\gamma\delta$ T cells is at least 50% V δ 1⁺ cells. In certain embodiments, the expanded population of $\gamma\delta$ T cells is at least 70% V δ 1⁺ cells. In select embodiments, the expanded population of $\gamma\delta$ T cells is at least 90% V δ 1⁺ cells.

[0069] In certain embodiments, the method further comprises expanding non-hematopoietic tissue-resident $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells). In some embodiments, the non-hematopoietic tissue-resident $\gamma\delta$ T

cells are expanded from a population of $\gamma\delta$ T cells that has been separated from non-hematopoietic tissue according to methods described above. In general, non-hematopoietic tissue-resident $\gamma\delta$ T cells are capable of spontaneously expanding upon removal of physical contact with stromal cells (e.g., skin fibroblasts). Thus, the scaffold-based culture methods described above can be used to induce such separation, resulting in de-repression of the $\gamma\delta$ T cells to trigger expansion. Accordingly, in some embodiments, no substantial TCR pathway activation is present during the expansion step (e.g., no exogenous TCR pathway activators are included in the culture). In various embodiments, the methods of expanding non-hematopoietic tissue-resident $\gamma\delta$ T cells omit contact with feeder cells, tumor cells, and/or antigen-presenting cells.

[0070] It will be understood that the amount of each of the above cytokines required to produce an expanded population of $\gamma\delta$ T cells will depend of the concentrations of one or more of the other cytokines. For example, if the concentration of IL-2 is increased or decreased, the concentration of IL-15 may be accordingly decreased or increased, respectively. As noted above, the amount effective to produce an expanded population refers herein to composite effect of all factors on cell expansion.

[0071] In certain embodiments, the $\gamma\delta$ T cells are exposed to certain factors prior to culture with other factors. For instance, the expansion culture can be gradually supplied with additional factors over the course of expansion, or, alternatively, the $\gamma\delta$ T cells can be transferred from a culture of one factor or group of factors to another.

[0072] In some embodiments, the $\gamma\delta$ T cells are expanded in culture for a period of several hours (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, or 21 hours) to about 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days). In one embodiment, the $\gamma\delta$ T cells are expanded for a period of 14 to 21 days. Thus, including a separation culture period (e.g., of 1 to 40 days, e.g., 14 to 21 days), the separation and expansion steps, in some embodiments, can last between 28 and 56 days, or about 41 days.

[0073] Methods of expansion provide an expanded population of $\gamma\delta$ T cells that is greater in number than a reference population. In some embodiments, the expanded population of $\gamma\delta$ T cells is greater in number than the separated population of $\gamma\delta$ T cells prior to the expansion step (e.g., at least 2-fold in number, at least 3-fold in number, at least 4-fold in number, at least 5-fold in number, at least 6-fold in number, at least 7-fold in number, at least 8-fold in number,

at least 9-fold in number, at least 10-fold in number, at least 15-fold in number, at least 20-fold in number, at least 25-fold in number, at least 30-fold in number, at least 35-fold in number, at least 40-fold in number, at least 50-fold in number, at least 60-fold in number, at least 70-fold in number, at least 80-fold in number, at least 90-fold in number, at least 100-fold in number, at least 200-fold in number, at least 300-fold in number, at least 400-fold in number, at least 500-fold in number, at least 600-fold in number, at least 700-fold in number, at least 800-fold in number, at least 900-fold in number, at least 1,000-fold in number at least 5,000-fold in number, at least 10,000-fold in number, or more relative to the separated population of $\gamma\delta$ T cells prior to the expansion step). Thus, the invention provides a means to produce large populations of non-hematopoietic tissue-derived $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) at high rates (e.g., by removing stromal cell contact and/or TCR stimulation, or by culturing in the presence of an effective amount of factors). In some embodiments, the expansion step described herein expands the $\gamma\delta$ T cells at a low population doubling time, which is given by the following equation:

$$\text{Doubling Time} = \frac{\text{Duration}^{\log 2}}{\text{Log (final concentration)} - \text{Log (initial concentration)}}$$

[0074] Given the information provided herein,, a skilled artisan will recognize that the invention provides methods of expanding non-hematopoietic tissue-derived $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) at a population doubling time of less than 5 days (e.g., less than 4.5 days, less than 4.0 days, less than 3.9 days, less than 3.8 days, less than 3.7 days, less than 3.6 days, less than 3.5 days, less than 3.4 days, less than 3.3 days, less than 3.2 days, less than 3.1 days, less than 3.0 days, less than 2.9 days, less than 2.8 days, less than 2.7 days, less than 2.6 days, less than 2.5 days, less than 2.4 days, less than 2.3 days, less than 2.2 days, less than 2.1 days, less than 2.0 days, less than 46 hours, less than 42 hours, less than 38 hours, less than 35 hours, less than 32 hours). In some embodiments, within 7 days of culture, the expanded population of $\gamma\delta$ T cells (e.g., the expanded population of V δ 1 T cells and/or DN T cells) comprises at least 10-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T-cells prior to expansion (e.g., at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at

least 800-fold, at least 900-fold, at least 1,000-fold, at least 2,000-fold, at least 3,000-fold, at least 4,000-fold, at least 5,000-fold, at least 6,000-fold, at least 7,000-fold, or at least 8,000-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion).

[0075] In some embodiments, within 14 days of culture, the expanded population of $\gamma\delta$ T cells (e.g., the expanded population of V δ 1 T cells and/or DN T cells) comprises at least 20-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion (e.g., at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 2,000-fold, at least 3,000-fold, at least 4,000-fold, at least 5,000-fold, at least 6,000-fold, at least 7,000-fold, at least 8,000-fold, at least 9,000-fold, or at least 10,000-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion). In some embodiments, within 21 days of culture, the expanded population of $\gamma\delta$ T cells (e.g., the expanded population of V δ 1 T cells and/or DN T cells) comprises at least 50-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion (e.g., at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 2,000-fold, at least 3,000-fold, at least 4,000-fold, at least 5,000-fold, at least 6,000-fold, at least 7,000-fold, at least 8,000-fold, at least 9,000-fold, or at least 10,000-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion).

[0076] In some embodiments, within 28 days of culture, the expanded population of $\gamma\delta$ T cells (e.g., the expanded population of V δ 1 T cells and/or DN T cells) comprises at least 100-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion (e.g., at least 110-fold, at least 120-fold, at least 130-fold, at least 140-fold, at least 150-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 2,000-fold, at least 3,000-fold, at least 4,000-fold, at least 5,000-fold, at least 6,000-fold, at least 7,000-fold, at least 8,000-fold, at least 9,000-fold, at least 10,000-fold, at least 12,000-fold, or at least

15,000-fold the number of $\gamma\delta$ T cells relative to the separated population of $\gamma\delta$ T cells prior to expansion).

[0077] Non-hematopoietic tissue-derived $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) expanded by the methods provided herein can have a phenotype well-suited for anti-tumor efficacy. In some embodiments, the expanded population of $\gamma\delta$ T cells (e.g., skin-derived V δ 1 T cells) has a greater mean expression of CD27 than a reference population (e.g., the separated population of $\gamma\delta$ T cells prior to the expansion step). In some embodiments, the expanded population of $\gamma\delta$ T cells has a mean expression of CD27 that is at least 2-fold relative to the separated population of $\gamma\delta$ T cells (e.g., at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 20,000-fold, or more, relative to the separated population of $\gamma\delta$ T cells).

[0078] An increase or decrease in expression of other markers can be additionally or alternatively used to characterize one or more expanded populations of non-hematopoietic tissue-derived $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells), including CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, CD2, NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64. In some instances, the expanded population of $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) has a greater mean expression of one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells, e.g., prior to expansion. Additionally or alternatively, the expanded population of $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) may have a greater frequency of cells expressing one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells. In some

embodiments, the expanded population of $\gamma\delta$ T cells (e.g., skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, e.g., V δ 1 T cells and/or DN T cells) has a lower mean expression of one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells. The expanded population may similarly have a lower frequency of cells expressing one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells.

[0079] A non-hematopoietic tissue-resident $\gamma\delta$ T cell produced by the method of the invention may thus have one or more of the following properties: (i) displays the phenotype CD69^{high}, TIM3^{high} and CD28^{low/absent}; (ii) upregulates one or more of CCR3, CD39, CD11b, and CD9; (iii) produces IFN- γ in response to an NKG2D ligand in the absence of TCR agonists; (iv) produces IL-13 in the absence of TCR agonists; (v) produces one or more of IFN- γ , TNF- α and GM-CSF in response to TCR activation; (vi) produces no or substantially no IL-17 in response to TCR activation; (vii) grows in culture medium containing IL-2 without additional growth factors; (viii) displays a cytotoxic T cell response in the absence of TCR agonists; and/or (ix) displays selective cytotoxicity for tumor cells over normal cells.

[0080] In some instances, a non-hematopoietic tissue-resident $\gamma\delta$ T cell produced by the method of the invention produces IL-13 in the absence of TCR agonists and/or produces IFN- γ in response to an NKG2D ligand in the absence of TCR agonists.

[0081] Numerous basal culture media suitable for use in the proliferation of $\gamma\delta$ T cells are available, in particular complete media, such as AIM-V, Iscoves medium and RPMI-1640 (Life Technologies). The medium may be supplemented with other media factors, such as serum, serum proteins and selective agents, such as antibiotics. For example, in some embodiments, RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 10 mM HEPES, pH 7.2, 1% penicillin-streptomycin, sodium pyruvate (1 mM; Life Technologies), non-essential amino acids (e.g. 100 μ M Gly, Ala, Asn, Asp, Glu, Pro and Ser; 1X MEM non-essential amino acids Life Technologies), and 10 μ L/L β -mercaptoethanol. Conveniently, cells are cultured at 37°C in a humidified atmosphere containing 5% CO₂ in a suitable culture medium. The $\gamma\delta$ T cells may be cultured as described herein in any suitable system, including stirred tank fermenters, airlift fermenters, roller bottles, culture bags or dishes, and other

bioreactors, in particular hollow fiber bioreactors. The use of such systems is well-known in the art. General methods and techniques for culture of lymphocytes are well-known in the art.

[0082] The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected.

[0083] In certain embodiments, disclosed herein are adoptive cell transfer (ACT) methods. In certain embodiments, the ACT methods comprise, T cell immunotherapy wherein subjects are infused with heterologous, autologous or allogeneic $\gamma\delta$ T cells to treat disease. In certain aspects, the ACT methods comprise infusion of subjects of $V\delta 2^- \gamma\delta$ T cells to treat disease. In certain aspects, the ACT methods comprise infusion of subjects of $V\delta 2^- \gamma\delta$ T cells that have been expanded *ex vivo* to treat disease. In certain aspects, the ACT methods comprise infusion of subjects of $V\delta 2^- \gamma\delta$ T cells that have been expanded *ex vivo* to treat cancer. In certain embodiments, $V\delta 2^- \gamma\delta$ T cells are selected and expanded *ex vivo* to enrich for $V\delta 2^- \gamma\delta$ T cells from a mixed population of immune cells.

[0084] In certain embodiments of the ACT methods, $\gamma\delta$ T cells are selected and/or engineered *ex vivo* to target specific antigens, such as tumor-associated antigens. In certain embodiments of the ACT methods, immune cells comprising $\gamma\delta$ T cells are obtained from tissue of donor subjects. In certain embodiments, $\gamma\delta$ T cells are obtained from non-hematopoietic tissue of donor subjects. In certain embodiments, $\gamma\delta$ T cells are obtained from blood of donor subjects. In certain embodiments, tumor infiltrating lymphocytes ("TIL"s) comprising $\gamma\delta$ T cells are obtained from donor subjects. In certain embodiments $\gamma\delta$ T cells are expanded in culture and selected for antigen specificity without altering their native specificity. In certain T cell immunotherapy methods, T lymphocytes comprising $\gamma\delta$ T cells obtained from the donor are engineered *ex vivo* by transduction with viral expression vectors, to express chimeric antigen receptors ("CAR"s) of predetermined specificity.

[0085] Concentrations of cytokines and growth factors can range from about 0.1 ng/mL to about 500 ng/mL, from about 1 ng/mL to about 200 ng/mL, from about 10 ng/ml to 100 ng/ml, depending on the type of cytokine and growth factor. Appropriate combinations and

concentrations of cytokines are disclosed in WO 2017/072367, which is incorporated herein by reference in its entirety.

6.3.1.3. Additional agents

[0086] In some embodiments, the methods further comprise administering to the subject at least one additional agent in an amount sufficient to activate V δ 2⁻ $\gamma\delta$ T cells. In select embodiments, the at least one additional agent is an agonist of DNAM-1.

6.3.1.4. Subjects

[0087] In various embodiments, the mammalian subject has cancer. In particular embodiments, the cancer has high infiltration of $\gamma\delta$ T cells. In a variety of these embodiments, the mammalian subject is a human patient.

[0088] In certain embodiments, the subject from whom $\gamma\delta$ 2⁻ T cells are obtained for culture (donor) is the same individual as the ultimate recipient of the cells, i.e., an autologous donor. Autologous $\gamma\delta$ T cells, which are removed and in some instances stored are transplanted back into the subject. In certain embodiments, the autologous $\gamma\delta$ T cells are subject to additional treatments prior to transplantation back into the recipient.

[0089] In some embodiments, for example, autologous $\gamma\delta$ T cells obtained from the subject are further treated or purified to remove any disease cells, such as cancer cells, prior to transplantation back into the subject. Such treatments and purification, also referred to as “purging,” of cell preparations to remove disease cell can include, among others, use of antibodies directed against cell surface markers expressed in disease cells and anti-cancer (*i.e.*, chemotherapeutic) treatments. In some embodiments, the autologous $\gamma\delta$ T cells are obtained following treatment of the subject for the underlying disease, thereby reducing the risk of presence of disease cells in the autologous $\gamma\delta$ T cell preparations. Also referred to as “in vivo purging,” the subject can be treated with an antibody therapeutic targeting the disease or treated with anti-cancer agent that does not destroy or suppress the hematopoietic system prior to obtaining the $\gamma\delta$ T cells for transplantation. In certain embodiments, the purged preparation of $\gamma\delta$ T cells can be further purified, such as by FACS or affinity selection (*e.g.*, chromatography), to select $\gamma\delta$ T cells or V δ 2⁻ $\gamma\delta$ T cells from other cells (*i.e.*, other immune cells or diseased cells).

[0090] In certain embodiments, the donor subject is not the same individual as the ultimate recipient of the cells, *i.e.*, a heterologous or allogeneic donor. In certain heterologous

embodiments, the cells obtained from the donor subject are not screened or matched for the recipient. As $\gamma\delta$ T cells are non-MHC restricted, they do not recognize a host into which they are transferred as foreign, which means that they are less likely to cause graft-versus-host disease. This means that they can be used “off the shelf” and transferred into any recipient, *e.g.*, for heterologous or allogeneic adoptive T cell therapy.

[0091] In certain allogeneic embodiments, allogeneic immune cells comprising $\gamma\delta$ T cells are obtained from donors who are selected based on matching at loci of the human lymphocyte antigen (HLA) complex. In some embodiments, the allogeneic immune cells comprising $\gamma\delta$ T cells can be further isolated or purified, and/or subject to further manipulation. In some embodiments, the allogeneic immune cells comprising $\gamma\delta$ T cells are subject to additional treatments to expand a population of immune cells or manipulated by recombinant methods to introduce heterologous genes or additional functionality to the allogeneic immune cells prior to transplantation into the recipient subject.

[0092] $\gamma\delta$ T cells obtained from a donor, either autologous, heterologous or allogeneic, can be subject to additional treatments prior to transplantation into a recipient subject. In some embodiments, the immune cells comprising $\gamma\delta$ T cells are treated to expand the population of $\gamma\delta$ T cells, for example by culturing in a suitable medium.

6.3.1.4.1. Selection of subjects with high $\gamma\delta$ T cell infiltration

[0093] In certain embodiments, the method further comprises the step of detecting $\gamma\delta$ T cell infiltration, or V δ 2⁺ $\gamma\delta$ T cell infiltration, of tissues in a mammalian subject and selecting subjects who have $\gamma\delta$ T cell infiltrates.

[0094] In certain embodiments, $\gamma\delta$ T cell infiltration or V δ 2⁺ $\gamma\delta$ T cell infiltration is detected in tumors of a mammalian subject. Detection of $\gamma\delta$ T cell or V δ 2⁺ $\gamma\delta$ T-cells may be performed by any method known in the art for the detection of subpopulations of immune cells in tissue. T cell infiltration in tissues (*e.g.*, tumor from tumor biopsies) can be determined by determining the estimated fraction of $\gamma\delta$ T cells to total leukocytes compared to a reference tissue such non-tumor tissue of a similar origin.

[0095] An increase or decrease in expression of other markers can be additionally or alternatively used to characterize $\gamma\delta$ T cells from tissue or $\gamma\delta$ T cells from tissue which have been expanded *ex-vivo* (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, *e.g.*, V δ 1 T cells and/or DN T cells), including CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39,

CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, CD2, NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64. In some instances, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, *e.g.*, V δ 1 T cells and/or DN T cells) has a greater mean expression of one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells, *e.g.*, prior to expansion. Additionally or alternatively, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, *e.g.*, V δ 1 T cells and/or DN T cells) may have a greater frequency of cells expressing one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells. In some embodiments, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non-V δ 2 T cells, *e.g.*, V δ 1 T cells and/or DN T cells) has a lower mean expression of one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells. The expanded population may similarly have a lower frequency of cells expressing one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells.

[0096] In certain embodiments, the composition is co-administered with an additional agent for treating cancer (*e.g.*, an anti-cancer agent). In an aspect, the composition is co-administered with an as yet to be described therapeutic for treating cancer. In co-administration procedures, the agents may be administered concurrently or sequentially. In another aspect, agents described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of, but not limited to, those described below. It is also anticipated that there could be two or more co-administered chemical agents, biological agents or radiation that may each be administered using different modes or different formulations.

6.3.1.4.2. Selection of subjects with high V δ 2⁻ $\gamma\delta$ T cells in peripheral blood

[0097] In certain embodiments, the method further comprises the step of detecting V δ 2⁻ $\gamma\delta$ in the blood of a mammalian subject, and selecting subjects who have high V δ 2⁻ $\gamma\delta$ T cells in the blood. In particular embodiments, the method comprises determining the ratio of V δ 1⁺ and V δ 2⁺ in a blood sample from a potential mammalian subject. In particular embodiments, the method comprises determining the total number of V δ 1⁺ and V δ 2⁺ in a blood sample from a potential mammalian subject. In certain aspects, the method further comprises comparing the ratio of V δ 1⁺ and V δ 2⁺ in a blood sample from a subject with cancer to a healthy individual. In certain aspects, the method further comprises comparing the total number of V δ 1⁺ and V δ 2⁺ in a blood sample from a subject with cancer to a healthy individual.

6.4. Methods of treating cancer

[0098] In another aspect, methods for treating cancers are presented.

6.4.1. Cancers with $\gamma\delta$ T cell infiltration

[0099] In various embodiments, the cancer has high infiltration of $\gamma\delta$ T cells. The methods comprise administering at least one TIGIT inhibitor to a patient with a cancer having high infiltration of $\gamma\delta$ T cells, in an amount sufficient to activate tissue-infiltrating $\gamma\delta$ T cells in the patient. In typical embodiments, the cancer-infiltrating $\gamma\delta$ T cells are V δ 2⁻ cells, and the amount of TIGIT inhibitor is sufficient to activate V δ 2⁻ cells. In some embodiments, the amount of TIGIT inhibitor is sufficient to activate V δ 1⁺ cells. In certain embodiments, the amount of TIGIT inhibitor is sufficient to activate double negative (DN) cells.

[00100] In various embodiments, the TIGIT inhibitor is an inhibitor as described in Section 6.6. In currently preferred embodiments, the TIGIT inhibitor is an antibody. In certain of these embodiments, the antibody binds specifically to TIGIT.

[00101] In some embodiments, the method further comprises the earlier step of selecting for treatment a patient whose cancer shows high levels of $\gamma\delta$ T cell infiltration.

[00102] In various embodiments, the $\gamma\delta$ T cell infiltration is detected by analysis of a tumor biopsy.

[00103] In typical embodiments, the analysis comprises an assay selected from immunohistochemistry, *in situ* hybridization, polymerase chain reaction, RNA seq, and

combinations thereof. *See, for example*, Gentles, *et al.*, 2015, incorporated herein by reference in its entirety.

[0100] In some embodiments, the cancer shows high levels of $V\delta 1^+$ or double negative (DN) cell infiltrate.

[0101] In certain embodiments, the method further comprises the prior step of detecting $\gamma\delta$ T cell infiltration, or $V\delta 2^- \gamma\delta$ T cell infiltration, of tissues in a mammalian subject and selecting subjects who have $\gamma\delta$ T cell infiltrates.

[0102] In certain embodiments, $\gamma\delta$ T cell infiltration or $V\delta 2^- \gamma\delta$ T cell infiltration is detected in tumors of a mammalian subject. Detection of $\gamma\delta$ T cell or $V\delta 2^- \gamma\delta$ T-cells may be performed by any method known in the art for the detection of subpopulations of immune cells in tissue. T cell infiltration in tissues (*e.g.*, tumor from tumor biopsies) can be determined by determining the estimated fraction of $\gamma\delta$ T cells to total leukocytes compared to a reference tissue such non-tumor tissue of a similar origin.

[0103] An increase or decrease in expression of other markers can be additionally or alternatively used to characterize $\gamma\delta$ T cells from tissue or $\gamma\delta$ T cells from tissue which have been expanded *ex-vivo* (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non- $V\delta 2$ T cells, *e.g.*, $V\delta 1$ T cells and/or DN T cells), including CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, CD2, NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64. In some instances, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non- $V\delta 2$ T cells, *e.g.*, $V\delta 1$ T cells and/or DN T cells) has a greater mean expression of one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells, *e.g.*, prior to expansion. Additionally or alternatively, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non- $V\delta 2$ T cells, *e.g.*, $V\delta 1$ T cells and/or DN T cells) may have a greater frequency of cells expressing one or more of the markers selected from the group consisting of CD124, CD215, CD360, CTLA4, CD1b, BTLA, CD39, CD45RA, Fas Ligand, CD25, ICAM-1, CD31, KLRG1, CD30, and CD2, relative to the separated population of $\gamma\delta$ T cells. In some embodiments, the expanded population of $\gamma\delta$ T cells (*e.g.*, skin-derived $\gamma\delta$ T cells and/or non- $V\delta 2$ T cells, *e.g.*, $V\delta 1$ T cells and/or DN T cells) has a lower mean expression of one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28,

CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells. The expanded population may similarly have a lower frequency of cells expressing one or more of the markers selected from the group consisting of NKp44, NKp46, ICAM-2, CD70, CD28, CD103, NKp30, LAG3, CCR4, CD69, PD-1, and CD64, relative to the separated population of $\gamma\delta$ T cells.

[0104] In certain embodiments, the TIGIT inhibitor is co-administered with an additional agent for treating cancer (*e.g.*, an anti-cancer agent). In an aspect, the composition is co-administered with an as yet to be described therapeutic for treating cancer. In co-administration procedures, the agents may be administered concurrently or sequentially. In another aspect, agents described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of, but not limited to, those described below. It is also anticipated that there could be two or more co-administered chemical agents, biological agents or radiation that may each be administered using different modes or different formulations.

6.4.2. Cancer patients with elevated $V\delta 2^-$ $\gamma\delta$ T cells in peripheral blood

[0105] In various embodiments, the method of treating cancer comprises administering a therapeutically effective amount of a TIGIT inhibitor to a cancer patient who has been determined to have an elevated representation of $V\delta 1^+$ cells in a sample of peripheral blood.

[0106] In some embodiments, the method further comprising the prior step of determining the representation of $V\delta 1^+$ cells in the peripheral blood sample. In some embodiments, the method further comprises the step, after determining the representation of $V\delta 1^+$ cells and before administering the TIGIT inhibitor, of selecting the patient for TIGIT inhibitor treatment if the patient's sample has been determined to have an elevated representation of $V\delta 1^+$ cells.

[0107] In particular embodiments, the method comprises determining the total number of $V\delta 1^+$ and $V\delta 2^+$ in a blood sample from a potential mammalian subject. In particular embodiments, the method comprises determining the ratio of $V\delta 1^+$ and $V\delta 2^+$ in a blood sample from a potential mammalian subject. In certain embodiments, the method further comprises comparing the total number of $V\delta 1^+$ and $V\delta 2^+$ in a blood sample from a subject with cancer to a healthy individual. In certain embodiments, the method further comprises comparing the ratio of $V\delta 1^+$ and $V\delta 2^+$ in a blood sample from a subject with cancer to a healthy individual.

[0108] In some embodiments, the elevated representation is an increase in $V\delta 1^+$ cells as a percentage of total lymphocytes in the sample. In certain embodiments, greater than 0.2% of total lymphocytes in the sample are determined to be $V\delta 1^+$ cells. In particular embodiments, greater than 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0% of total lymphocytes in the sample are determined to be $V\delta 1^+$ cells. In some embodiments, greater than 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, or 5.0% of total lymphocytes in the sample are determined to be $V\delta 1^+$ cells.

[0109] In some embodiments, the elevated representation is an increase in $V\delta 1^+$ cells as a percentage of total $\gamma\delta$ T cells in the sample. In certain embodiments, greater than 10% of total $\gamma\delta$ T cells in the sample are $V\delta 1^+$ cells. In particular embodiments, greater than 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% of total $\gamma\delta$ T cells in the sample are $V\delta 1^+$ cells.

[0110] In some embodiments, the elevated representation is an increase in the ratio of $V\delta 1^+$ cells to $V\delta 2^+$ cells in the sample. In certain embodiments, the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:9. In particular embodiments, the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:8, 1:7, 1:6, or 1:5.

[0111] In typical embodiments, the TIGIT inhibitor is an antibody or antigen-binding antibody fragment that binds specifically to TIGIT.

6.4.3. Additional therapeutic agents

[0112] In various embodiments, the method further comprises administering at least one additional anti-cancer therapy. In some embodiments, at least one of the at least one additional anti-cancer therapy is a non-TIGIT checkpoint inhibitor. In particular embodiments, the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PD-1. In particular embodiments, the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PDL1.

[0113] In various embodiments, the cancer is a solid, non-hematopoietic, cancer. In particular embodiments, the cancer is a breast cancer.

[0114] In various embodiments, the cancer is a hematological cancer.

[0115] In various embodiments, the method further comprises administering at least one additional therapeutic agent.

[0116] In some embodiments, the at least one additional therapeutic agent is an agonist of DNAM-1.

[0117] In some embodiments, the at least one or more additional agent is an anti-cancer agent. In certain embodiments, the anti-cancer agent is selected from the group consisting of radiation, a chemotherapeutic or growth inhibitory agent, a targeted therapeutic agent, a small molecule inhibitor, a T cell expressing a chimeric antigen receptor, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate, an angiogenesis inhibitor, an antineoplastic agent, a cancer vaccine, an adjuvant, and combinations thereof. In particular embodiments, the anti-cancer agent is a chemotherapeutic agent. In particular embodiments, the anti-cancer agent is a targeted therapeutic agent. In certain embodiments, the anti-cancer agent is an antibody.

[0118] In various embodiments, the anti-cancer agent is administered continuously. In some embodiments, the anti-cancer agent is administered intermittently.

[0119] In some embodiments, the TIGIT inhibitor is administered before the anti-cancer agent. In some embodiments, the TIGIT inhibitor is administered simultaneous with the anti-cancer agent. In some embodiments, the TIGIT inhibitor is administered after the anti-cancer agent.

[0120] In some embodiments, the additional therapeutic agent is a checkpoint inhibitor.

6.5. Methods of increasing the number of tissue-infiltrating $\gamma\delta$ T cells in a mammalian subject

[0121] In another aspect, methods of increasing the number of tissue-infiltrating $\gamma\delta$ T cells in a mammalian subject recipient are presented. The methods comprise (i) obtaining $\gamma\delta$ T cells from tissue of a donor, (ii) expanding the $\gamma\delta$ T cells *ex vivo*, (iii) transplanting the expanded $\gamma\delta$ T cells into a recipient, and (iv) administering at least one TIGIT inhibitor to the recipient.

[0122] In typical embodiments, the obtained and/or expanded $\gamma\delta$ T cells are $V\delta 2^-$. In certain embodiments, the $V\delta 2^-$ cells are $V\delta 1^+$ or DN T cells. In certain embodiments, $V\delta 2^-$ cells comprise both $V\delta 1^+$ and DN T cells.

[0123] In some embodiments, the $\gamma\delta$ T cells are obtained from hematopoietic tissue described herein in Section 6.3.1.1.

[0124] In some embodiments, the $\gamma\delta$ T cells are obtained from non-hematopoietic tissue, described herein in Section 6.3.1.2.

[0125] In typical embodiments, the TIGIT inhibitor is an inhibitor described herein in Section 6.6.

[0126] In some embodiments, the *ex vivo* expanded $V\delta 2^- \gamma\delta$ T cells were obtained by: culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture. In various embodiments, the methods culture and expansion methods are selected from those described in Section 6.3 above.

6.6. TIGIT Inhibitors

[0127] In various embodiments of the methods described herein, the TIGIT inhibitor is selected from an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVRL3 binding to TIGIT, and combinations thereof.

[0128] In some embodiments, the TIGIT inhibitor is an antagonist of TIGIT interaction with PVR. In certain embodiments, the TIGIT inhibitor is an agent that inhibits and/or blocks the interaction of TIGIT with PVR. The TIGIT inhibitor may block the interaction of PVR with TIGIT, leading to sequestration of TIGIT and increased binding of PVR to DNAM1. In particular embodiments, the TIGIT inhibitor is an antibody that binds specifically to TIGIT or to PVR. In particular embodiments, the TIGIT inhibitor is an antigen-binding fragment of an antibody that binds specifically to TIGIT or to PVR.

[0129] In certain embodiments, the TIGIT inhibitor is an antigen binding antibody fragment selected from Fab, Fab', F(ab')₂, F_d, F_v, complementarity determining region (CDR) fragment, or single-chain antibody (scFv). In certain embodiments, the TIGIT inhibitor is a single

domain antibody or single-chain antibody. In particular embodiments, the single domain antibody is a V_{HH} domain.

[0130] In particular embodiments, the TIGIT inhibitor is an inhibitor described in WO 2017/053748, WO 2016/073282, WO/2016/011264 or WO 2015/009856, the disclosures of which are incorporated herein by reference in their entireties. In particular embodiments, the TIGIT inhibitor is an inhibitor described in WO 2017/030823, WO 2016/028656, WO 2016/106302, WO 2016/191643, WO 2017/059095, WO 2017/037707, of WO 2017/048824, the disclosures of which are incorporated herein by reference in their entireties.

[0131] In certain aspects, the TIGIT inhibitor is a human antibody. In certain aspects, the human antibody has variable regions in which both the framework and CDR regions are sequences identical to those of human origin.

[0132] In certain aspects, the TIGIT inhibitor is a humanized antibody. In certain aspects, the humanized antibody is a non-human (*e.g.*, murine) antibody that is a chimeric antibody and contains minimal sequence derived from non-human immunoglobulin. In one embodiment, the humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin variable domain are replaced by corresponding non-human residues. These modifications may be made to further refine antibody performance. Furthermore, in a specific embodiment, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. In an embodiment, the humanized antibodies do not comprise residues that are not found in the recipient antibody or in the donor antibody. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In an embodiment where the humanized antibodies do comprise residues that are not found in the recipient antibody or in the donor antibody, the Fc regions of the antibodies are modified.

[0133] In certain aspects, the TIGIT inhibitor is a small molecule inhibitor. In certain aspects, the TIGIT inhibitor specifically binds TIGIT. In certain aspects, the TIGIT inhibitor binds PVR.

6.7. Dosage regimens

[0134] In the methods of activating V δ 2 $\cdot$ γ δ T cells and methods of treating cancer described herein, various routes of administration of the TIGIT inhibitor and/or additional therapeutic agents are permissible.

[0135] Methods of delivery include, but are not limited to, intra-arterial, intra-muscular, intravenous, intranasal, and oral routes. Methods to enhance uptake can include, but are not limited to, encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis, and the like. In specific embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, injection, or by means of a catheter. It is contemplated that the agents identified can be administered to subjects or individuals susceptible to or at risk of developing a variety of conditions benefiting from activation of γ δ T cells. When the agent is administered to a subject, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject.

[0136] Therapeutic amounts are empirically determined and vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the agent. When delivered to an animal, the method is useful to further confirm efficacy of the agent.

[0137] In some embodiments, *in vivo* administration is effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and vary with the composition used for therapy, the purpose of therapy, the target cell being treated, and the subject being treated. Single or multiple administrations are carried out with the dose level and pattern being selected by the treating physician.

[0138] Suitable dosage formulations and methods of administering the agents are readily determined by those of skill in the art. Preferably, the compounds are administered at about 0.01 mg/kg to about 200 mg/kg, more preferably at about 0.1 mg/kg to about 100 mg/kg,

even more preferably at about 0.5 mg/kg to about 50 mg/kg. When the compounds described herein are co-administered with another agent, the effective amount may be more or less than when the agent is used alone.

[0139] The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take the form of suspensions, solutions and emulsions of the active ingredient in aqueous diluents, nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

[0140] More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including, but not limited to, oral, rectal, nasal, topical (including, but not limited to, transdermal, aerosol, buccal and sublingual), vaginal, parental (including, but not limited to, subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It is also appreciated that the preferred route varies with the condition and age of the recipient, and the disease being treated.

[0141] Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient.

[0142] Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a lower total dosage of each component antiviral agent than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

[0143] The present invention also includes methods involving co-administration of the TIGIT inhibitors described herein with one or more additional active agents. Indeed, it is a further aspect of this invention to provide methods for enhancing prior art therapies and/or pharmaceutical compositions by co-administering the agents of this invention. In co-administration procedures, the agents may be administered concurrently or sequentially. In one embodiment, the agents described herein are administered prior to the other active agent(s).

The pharmaceutical formulations and modes of administration may be any of those described above. In addition, the two or more co-administered chemical agents, biological agents or radiation may each be administered using different modes or different formulations.

[0144] In certain embodiments of the methods provided herein, one or more agents provided herein and one or more therapeutic agents, TIGIT inhibitors, T-cell checkpoint inhibitors or anti-cancer agents are administered to a subject under one or more of the following conditions: at different periodicities, at different durations, at different concentrations, by different administration routes, *etc.* In certain embodiments, the TIGIT inhibitor is administered prior to an additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent, *e.g.*, 0.5, 1, 2, 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, or 1, 2, 3, or 4 weeks prior to the administration of therapeutic or anti-cancer agent. In certain embodiments, the TIGIT inhibitor is administered after an additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent, *e.g.*, 0.5, 1, 2, 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, or 1, 2, 3, or 4 weeks after the administration of an additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent. In certain embodiments, the TIGIT inhibitor and an additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent, are administered concurrently but on different schedules, *e.g.*, the TIGIT inhibitor is administered daily while the additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent, is administered once a week, once every two weeks, once every three weeks, or once every four weeks. In other embodiments, the TIGIT inhibitor is administered once a week while the additional TIGIT inhibitor, T-cell checkpoint inhibitor, therapeutic or anti-cancer agent is administered daily, once a week, once every two weeks, once every three weeks, or once every four weeks.

[0145] In certain embodiments TIGIT inhibitors are administered in combination with an additional T-cell checkpoint inhibitor. In certain aspects, the T-cell checkpoint inhibitor or checkpoint inhibitor is any molecule that inhibits the activity of a T-cell checkpoint. In certain aspects the T-cell checkpoint inhibitor inhibits a protein or gene that functions to inhibit or suppress the activation of a T-cell. In certain aspects, the T-cell checkpoint inhibitor inhibits the expression or activity of, but not limited to: A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA and combinations thereof.

[0146] In certain embodiments TIGIT inhibitors are administered in combination with anti-cancer agents. A number of suitable therapeutic or anticancer agents are contemplated for use

in the methods provided herein. Indeed, the methods provided herein can include but are not limited to, administration of numerous therapeutic agents such as: agents that induce apoptosis; polynucleotides (*e.g.*, anti-sense, ribozymes, siR A); polypeptides (*e.g.*, enzymes and antibodies); biological mimetics; alkaloids; alkylating agents; antitumor antibiotics; anti-metabolites; hormones; platinum compounds; monoclonal or polyclonal antibodies (*e.g.*, antibodies conjugated with anticancer drugs, toxins, defensins), toxins; radionuclides; biological response modifiers (*e.g.*, interferons (*e.g.*, IFN- α) and interleukins (*e.g.*, IL-2)); adoptive immunotherapy agents; hematopoietic growth factors; agents that induce tumor cell differentiation (*e.g.*, all-trans-retinoic acid); gene therapy reagents (*e.g.*, antisense therapy reagents and nucleotides); tumor vaccines; angiogenesis inhibitors; proteasome inhibitors; NF-KB modulators; anti-CDK compounds; HDAC inhibitors; and the like. Numerous other examples of therapeutic agents such as chemotherapeutic compounds and anticancer therapies suitable for co-administration with the disclosed compounds are known to those skilled in the art.

6.8. Pharmaceutical compositions

[0147] The TIGIT inhibitors for use in the methods described herein can be formulated in pharmaceutical compositions. The *ex vivo* cultured, and optionally expanded, $\gamma\delta$ T cells for use in certain of methods described herein may be formulated as a medicament.

[0148] Pharmaceutical compositions comprising TIGIT inhibitors can comprise, in addition to one or more of the TIGIT inhibitors, one or more additional checkpoint inhibitors, and a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, *e.g.* oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[0149] Pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet can include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can be included.

[0150] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required.

[0151] The pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a “therapeutically effective amount” or “prophylactically effective amount”(as the case can be, although prophylaxis can be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the cancer being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

[0152] A composition can be administered alone or in combination with other treatments (e.g., additional TIGIT inhibitors, T-Cell checkpoint inhibitors and/or anti-cancer agents), either simultaneously or sequentially dependent upon the cancer to be treated.

7. EXAMPLES

[0153] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, *etc.*), but some experimental error and deviation should, of course, be allowed for.

[0154] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art.

Methods

T cell isolation from Human Peripheral Blood

[0155] Peripheral Blood (PB) from healthy volunteers was used to isolate Peripheral Blood Mononuclear Cells (PBMCs) by layering whole blood onto Ficoll (Ficoll-Paque PLUS, GE Healthcare Life Sciences) followed by centrifugation at 400 g for 20 minutes to separate red blood cells, blood plasma, and white lymphocytes/monocytes. White blood cells were carefully harvested through a stripette and washed four times in cold PBS.

The PBMCs were used either immediately for immunostaining and phenotypic analysis of PB-derived $\gamma\delta^+$ T cells or cultured to have PB-derived $\gamma\delta^+$ T cell line for functional assays. To this second aim, cells were resuspended in RPMI-1640 medium (Life Technologies) with 10% heat-inactivated foetal bovine serum (Life Technologies), L-glutamine (292 $\mu\text{g/ml}$; Life Technologies), penicillin (100 units/ml; Life Technologies), streptomycin (100 $\mu\text{g/ml}$; Life Technologies), N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES; 0.01 M; Life Technologies), sodium pyruvate (1 mM; Life Technologies), minimal essential media (MEM) non-essential amino acids (1X; Life Technologies) at a density of 1×10^6 per mL and supplemented with human recombinant IL-2 (100 IU/ml; PROLEUKIN®; Novartis Pharmaceutical UK Ltd) and human recombinant IL-15 (20 ng/ml; Biolegend). Cells were transferred into a 24 well plate that was coated with α -pan $\gamma\delta$ TCR monoclonal Antibody (mAb) (20 $\mu\text{g/ml}$; clone B1; Biolegend) 90 minutes prior to cell transfer. Cells were grown for 14 days, at 37°C and 5% CO₂, media changed ever 2-3 days and fresh cytokines added. Upon reaching confluence, cells were split 1:1. Under these conditions, after 14 days, the original minor population of $\gamma\delta^+$ T cells is normally highly activated through their TCR (as indicated by upregulation of CD69 and CD25) and largely enriched consisting of mainly V δ 2⁺ T cells but also V δ 1⁺ T cells (up to 30% of all $\gamma\delta^+$ T cells).

[0156] When cell counts were required, lymphocytes were counted by either; (1) trypan blue stain (0.4%) (Life Technologies) and haemocytometer, or (2) CASY® Model TT cell counter and analyzer (Roche).

Isolation of non-haematopoietic tissue-resident T cells from Human Skin

[0157] A three-dimensional skin explant protocol was established using the Clark protocol. Cellfoam Matrices (Cytomatrix Pty Ltd) having dimensions of 9 mm x 9 mm x 1.5 mm, were autoclaved and incubated in a solution of 100 mg/ml rat tail collagen I (BD Biosciences) in PBS for 30 minutes at room temperature, followed by one rinse in PBS. Samples of adult human skin were obtained within 3-6 hours of cutaneous surgery. Subcutaneous fat was removed and the remaining skin tissue was minced into fragments measuring approximately 1 mm x 1 mm. Approximately five skin fragments/explants were placed and pressed down onto the surface of each matrix. Each matrix was placed into a separate well of a 24-well plate (Corning) containing 2 ml of Skin-T media (Iscove's Modified Dulbecco's Medium (IMDM; Life Technologies) with 10% heat-inactivated fetal bovine serum (Life Technologies), L-glutamine (292 µg/ml; Life Technologies), penicillin (100 units/ml; Life Technologies), streptomycin (100 µg/ml; Life Technologies), N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES; 0.01 M; Life Technologies), sodium pyruvate (1 mM; Life Technologies), minimal essential media (MEM) non-essential amino acids (1x; Life Technologies) and 3.5 µl/L 2-mercaptoethanol (Life Technologies). For the first 7 days of culture Amphotericin (2.5 µg/ml; Life Technologies) was added to the media. The culture was kept at 37°C and 5% CO₂ and media were refreshed three times per week by aspirating the upper 1 mL of media from each well and adding 1 ml of fresh medium. Human recombinant IL-2 (100 IU/mL; PROLEUKIN®; Novartis Pharmaceutical UK Ltd) and human recombinant IL-15 (20 ng/ml; Biolegend) were added at the initiation of culture and on each media refresh until the isolation of lymphocytes after 21-35 days. Up to 96 wells (four 24-well plates) were set up in culture for each donor.

[0158] To isolate the lymphocytes, the matrices and media were transferred to a 50 ml centrifuge tube (Corning) containing 10 ml Hanks Balanced Salt Solution (HBSS; Life Technologies) with 0.01 mM HEPES (up to 12 matrices/tube). The matrices were rinsed with the cell suspension using a 10 ml pipette, and the cell suspension was passed through a 70 µm filter (BD Biosciences) into a fresh 50 ml centrifuge tube. The rinsing of the matrices was repeated two additional times. The media from the culture well was also aspirated and passed through a 70 µm filter into a fresh 50 ml centrifuge tube. The wells were washed two further times with 1 ml of 0.01 mM HEPES/HBSS and passed through a 70 µm filter. Cells were subsequently isolated by centrifugation (1600 rpm for 15 minutes). The pellet was re-suspended in Skin-T media. The final cell pellet was re-suspended in Skin-T media for

subsequent flow cytometry analysis or functional studies. When cell counts were required, lymphocytes were counted at this stage by either; (1) trypan blue stain (0.4%) (Life Technologies) and haemocytometer, or (2) CASY® Model TT cell counter and analyzer (Roche).

$\gamma\delta^+$ T cell phenotypic analysis

[0159] Lymphocytes (0.5×10^6 cells/well) were plated in a 96-U bottom well plate and washed in PBS. First, a live/dead staining was done with the Fixable Blue Dead Cell Stain Kit (Life Technologies) or the Fixable Viability Dye eFluor 770 NIR (eBioscience) according to the manufacture instructions. Then the cells were washed in FACS buffer (PBS, 0.5% BSA, 2 mM EDTA) and stained in ice with a mix of the following mAbs: BV510 conjugated α -CD3 (clone OKT3; Biolegend), PC7-conjugated α -pan $\gamma\delta$ (IMMU510 clone; Beckman Coulter), FITC-conjugated α -V δ 1 (TS8.2 clone; Life Technologies), PE-conjugated α -V δ 2 (B6 clone; Biolegend), APC-conjugated α -DNAM1 (11A8 clone; Biolegend), PerCP eFluor710-conjugated α -TIGIT (MBSA43 clone; eBioscience). Finally, the cells were fixed in fixation buffer (eBioscience), resuspended in FACS buffer and acquired by a FACS Fortessa machine. Data were analysed through the Flowjo 9.9 Software.

T cell cytokine production assay

[0160] PB-derived $\gamma\delta^+$ T cell line or Skin-derived lymphocytes or sorted pan $\gamma\delta^+$ T cells were plated in a 96-flat bottom well plate with a density of 0.2×10^6 cells/well. The plate was previously coated with different combination of the following proteins: α -CD3 Ab (5 μ g/ml) (HIT3a clone; Biolegend) or its isotype control (MOPC-173; Biolegend), recombinant human PVR Fc Chimera Protein (rhPVR) (20 μ g/ml) (Life Technologies), recombinant human MICA Fc Chimera Protein (rhMICA) (10 μ g/ml) (R&D Systems) or their hIgG1 control (Biolegend). The blocking α -DNAM1 Ab (DX11 clone; BD Biosciences) was added to the cell suspension (10 μ g/ml). The cells were stimulated for 6 hours in RPMI 10% FCS supplemented with the intracellular protein transport inhibitor Brefeldin A (20 μ g/ml)(Sigma-Aldrich), at 37°C and 5% CO₂.

[0161] At the end of the stimulation, the cells were harvested and moved to a 96-U bottom well plate, extracellular stained and fixed as previously described for the phenotypic analysis. The intracellular staining was performed in Permeabilization Buffer (eBioscience) according the manufacture instructions and the cytokine production was assessed using the following mAbs: eFluor450-conjugated α -IFN γ (4S.B3; eBioscience) APC-conjugated α -TNF α (MAB11; Biolegend). The cells were resuspended in FACS buffer and acquired by a FACS Fortessa machine. Data were analysed through the Flowjo 9.9 Software.

T cell proliferation assay

[0162] Pan $\gamma\delta^+$ T cells were purified from Skin-derived lymphocytes using a α -pan $\gamma\delta$ mAb (IMMU510 clone; Beckman Coulter) and a FACS Aria cell sorting. They were then stained with the CellTrace Violet (CTV) Cell Proliferation Kit (Life Technologies) and cultured in a 96-flat bottom well plate for 1 week in complete medium supplemented with IL-2. The plate was previously coated with different combination of the following proteins: α -CD3 Ab (50 ng/ml) (HIT3a clone; Biolegend) or its isotype control (MOPC-173; Biolegend), rhPVR (10 μ g/ml) (Life Technologies) or its hIgG1 control (Biolegend). At the end of the stimulation, the cells were harvested and moved to a 96-U bottom well plate, extracellular stained and fixed as previously described for the phenotypic analysis. The cells were resuspended in FACS buffer and acquired by a FACS Fortessa machine. Data were analysed through the Flowjo 9.9 Software.

Expansion of skin-derived $\gamma\delta$ T cells

[0163] Mixed lymphocytes were harvested after three to four weeks of scaffold culture, washed in PBS, spun down, and re-suspended in Roswell Park Memorial Institute 1640 medium (RPMI-1640; Life Technologies) with filtered 10% heat-inactivated fetal bovine serum (Life Technologies), L-glutamine (292 μ g/ml; Life Technologies), penicillin (100 units/ml; Life Technologies), streptomycin (100 μ g/ml; Life Technologies), N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES; 0.01 M; Life Technologies), sodium pyruvate (1 mM; Life Technologies), minimal essential media (MEM) non-essential amino acids (1X; Life Technologies) and 50 μ M 2-mercaptoethanol (Life Technologies) at a

concentration of 1×10^6 cells/ml. The initial population of $V\delta 1^+$ cells was 1.12% of lymphocytes. Cells were seeded at 2×10^5 cells/well into 96 well flat bottom plates (Corning) or at 2×10^6 cells/well into 24 well plates (Corning) for expansion and supplemented with factors at the following concentrations: IL-2 (100 U/ml), IL-15 (10 ng/ml), IL-4 (5 ng/ml) and IL-21 (10 ng/ml). Cells were monitored daily by microscopy and provided with fresh media and cytokines three times per week. Upon full confluence and cell aggregation, cells were split 1: 1 into additional wells and plates, as required. After 21 days, cells were harvested using ACCUTASE® (eBioscience), counted, and analyzed using flow cytometry.

Example 1: $\gamma\delta^+$ T cell subpopulations in human PBMC and skin

[0164] Within the human adaptive, cell based immune system, there are 3 types of lymphocytes using somatic gene rearrangement to generate diverse surface receptors, B cells, $\alpha\beta$ T cells and $\gamma\delta$ T cells respectively. Only $\gamma\delta$ T cells show compartmentalization, with V $\delta 2$ TCR positive cells dominating the human blood whilst T cells recombining other TCR genes (eg. V $\delta 1$, $\delta 3$, $\delta 4$, $\delta 5$, $\delta 6$, $\delta 7$, $\delta 8$) are predominantly found in human tissues such as the skin, the gut, the lungs, the liver and others (Figure 1A).

[0165] Peripheral blood mononuclear cells from 11 donors and human skin derived lymphocytes from 27 donors were isolated as described above. Flow cytometry was performed using the following antibody-fluorochrome conjugates: V $\delta 1$ -FITC, CD3-BV510 and V $\delta 2$ -PE. Samples were also stained for viability using the eFluor 770 NIR live/dead dye. All T lymphocytes present were gated using the marker CD3, and within this population the presence of V $\delta 2^+$ $\gamma\delta$ T cells or V $\delta 1^+$ $\gamma\delta$ T cells made visible using the according antibodies. The results show that V $\delta 2$ TCR positive cells are dominating in the human blood with almost no V $\delta 1$ T cells being present. In human skin, the situation is inverted with V $\delta 1$ TCR positive cells being the signature $\gamma\delta$ T cell population and almost no V $\delta 2$ TCR positive cells can be found (Figure 1B).

Example 2: Human V δ 1⁺/DN and V δ 2⁺ T cells have very distinct expression patterns of DNAM1 and TIGIT

[0166] PB-derived $\gamma\delta$ ⁺ T cells and Skin-derived $\gamma\delta$ ⁺ T cells were tested by FACS analysis for the presence of DNAM1 and TIGIT on their surface. On a first analysis, DNAM1 and TIGIT expression was evaluated on a pan $\gamma\delta$ ⁺ T cell population. DNAM1 was expressed by both PB-derived $\gamma\delta$ ⁺ T cells and Skin-derived $\gamma\delta$ ⁺ T cells. As was reported for $\alpha\beta$ T cells, PB- $\gamma\delta$ ⁺ T cells expressed TIGIT only upon activation, obtained in this case with PHA treatment. On the contrary, TIGIT was already expressed by unstimulated Skin-derived $\gamma\delta$ ⁺ T cells.

[0167] In a second step, DNAM1 and TIGIT expression was assessed by staining PB-derived and Skin-derived lymphocytes with anti-V δ 1 and anti-V δ 2 Abs, in addition to the pan $\gamma\delta$ Ab. This staining strategy allowed to gate three different populations into the pan $\gamma\delta$ ⁺ gate: V δ 1⁺, V δ 2⁺ and V δ 1⁺V δ 2⁻ (named here Double Negative, DN). Like previously said, PB- $\gamma\delta$ ⁺ T cells are mostly represented by the V δ 2⁺ population, and in a smaller percentage by V δ 1⁺ or DN. The V δ 2⁺ population largely expressed DNAM1, more than 70% in all donors, while only a small proportion, less than 25%, or none of them expressed TIGIT. The V δ 1⁺ and DN populations present in the PB expressed DNAM1, even if to a lower level compare to the V δ 2⁺ population, and shared TIGIT constitutive expression as the Skin-derived $\gamma\delta$ ⁺ T cells. Indeed, Skin-derived $\gamma\delta$ ⁺ T cells are composed by V δ 1⁺ and DN cells, and even in this second more detailed staining, we confirmed that V δ 1⁺ and DN cells constitutively expressed DNAM1 and TIGIT. Specifically, DNAM1 is expressed by more than 70% of V δ 1⁺ and DN cells in all donors, and TIGIT is expressed by more than 50% of V δ 1⁺ cells. DN T cells showed a more variable TIGIT expression because it is a heterogeneous population and it is represented by populations expressing TIGIT and populations not expressing TIGIT, in a different proportion in every donor. Figure 3 shows $\gamma\delta$ T cells derived either from PBMCs or skin tissue express DNAM1. $\gamma\delta$ T cells isolated from human PBMCs (Figure 4) and lymphocytes isolated from human skin tissue (Figure 5) were stained with anti-TIGIT antibodies and antibodies specific for either the γ chain or δ chain TCR and subjected to FACS analysis. Only 1.57% of the δ T cells isolated from unstimulated PBMCs expressed TIGIT, whereas 30.3% of δ T cells isolated from PBMCs stimulated with phytohaemagglutinin (PHA) expressed TIGIT (Figure 4). However, nearly all of the $\gamma\delta$ T cells isolated from unstimulated skin leukocytes expressed TIGIT (Figure 5). $\gamma\delta$ T cells isolated from human PBMCs (Figures 6 and 7) and lymphocytes isolated from human skin tissue (Figures 8 and 9) were stained for

DNAM1, TIGIT, γ chain or δ chain TCR, $V\delta 1$, and $V\delta 2$ and subjected to FACS analysis. $V\delta 2^+ \gamma\delta$ T cells isolated from PBMCs expressed high levels of DNAM1 (Figure 6) and low levels of TIGIT (Figure 7) while $V\delta 1^+ \gamma\delta$ T cells expressed low levels of DNAM1 (Figure 6) and high levels of TIGIT (Figure 7). DN cells ($V\delta 1^- V\delta 2^-$) expressed moderate levels of DNAM1 (Figure 8) and high levels of TIGIT. $V\delta 1^+ \gamma\delta$ T cells and DN cells isolated from human skin leukocytes expressed high levels of DNAM1 and TIGIT (Figures 8 and 9).

[0168] In summary, all $\gamma\delta^+$ subpopulations express DNAM1. Skin-derived $V\delta 1^+$ and DN T cells express higher DNAM1 compare to PB-derived $V\delta 1^+$ and DN T cells, very likely for the natural presence of IL15 in the skin that upregulates DNAM1. Of note, $V\delta 1^+$ and DN T cells, in PB and Skin, already express TIGIT in unstimulated conditions, while $V\delta 2^+$ T cells do not. .

Example 3: α CD3-induced cytokine production is inhibited by PVR in $V\delta 1^+$ and DN $\gamma\delta$ T cells.

[0169] To confirm TIGIT functionality in suppressing TCR stimulation in DN and $V\delta 1^+ \gamma\delta$ T cells, soluble PVR was added to α CD3-stimulated DN and $V\delta 1^+ \gamma\delta$ T cells isolated from either human PBMCs (figure 10 and 11) or human skin lymphocytes (Figures 11). PB-derived and Skin-derived lymphocytes were stimulated for 6 hours by plate-bound α CD3 Ab in combination with rhPVR or its isotype control, and cytokine production, specifically IFN γ and TNF α , was assessed by intracellular staining and FACS analysis gating on $V\delta 1^+$, $V\delta 2^+$, or DN populations.

[0170] The cytokine production induced by TCR stimulation was strongly inhibited in $V\delta 1^+$ and DN cells when rhPVR was also present, while the $V\delta 2^+$ population, that is TIGIT $^-$, was unaffected. We didn't notice any difference whether we performed the assay with PB-derived or Skin-derived $\gamma\delta$ T cells. Moreover, with 2 donors, we could perform the experiment with sorted pan $\gamma\delta^+$ T cells and we confirmed the PVR-induced inhibition of the cytokine production, suggesting an intrinsic $\gamma\delta^+$ T cell mechanism that doesn't need the presence of other immune cells. Figure 10 shows IFN γ and TNF α production in a representative donor of PB-derived lymphocytes. Figure 11 shows collective plots for IFN γ and TNF α production in $\gamma\delta$ T cells derived from PB and Skin. These results very strongly suggest that rhPVR engages TIGIT on $V\delta 1^+$ and DN cells, triggering its inhibitory function and weakening their activation.

Example 4: α CD3-induced proliferation is inhibited by PVR in V δ 1⁺ and DN $\gamma\delta$ T cells

[0171] Skin-derived $\gamma\delta$ ⁺ T cells, previously sorted and CTV-stained, were stimulated with plate-bound α CD3 Ab in combination with rhPVR or its isotype control. After 7 days of stimulation, the proliferation of V δ 1⁺ and DN $\gamma\delta$ T cells was evaluated by FACS analysis of the CTV dilution. Figures 12 and 13 show increased dye concentrations retained in a much higher percentage of DN and V δ 1⁺ $\gamma\delta$ T cells after exposure to PVR upon stimulation by α CD3 as compared to cells exposed to isotype control, demonstrating reduced cell proliferation in cells with higher dye concentrations. The α CD3-induced proliferation of V δ 1⁺ and DN $\gamma\delta$ T cells was impaired in the presence of PVR compare to its control, further confirming the inhibitory role of the PVR/TIGIT axis on the TCR-induced activation of V δ 1⁺ and DN T cells.

Example 5: PVR specifically inhibits TCR signaling

[00104] To confirm that PVR mediated inhibition of stimulation DN and V δ 1⁺ $\gamma\delta$ T cells was due specifically to inhibition of TCR signalling; skin-derived lymphocytes were stimulated by plate-bound isotype control or α CD3 Ab or MHC class I polypeptide-related sequence A (rhMICA), (ligand for the NKG2D receptor) in the presence of rhPVR or its control. After 6 hours of stimulation, V δ 1⁺ and DN cell cytokine production was assessed by intracellular staining and FACS analysis gating on V δ 1⁺, V δ 2⁺, or DN populations. Like already demonstrated, α CD3-induced IFN γ and TNF α production is inhibited by the presence of PVR (Figure 14). In parallel, the same donor was stimulated with plate-bound MICA, an innate TCR-independent activator for V δ 1⁺ and DN T cells. Of note, the presence of PVR does not inhibit MICA-induced cytokine production, rather increase the production of IFN γ and TNF α by V δ 1⁺ and DN T cells (Figures 15 and 16). Indeed, PVR, when is not in the context of a TCR stimulation, doesn't work as an inhibitor through TIGIT but works as a costimulator through DNAM1, as demonstrated by the stimulation with PVR only: V δ 1⁺ and DN T cells are induced to produce, even if in a small amount, IFN γ and TNF α and this production is reverted by a blocking α DNAM1 mAb (Figures 15 and 16).

[00105] Production of another cytokine associated with TCR signaling, granulocyte-macrophage colony-stimulating factor (GM-CSF), was also analyzed in response to PVR

(Figure 17). In addition to IFN γ and TNF α , GMCSF also was inhibited by PVR, demonstrating that PVR is a general inhibitor of TCR stimulation.

Example 6: PVR inhibitory effect is lost on V δ 1⁺ and V δ 3⁺ cells not expressing TIGIT

[00106] Tissue-derived V δ 1⁺ and V δ 3⁺ cells cultured and expanded *ex-vivo* in the presence of IL-2 and IL-15 (culture method 1) expressed high amounts of TIGIT, whereas V δ 1⁺ and V δ 3⁺ cells cultured in the presence of IL-2, IL-4, IL-15 and IL-21 (culture method 2), expressed low levels of TIGIT (Figure 18). FACS analysis of tissue-derived V δ 1⁺ and V δ 3⁺ cells from individual donors cultured in the presence of IL-2, IL-4, IL-15 and IL-21, demonstrated that PVR inhibition of IFN γ and TNF α production was lost in V δ 1⁺ and V δ 3⁺ cells expressing low levels of TIGIT (Figures 19 and 20).

[0172] In conclusion, the TCR stimulation is a strong activator of V δ 1⁺ and DN T cells, in terms of production of inflammatory cytokines like IFN γ and TNF α but also in terms of proliferation. On the other side, the TCR-induced activation of V δ 1⁺ and DN T cells is normally kept under control by the PVR/TIGIT axis. Indeed TIGIT is constitutively expressed by PB- and Skin-derived V δ 1⁺ and DN T cells. The inhibitory receptor TIGIT, like has been extensively demonstrated, has greater affinity for its ligand PVR compare to the activator receptor DNAM1. In the context of a TCR stimulation, PVR preferentially binds TIGIT and triggers its inhibitory signaling into the cells dampening the activation of V δ 1⁺ and DN T cells. On the other hand, when not in a TCR activation context, PVR is available to bind the costimulatory receptor DNAM1 and help the activation of V δ 1⁺ and DN T cells. By using molecules or mAbs blocking TIGIT, and its interaction with PVR and its other ligands PVRL2 and PVRL3, we could dampen its negative signalling and promote V δ 1⁺ and DN T cell activation.

[0173] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0174] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

8. REFERENCES CITED

[0175] Gentles, A. J., et al., “The prognostic landscape of genes and infiltrating immune cells across human cancers”; Nature Medicine ; Vol 21; No. 8; August 2015; pp. 938-945.

CLAIMS

1. A method of activating V δ 2⁻ gamma-delta T cells ($\gamma\delta$ T cells) in a mammalian subject, comprising:
antagonizing TIGIT activity of V δ 2⁻ $\gamma\delta$ T cells present in the subject to an extent sufficient to activate the V δ 2⁻ $\gamma\delta$ T cells *in vivo*.
2. The method of claim 1, wherein the V δ 2⁻ $\gamma\delta$ T cells are V δ 1⁺ cells.
3. The method of claim 1, wherein the V δ 2⁻ $\gamma\delta$ T cells are V δ 1⁻ V δ 2⁻ double negative (DN) cells.
4. The method of any one of claims 1 - 3, wherein antagonizing TIGIT activity comprises administering at least one TIGIT inhibitor to the subject.
5. The method of claim 4, wherein the at least one TIGIT inhibitor is administered adjunctively with V δ 2⁻ $\gamma\delta$ T cells that have been cultured *ex vivo*.
6. The method of claim 5, wherein the at least one TIGIT inhibitor is added to the V δ 2⁻ $\gamma\delta$ T cells during culturing *ex vivo*.
7. The method of claim 5 or 6, wherein the cultured V δ 2⁻ $\gamma\delta$ T cells have been expanded *ex vivo*.
8. The method of any one of claims 5 - 7, wherein the at least one TIGIT inhibitor is administered in a composition comprising the cultured $\gamma\delta$ T cells.
9. The method of any one of claims 5 - 7, wherein the at least one TIGIT inhibitor is administered separately from the *ex vivo* expanded $\gamma\delta$ T cells.
10. The method of any one of claims 4 - 9, further comprising administering to the subject at least one additional agent in an amount sufficient to activate V δ 2⁻ $\gamma\delta$ T cells.
11. The method of claim 10, wherein the at least one additional agent is an agonist of DNAM-1.
12. The method of any one of claims 4 - 11, wherein the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with Poliovirus receptor (PVR), an agent that inhibits and/or blocks the interaction

of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling within a V δ 2⁺ $\gamma\delta$ T cell mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a V δ 2⁺ $\gamma\delta$ T cell mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a V δ 2⁺ $\gamma\delta$ T cell mediated by PVRL3 binding to TIGIT, and combinations thereof.

13. The method of claim 12, wherein the TIGIT inhibitor is an antibody.
14. The method of claim 13, wherein the antibody binds specifically to TIGIT.
15. The method of claim 13, wherein the antibody binds to PVR to specifically inhibit PVR interaction with TIGIT.
16. The method of any one of claims 13 - 15, wherein the antibody is a full length IgG antibody.
17. The method of any one of claims 13 - 16, wherein the antibody is an antigen-binding antibody fragment.
18. The method of any one of claims 13 - 16, wherein the antibody is a single domain antibody.
19. The method of any one of claims 13 - 19 wherein the antibody is fully human.
20. The method of any one of claims 13 - 20, wherein the antibody is humanized.
21. The method of claim 12, wherein the TIGIT inhibitor is a small molecule inhibitor.
22. The method of claim 7 or any one of claims 8 – 21 as depend from claim 7, wherein the *ex vivo* expanded V δ 2⁺ $\gamma\delta$ T cells were obtained by:

culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture.

23. The method according to claim 22, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2.

24. The method according to claim 22 or 23, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of interleukin-15 (IL-15).
25. The method according to any one of claims 22 - 34, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2 and IL-15.
26. The method according to any one of claims 22 - 25, wherein the culturing step comprises culturing the lymphocytes in the absence of TCR activation or co-stimulation signals.
27. The method according to any one of claims 22 - 26, wherein the culturing step comprises culturing the lymphocytes in the absence of a T cell receptor pathway agonist.
28. The method according to any one of claims 22 - 27, wherein the culturing step comprises culturing the lymphocytes in the absence of stromal or epithelial cells.
29. The method according to claim 28, wherein stromal or epithelial cells are removed prior to culture.
30. The method according to claim 28, wherein the lymphocytes are cultured in the absence of fibroblasts.
31. The method according to any one of claims 22 to 30, wherein the lymphocytes have been obtained from skin, the gastrointestinal tract (e.g., colon), mammary gland tissue, lung, liver, pancreas, prostate or reproductive tissue.
32. The method of any one of claims 22 – 31, wherein the *ex vivo* expanded V δ 2⁺ $\gamma\delta$ T cells were obtained by:

culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of IL-2, IL-15, and a factor selected from the group consisting of IL-4, IL-21, IL-6, IL-7, IL-8, IL-9, IL-12, IL-18, IL-33, IGF-1, IL-1 β , human platelet lysate (HPL), and stromal cell-derived factor-1 (SDF-1) for at least 5 days to produce an expanded population of $\gamma\delta$ T cells.

33. The method of any one of claims 22 - 32, wherein, within 14 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 20-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue.

34. The method of claim 33, wherein, within 7 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 2-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue.

35. The method of any one of claims 22 - 34, wherein the expanded population of $\gamma\delta$ T cells is at least 50% V δ 1⁺ cells.

36. The method of claim 35, wherein the expanded population of $\gamma\delta$ T cells is at least 70% V δ 1⁺ cells.

37. The method of claim 36, wherein the expanded population of $\gamma\delta$ T cells is at least 90% V δ 1⁺ cells.

38. The method of any one of claims 1 – 37, wherein the subject has cancer.

39. The method of claim 38, wherein the cancer has high infiltration of $\gamma\delta$ T cells.

40. A method of treating cancers having high infiltration of $\gamma\delta$ T cells, comprising:
administering at least one TIGIT inhibitor to a patient with a cancer having high infiltration of $\gamma\delta$ T cells, in an amount sufficient to activate tissue-infiltrating $\gamma\delta$ T cells in the patient.

41. The method of claim 40, wherein the cancer-infiltrating $\gamma\delta$ T cells are V δ 2⁻ cells.

42. The method of claim 40 or 41, wherein the V δ 2⁻ cells are V δ 1⁺ or DN cells.

- 43.** The method of any one of claims 40 - 42, wherein the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^- \gamma\delta$ T cell mediated by PVRL3 binding to TIGIT, and combinations thereof.
- 44.** The method of any one of claims 40 - 43, wherein the TIGIT inhibitor is an antibody.
- 45.** The method of claim 44, wherein the antibody binds specifically to TIGIT.
- 46.** The method of claim 44, wherein the antibody binds to PVR to specifically inhibit PVR interaction with TIGIT.
- 47.** The method of any one of claims 44 - 46, wherein the antibody is a full length IgG antibody.
- 48.** The method of any one of claims 44 - 46, wherein the antibody is an antigen-binding antibody fragment.
- 49.** The method of any one of claims 44 - 46, wherein the antibody is a single domain antibody.
- 50.** The method of any one of claims 44 - 49, wherein the antibody is fully human.
- 51.** The method of any one of claims 44 - 49, wherein the antibody is humanized.
- 52.** The method of any one of claims 40 - 43, wherein the TIGIT inhibitor is a small molecule inhibitor.
- 53.** The method of any one of claims 40 - 52, further comprising the antecedent step of selecting for treatment a patient whose cancer shows high levels of $\gamma\delta$ T cell infiltration.
- 54.** The method of claim 53, wherein the $\gamma\delta$ T cell infiltration is detected by analysis of a tumor biopsy by a method comprising an assay selected from the group consisting of immunohistochemistry, polymerase chain reaction, in situ hybridization, or combinations thereof.

55. The method of claim 53 or 54, wherein the $\gamma\delta$ T cell infiltration comprises $V\delta 1^+$ or double negative (DN) cells.
56. The method of any one of claims 40 - 55, wherein at least one additional therapeutic agent is administered.
57. The method of claim 56, wherein the at least one or more additional agent comprises an agonist of DNAM-1.
58. The method of claim 57, wherein the at least one or more additional agent comprises an anti-cancer agent.
59. The method of claim 58, wherein the anti-cancer agent is selected from the group consisting of radiation, a chemotherapeutic or growth inhibitory agent, a targeted therapeutic agent, a small molecule inhibitor, a T cell expressing a chimeric antigen receptor, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate, an angiogenesis inhibitor, an antineoplastic agent, a cancer vaccine, an adjuvant, and combinations thereof.
60. The method of claim 59, wherein the anti-cancer agent is a chemotherapeutic.
61. The method of claim 59, wherein the anti-cancer agent is a small molecule inhibitor.
62. The method of claim 59, wherein the anti-cancer agent is an antibody.
63. The method of any one of claims 40 - 62, wherein the TIGIT inhibitor is administered continuously.
64. The method of any one of claims 40 - 62, wherein the TIGIT inhibitor is administered intermittently.
65. The method of any one of claims 58 - 64, wherein the anti-cancer agent is administered continuously.
66. The method of any one of claims 58 - 64, wherein the anti-cancer agent is administered intermittently.
67. The method of any one of claims 58 - 64, wherein the TIGIT inhibitor is administered before the anti-cancer agent.
68. The method of any one of claims 58 - 64, wherein the TIGIT inhibitor is administered simultaneous with the anti-cancer agent.

69. The method of any one of claims 58 - 64, wherein the TIGIT inhibitor is administered after the anti-cancer agent.
70. A method of increasing the number of tissue infiltrating $\gamma\delta$ T cells in a mammalian subject recipient, comprising:
- obtaining $\gamma\delta$ T cells from tissue comprising $\gamma\delta$ T cells from a donor;
 - expanding the $\gamma\delta$ T cells *ex vivo*;
 - transplanting the expanded $\gamma\delta$ T cells into a recipient, and administering at least one TIGIT inhibitor to the recipient.
71. The method of claim 70, wherein the obtained and/or expanded $\gamma\delta$ T cells are $V\delta 2^-$.
72. The method of claim 71, wherein the $V\delta 2^-$ cells are $V\delta 1^+$ or DN.
73. The method of any one of claims 70 - 72, wherein the at least one TIGIT inhibitor is selected from the group consisting of an antagonist of TIGIT activity, an antagonist of TIGIT interaction with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVR, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL2, an agent that inhibits and/or blocks the interaction of TIGIT with PVRL3, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVR binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVRL2 binding to TIGIT, an agent that inhibits and/or blocks the intracellular signaling within a $V\delta 2^-$ $\gamma\delta$ T cell mediated by PVRL3 binding to TIGIT, and combinations thereof.
74. The method of any one of claims 70 - 73, wherein the at least one TIGIT inhibitor is an antibody.
75. The method of claim 74, wherein the antibody binds specifically to TIGIT.
76. The method of claim 74, wherein the antibody binds to PVR.
77. The method of any one of claims 74 - 76, wherein the antibody is a full length IgG antibody.
78. The method of any one of claims 74 - 76, wherein the antibody is an antigen-binding antibody fragment.
79. The method of any one of claims 74 - 76, wherein the antibody is a single domain antibody.

80. The method of any one of claims 74 - 79, wherein the antibody is fully human.
81. The method of any one of claims 74 - 79, wherein the antibody is humanized.
82. The method of any one of claims 70 - 73, wherein the at least one TIGIT inhibitor comprises a small molecule inhibitor.
83. The method of any one of claims 71 - 82, wherein the *ex vivo* expanded V δ 2⁺ γ δ T cells were obtained by:
- culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture.
84. The method according to claim 83, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2.
85. The method according to claim 83 or 84, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of interleukin-15 (IL-15).
86. The method according to any one of claims 81 - 85, wherein the culturing step comprises culturing the lymphocytes obtained from human or non-human animal non-hematopoietic tissue in the presence of IL-2 and IL-15.
87. The method according to any one of claims 83 - 86, wherein the culturing step comprises culturing the lymphocytes in the absence of TCR activation or co-stimulation signals.
88. The method according to any one of claims 83 - 87, wherein the culturing step comprises culturing the lymphocytes in the absence of a T cell receptor pathway agonist.
89. The method according to any one of claims 83 - 88, wherein the culturing step comprises culturing the lymphocytes in the absence of stromal or epithelial cells.

90. The method according to claim 89, wherein stromal or epithelial cells are removed prior to culture.
91. The method according to claim 89, wherein the lymphocytes are cultured in the absence of fibroblasts.
92. The method according to any one of claims 83 - 91, wherein the lymphocytes have been obtained from skin, the gastrointestinal tract (e.g., colon), mammary gland tissue, lung, liver, pancreas or prostate.
93. The method of any one of claims 83 - 92, wherein the *ex vivo* expanded V δ 2⁻ $\gamma\delta$ T cells were obtained by:
- culturing lymphocytes obtained from non-hematopoietic tissue of humans or non-human animals in the presence of IL-2, IL-15, and a factor selected from the group consisting of IL-4, IL-21, IL-6, IL-7, IL-8, IL-9, IL-12, IL-18, IL-33, IGF-1, IL-1 β , human platelet lysate (HPL), and stromal cell-derived factor-1 (SDF-1) for at least 5 days to produce an expanded population of $\gamma\delta$ T cells.
94. The method of any one of claims 90 - 93, wherein, within 14 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 20-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue.
95. The method of claim 94, wherein, within 7 days of culture, the expanded population of $\gamma\delta$ T cells comprises at least 2-fold the number of $\gamma\delta$ T cells as the $\gamma\delta$ T cells obtained from a non-hematopoietic tissue.
96. The method of any one of claims 83 - 95, wherein the expanded population of $\gamma\delta$ T cells is at least 50% V δ 1⁺ cells.
97. The method of claim 96, wherein the expanded population of $\gamma\delta$ T cells is at least 70% V δ 1⁺ cells.

- 98.** The method of claim 97, wherein the expanded population of $\gamma\delta$ T cells is at least 90% V δ 1⁺ cells.
- 99.** The method of any one of claims 70 - 98, wherein the administration of the at least one TIGIT inhibitor is performed prior to, during, after the transplantation, or combinations thereof.
- 100.** The method of any one of claims 70 - 99, wherein at least one additional agent is administered to the recipient.
- 101.** The method of claim 100, wherein the at least one additional agent is an anti-cancer agent.
- 102.** The method of claim 101, wherein the anti-cancer agent is selected from the group consisting of radiation, a chemotherapeutic or growth inhibitory agent, a targeted therapeutic agent, a small molecule inhibitor, a T cell expressing a chimeric antigen receptor, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate, an angiogenesis inhibitor, an antineoplastic agent, a cancer vaccine, an adjuvant, and combinations thereof.
- 103.** The method of any one of claims 70 - 102, wherein the recipient has cancer.
- 104.** The method of any one of claims 70 - 103, wherein the $\gamma\delta$ T cells are obtained from an autologous donor.
- 105.** The method of any one of claims 83 - 104, wherein the non-hematopoietic tissue is tumor tissue.
- 106.** The method of any one of claims 83 - 104, wherein the non-hematopoietic tissue is skin tissue.
- 107.** The method of any one of claims 70 - 106, further comprising separating V δ 1⁺ $\gamma\delta$ T cells or DN cells from the $\gamma\delta$ T cells prior to transplantation.
- 108.** The method of any one of the preceding claims, wherein at least one additional checkpoint inhibitor is administered.
- 109.** The method of claim 108, wherein the additional checkpoint inhibitor does not inhibit PD1.
- 110.** The method of claim 108, wherein the additional checkpoint inhibitor inhibits TIM-3

- 111.** The method of claim 109, wherein the additional checkpoint inhibitor inhibits LAG-3.
- 112.** The method of any one of the preceding claims, wherein the administration of the TIGIT inhibitor results in elevated release of cytokines from the $\gamma\delta$ T cells, selected from the group consisting of IFN- γ , TNA- α , interleukins, and combinations thereof.
- 113.** A method of treating cancer, comprising:

administering a therapeutically effective amount of a TIGIT inhibitor to a cancer patient who has been determined to have an elevated representation of $V\delta 1^+$ cells in a sample of peripheral blood.
- 114.** The method of claim 113, further comprising the prior step of determining the representation of $V\delta 1^+$ cells in the peripheral blood sample.
- 115.** The method of claim 114, further comprising the step, after determining the representation of $V\delta 1^+$ cells and before administering the TIGIT inhibitor, of selecting the patient for TIGIT inhibitor treatment if the patient's sample has been determined to have an elevated representation of $V\delta 1^+$ cells.
- 116.** The method of any one of claims 113 - 115, wherein the elevated representation is an increase in $V\delta 1^+$ cells as a percentage of total lymphocytes in the sample.
- 117.** The method of claim 116, wherein greater than 0.5% of total lymphocytes in the sample are determined to be $V\delta 1^+$ cells.
- 118.** The method of claim 117, wherein greater than 1.0% of total lymphocytes are determined to be $V\delta 1^+$ cells.
- 119.** The method of claim 118, wherein greater than 2.0% of total lymphocytes are determined to be $V\delta 1^+$ cells.
- 120.** The method of any one of claims 113 - 115, wherein the elevated representation is an increase in $V\delta 1^+$ cells as a percentage of total $\gamma\delta$ T cells in the sample.

- 121.** The method of claim 120, wherein greater than 10% of total $\gamma\delta$ T cells in the sample are $V\delta 1^+$ cells.
- 122.** The method of claim 121, wherein greater than 15% of total $\gamma\delta$ T cells in the sample are $V\delta 1^+$ cells.
- 123.** The method of any one of claims 113 - 115, wherein the elevated representation is an increase in the ratio of $V\delta 1^+$ cells to $V\delta 2^+$ cells in the sample.
- 124.** The method of claim 123, wherein the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:9.
- 125.** The method of claim 124, wherein the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:8.
- 126.** The method of claim 125, wherein the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:7.
- 127.** The method of claim 126, wherein the ratio of $V\delta 1^+$: $V\delta 2^+$ cells is greater than 1:6.
- 128.** The method of any one of claims 113 - 127, wherein the TIGIT inhibitor is an antibody or antigen-binding antibody fragment that binds specifically to TIGIT.
- 129.** The method of any one of claims 113 - 128, further comprising administration of at least one additional anti-cancer therapy.
- 130.** The method of claim 129, wherein at least one of the at least one additional anti-cancer therapy is administration of a non-TIGIT checkpoint inhibitor.
- 131.** The method of claim 130, wherein the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PD-1.
- 132.** The method of claim 130, wherein the non-TIGIT checkpoint inhibitor is an antibody that binds specifically to PDL1.

133. The method of any one of claims 1 - 132, wherein the cancer is a solid, non-hematopoietic, cancer.

134. The method of claim 133, wherein the cancer is a breast cancer.

135. The method of any one of claims 113 - 132, wherein the cancer is a hematological cancer.

136. The method of claim 7 or a

ny one of claims 8 – 21 as depend from claim 7, wherein the *ex vivo* expanded V δ 2⁺ γ δ T cells were obtained by:

culturing lymphocytes obtained from hematopoietic tissue of humans or non-human animals in the presence of interleukin-2 (IL-2) and/or interleukin-15 (IL-15), and not in direct contact with stromal or epithelial cells during culture.

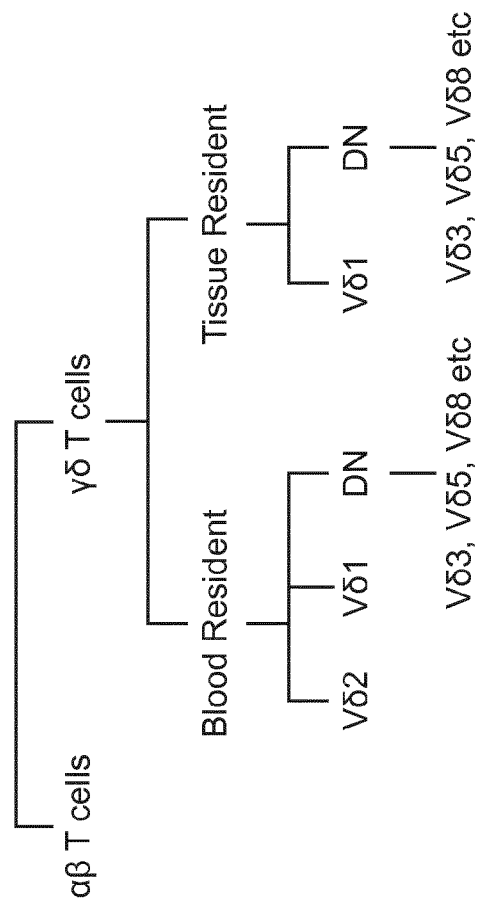
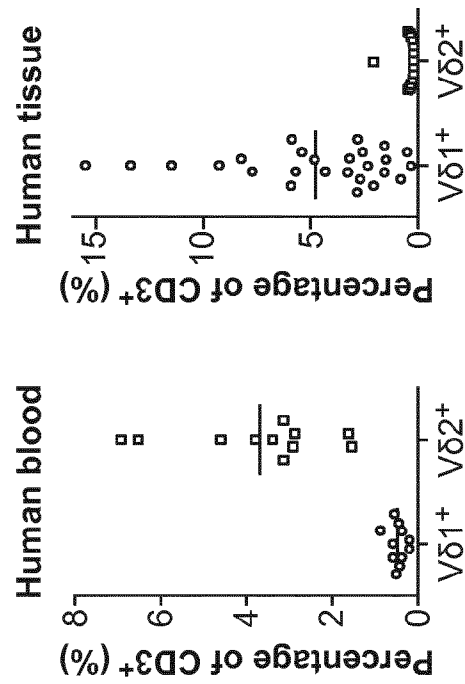


Figure 2

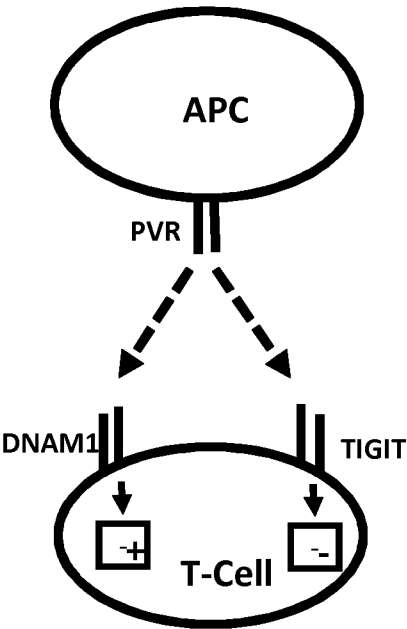


Figure 3

Expression of DNAM1 on $\gamma\delta$ T cells derived from Human PBMC and Skin

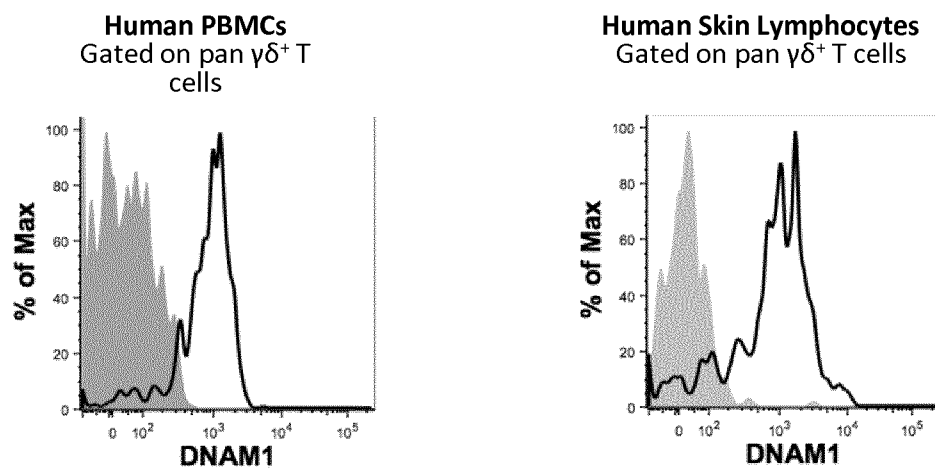


Figure 4

Expression of TIGIT on $\gamma\delta$ T cells derived from Human PBMC

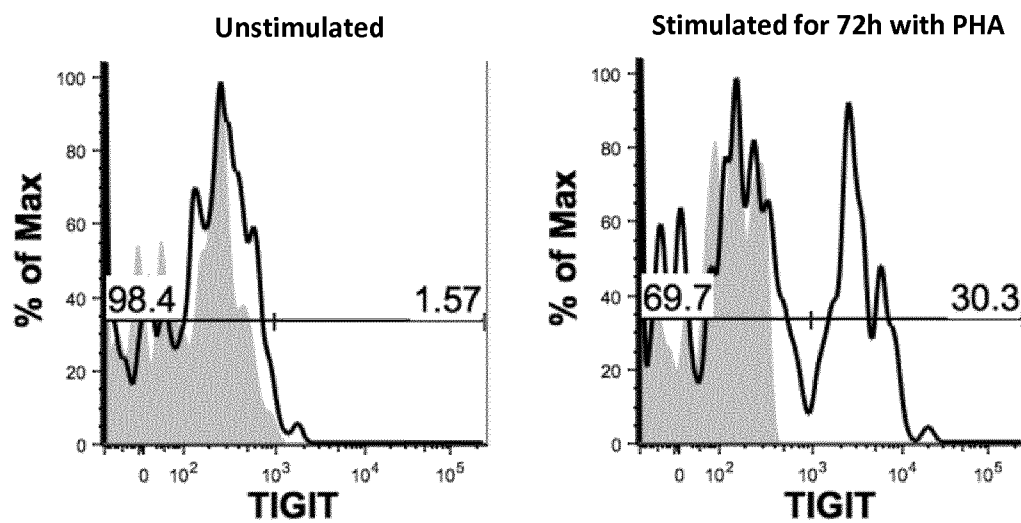
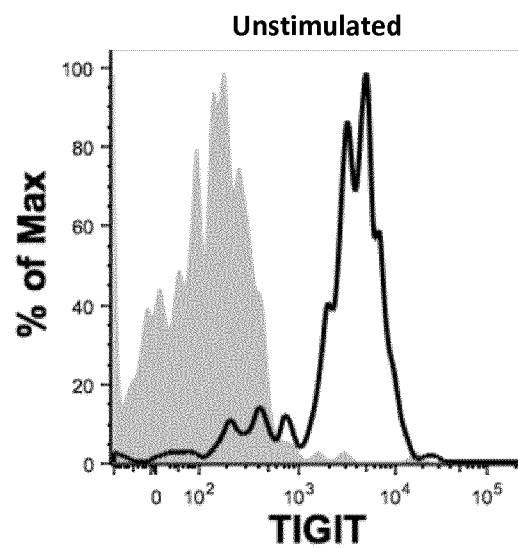


Figure 5

Expression of TIGIT on $\gamma\delta$ T cells derived from Human skin



Expression of DNAM1 and TIGIT on Vδ1+, DN and Vδ2+ T cells
from Human PBMCs

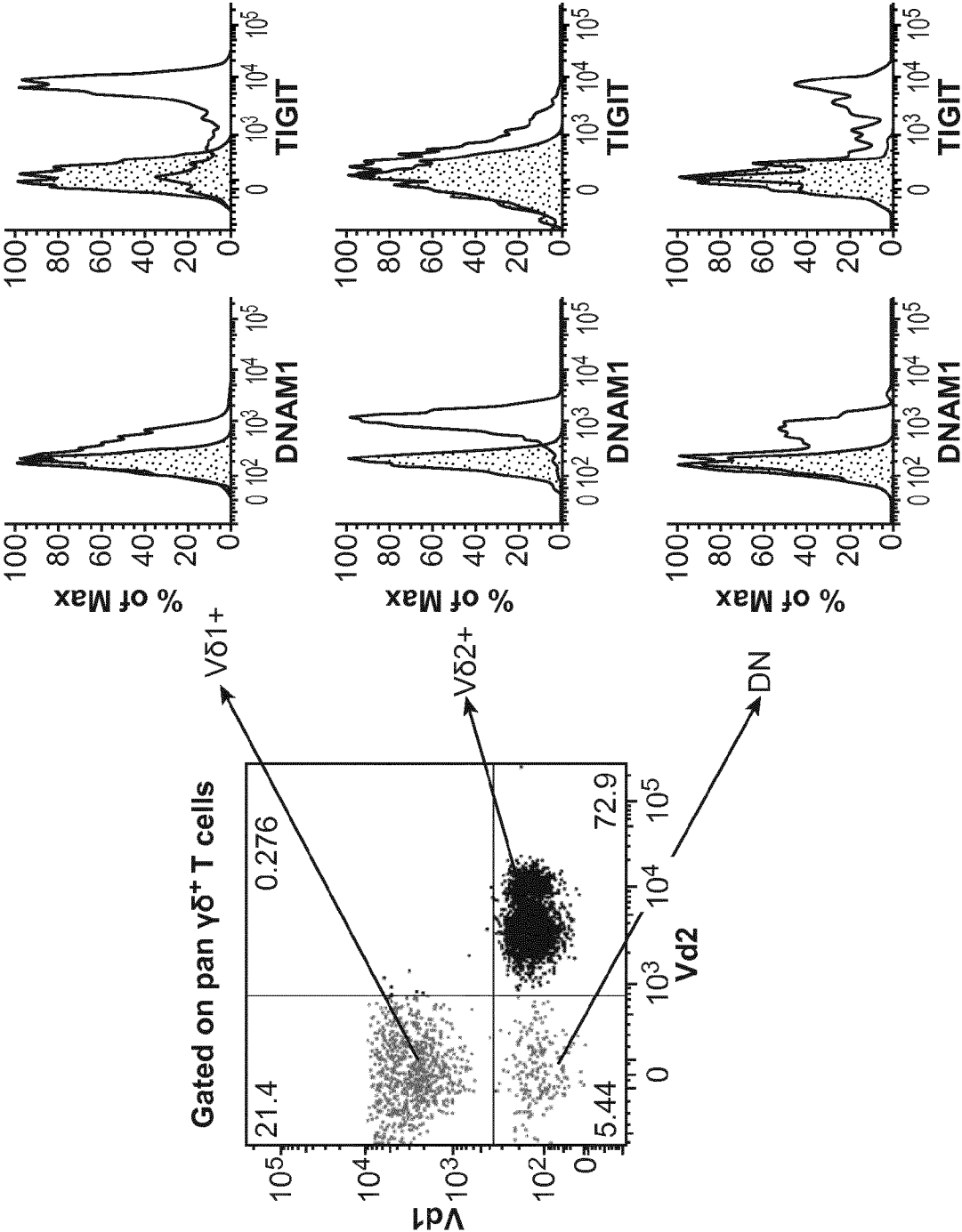


FIG. 6

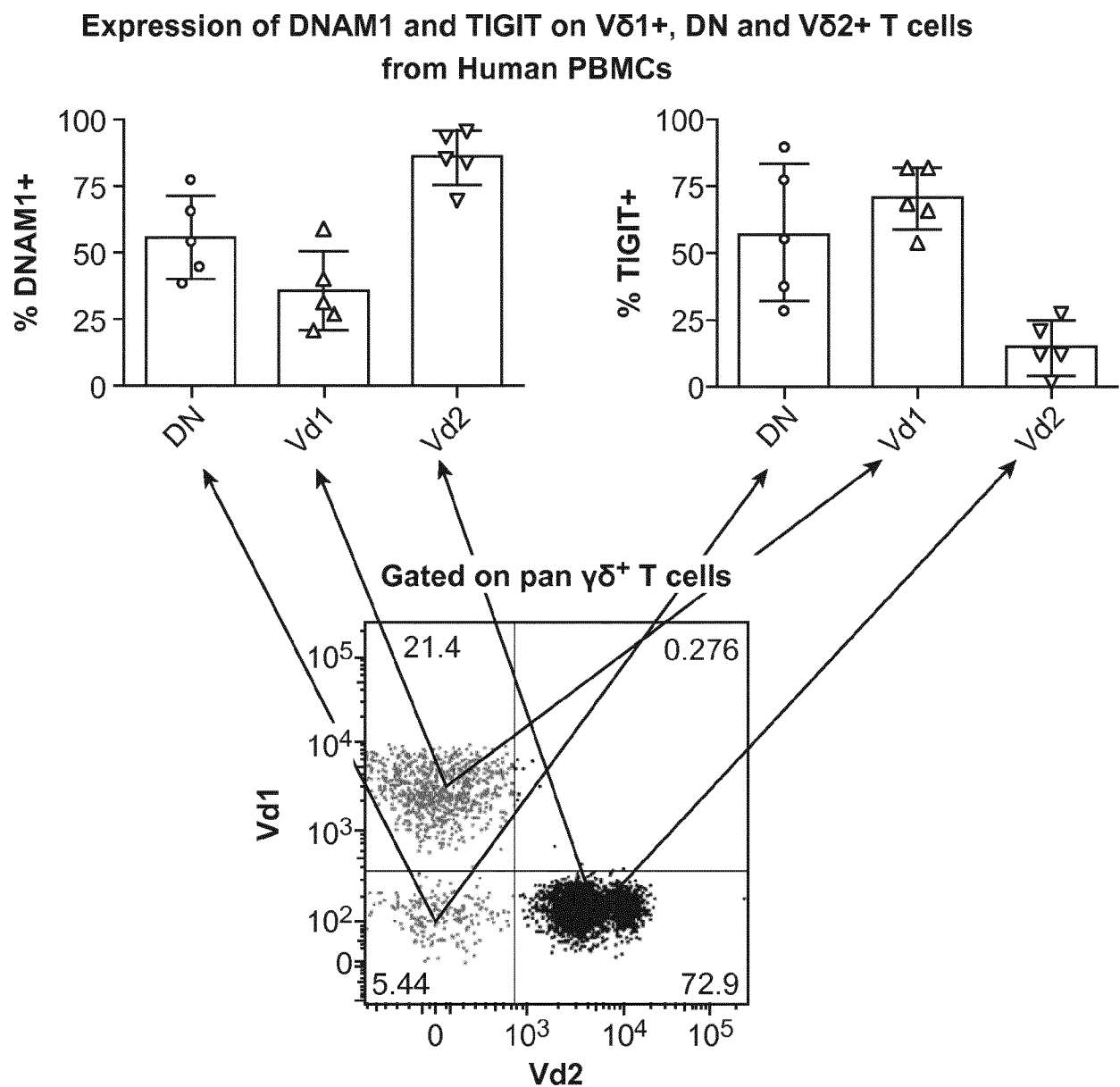


FIG. 7

Expression of DNAM1 and TIGIT on $V\delta 1+$ and DN T cells from Human Skin

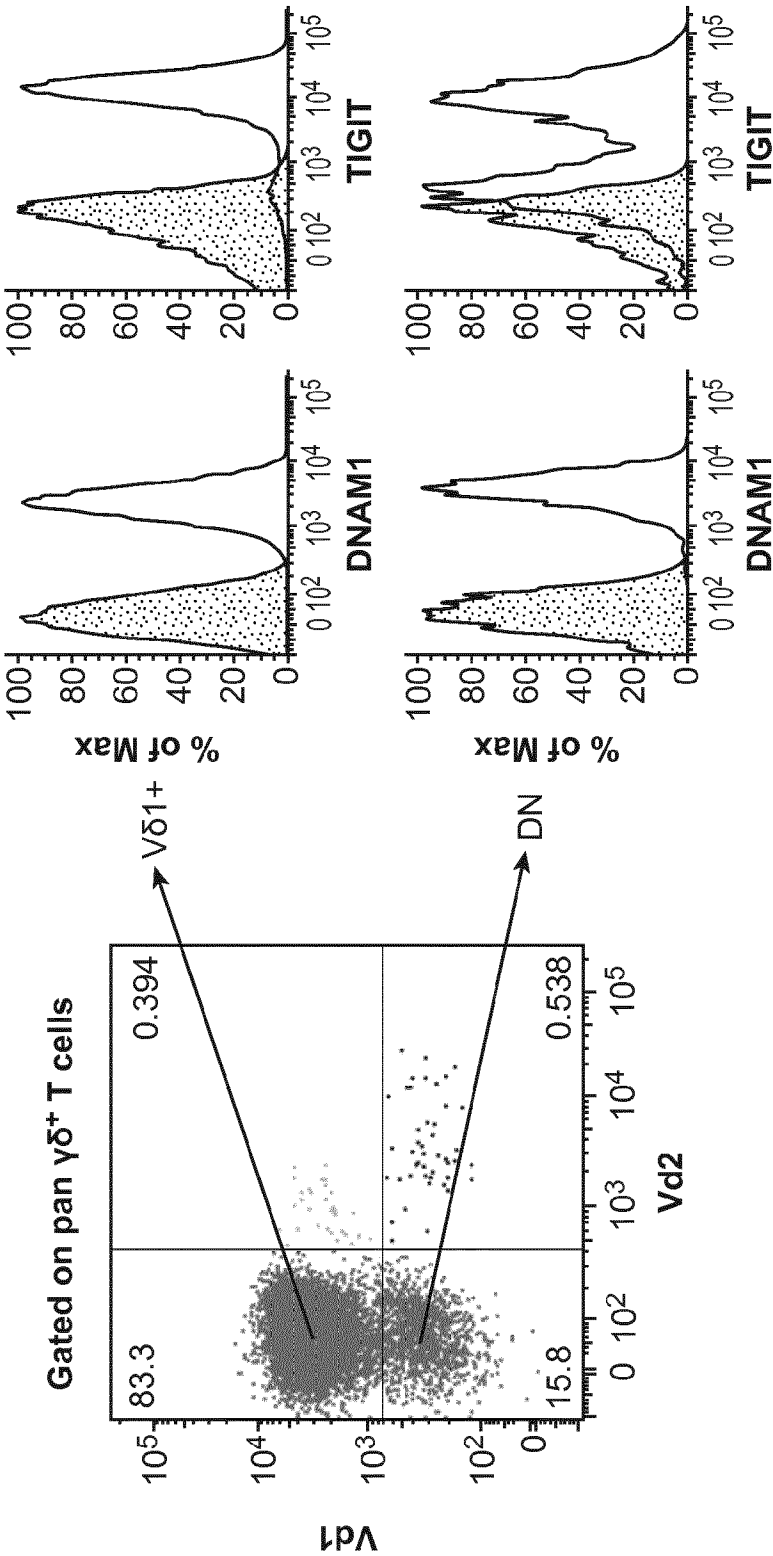
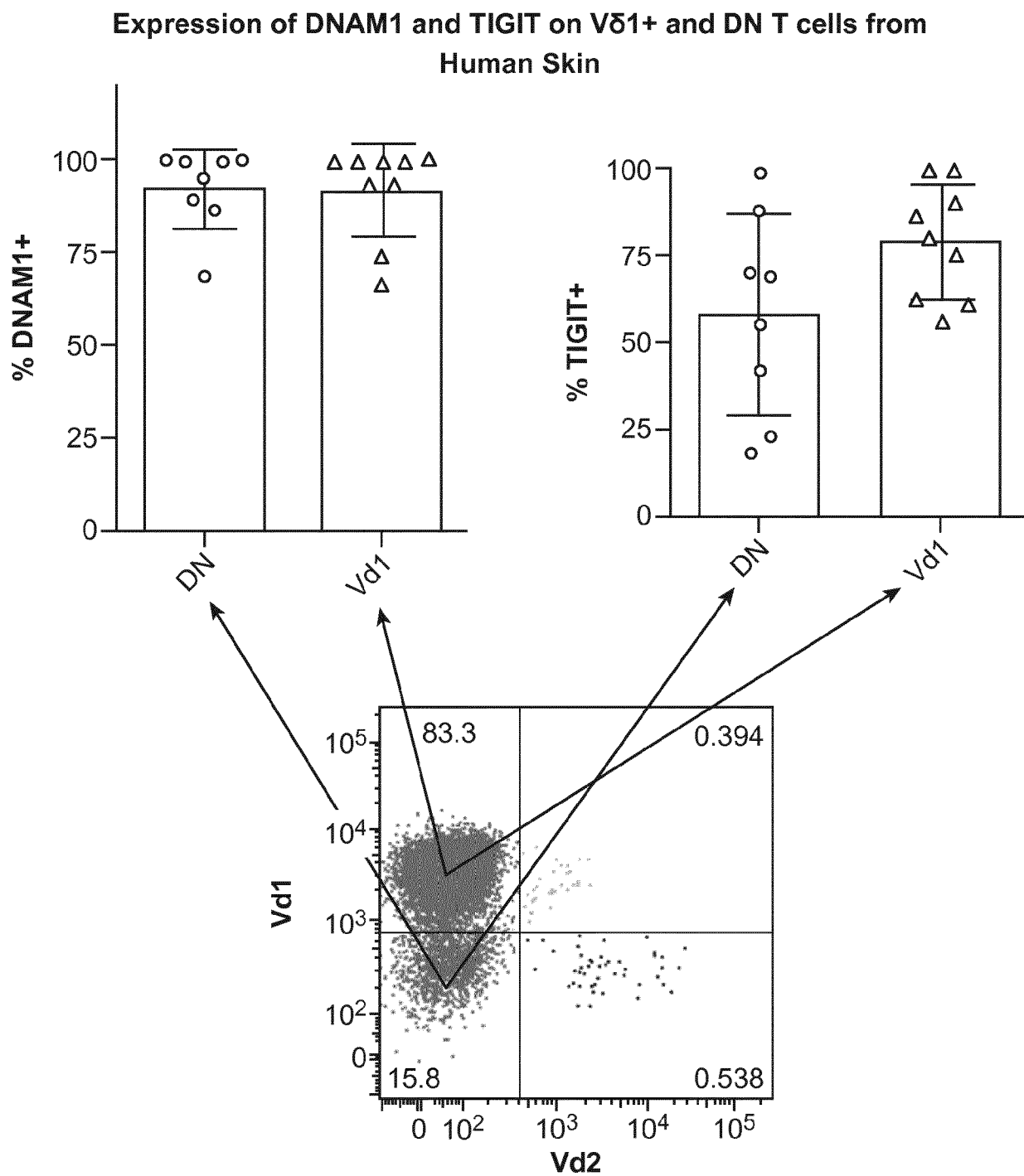


FIG. 8

**FIG. 9**

PVR inhibits TCR stimulation in DN/ $\delta 1$ + T cells from Human PBMCs

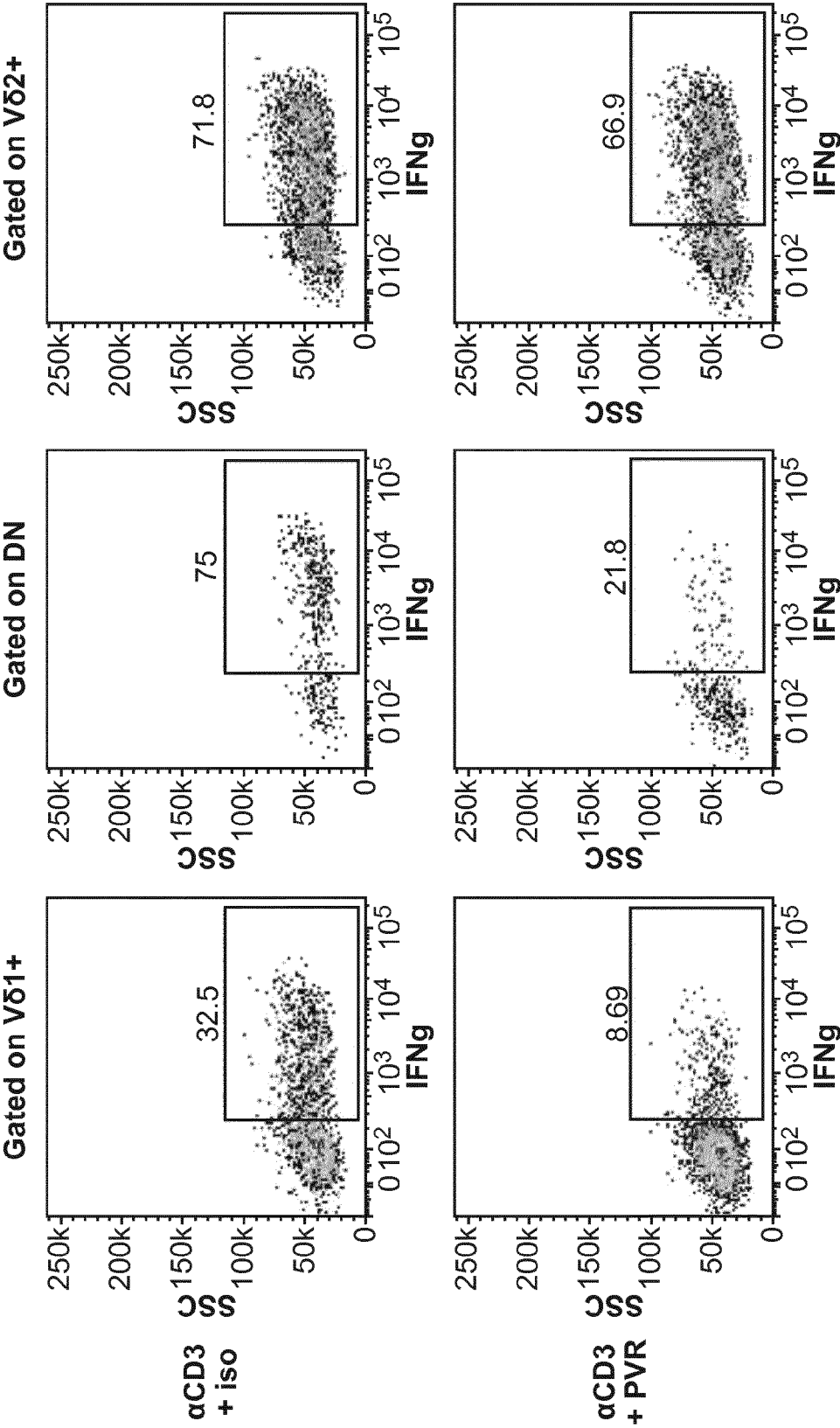


FIG. 10

PVR inhibits TCR stimulation in DN/Vδ1+ T cells

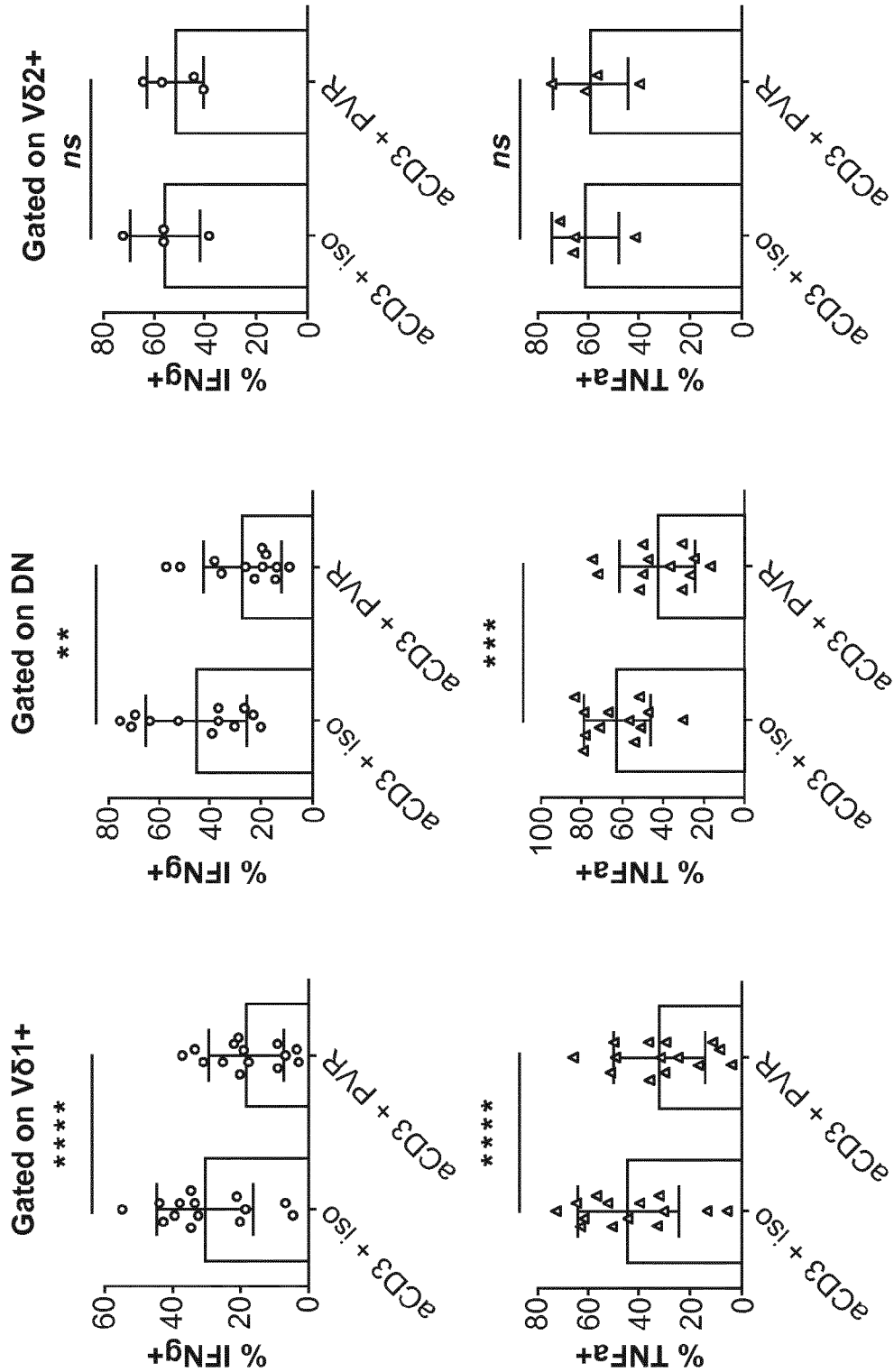


FIG. 11

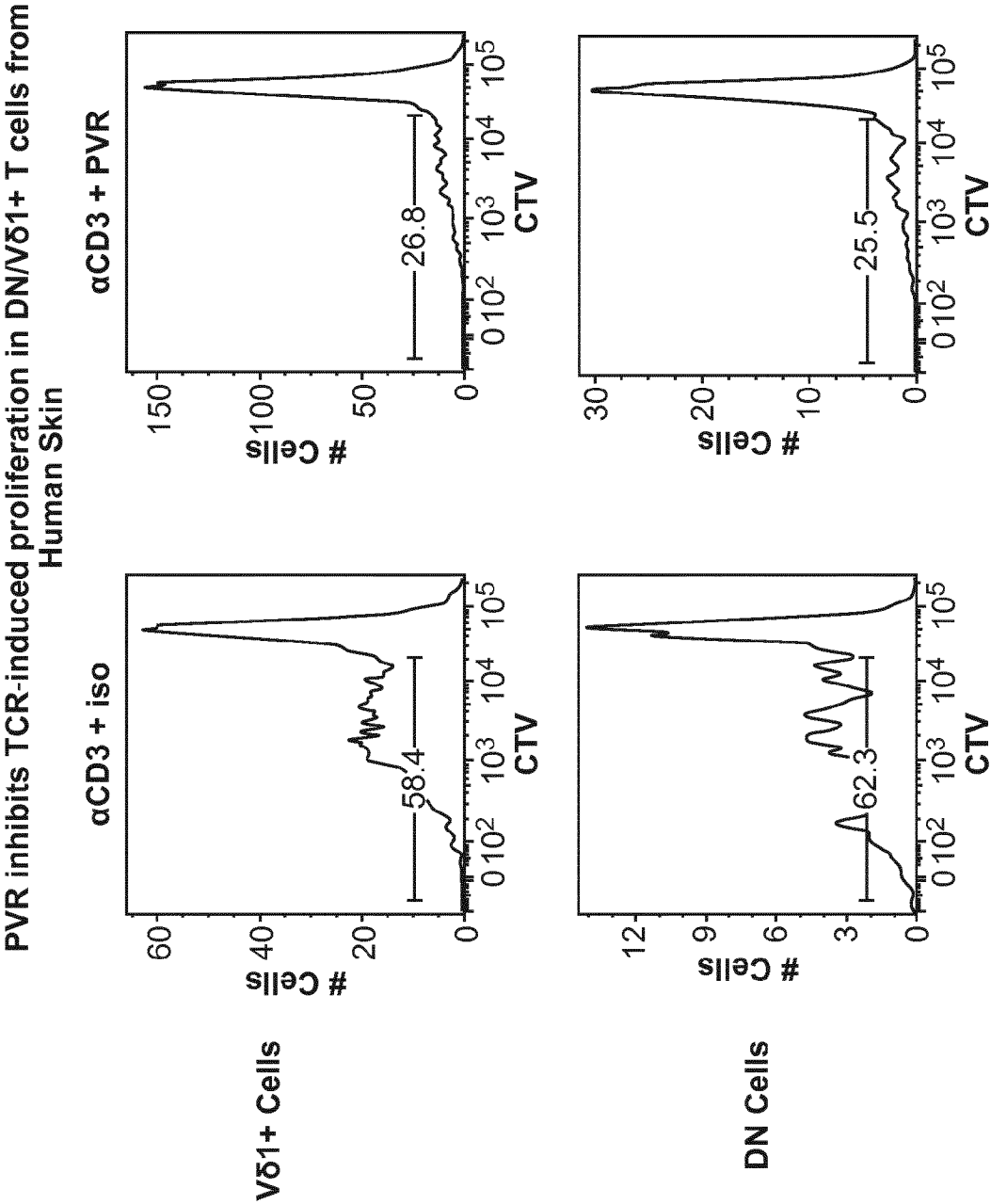


FIG. 12

Figure 13

PVR inhibits TCR-induced proliferation in DN/V δ 1+ T cells from Human Skin

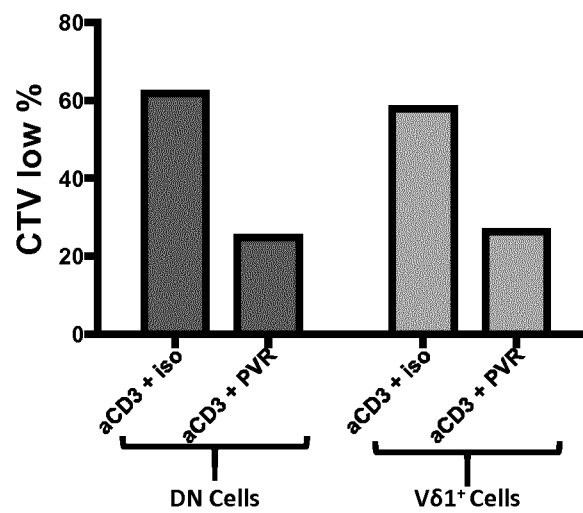


Figure 14

PVR specifically inhibits TCR signalling

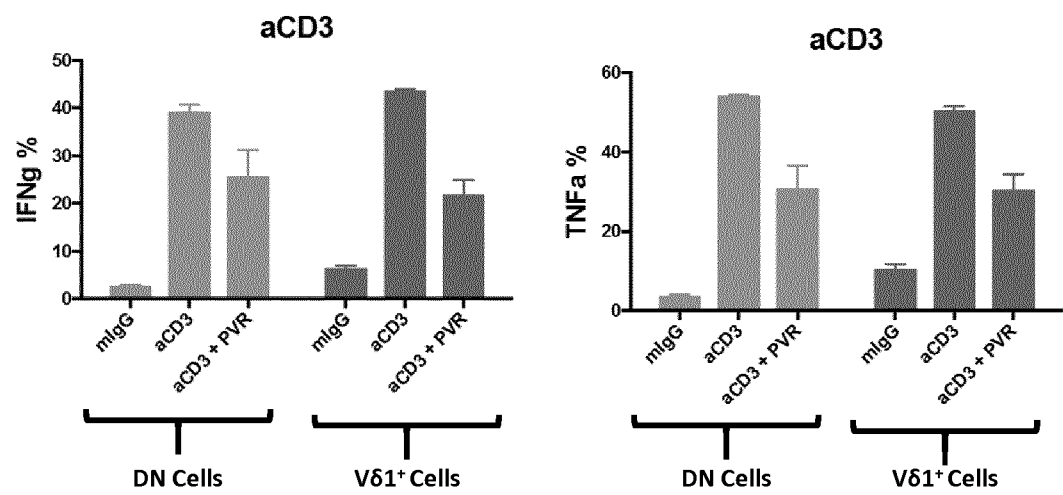


Figure 15 PVR specifically inhibits TCR signalling: INFg Expression

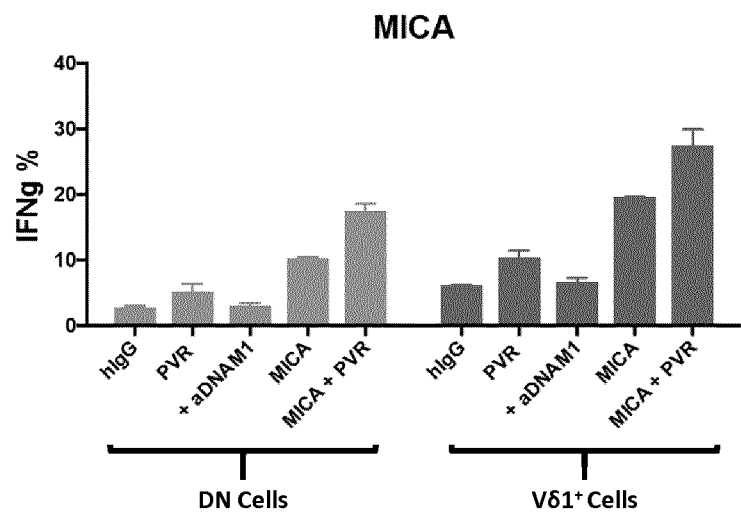


Figure 16

PVR specifically inhibits TCR signalling: TNF α Expression

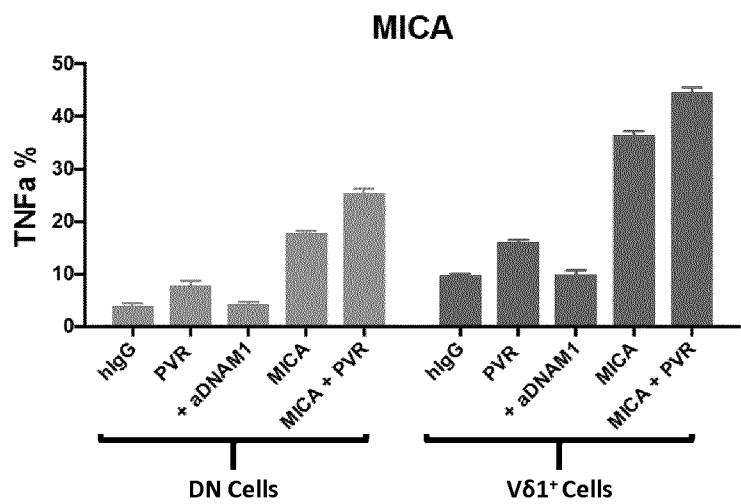
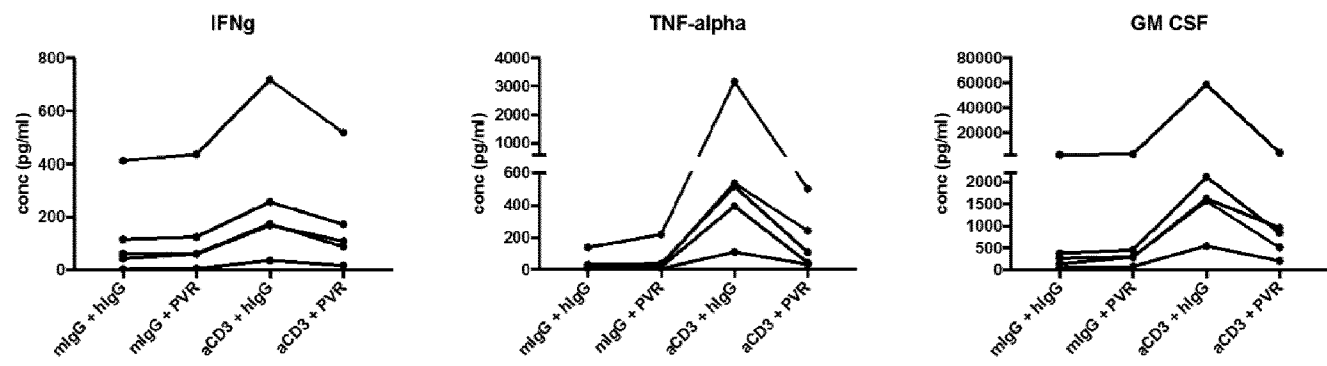


Figure 17



We can modulate TIGIT expression on Vδ1+/Vδ3+ cells using different culture conditions

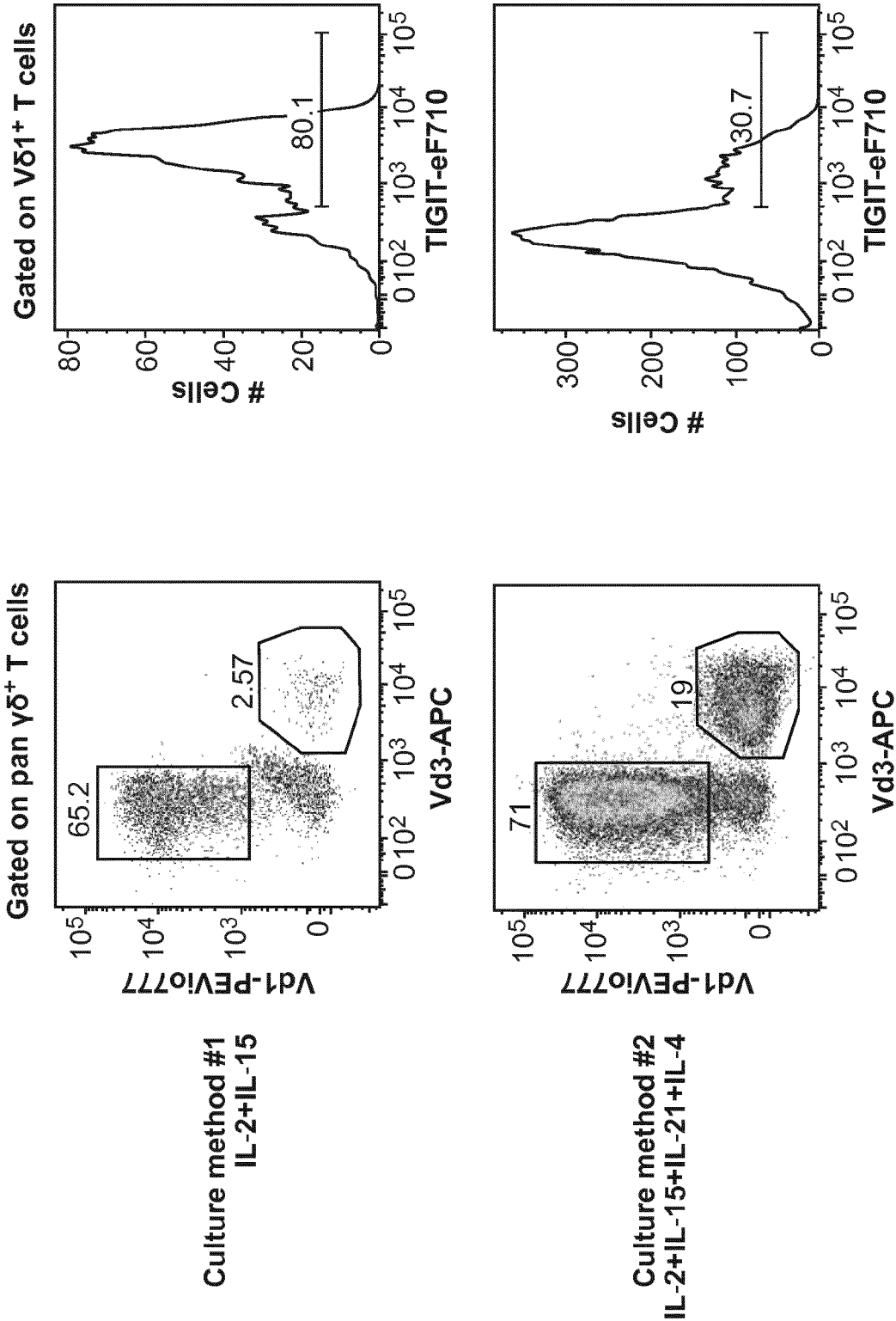


FIG. 18

Figure 19

PVR inhibitory effect is lost on Vδ1+/Vδ3+ cells not expressing TIGIT

Donor 1
Culture method #2

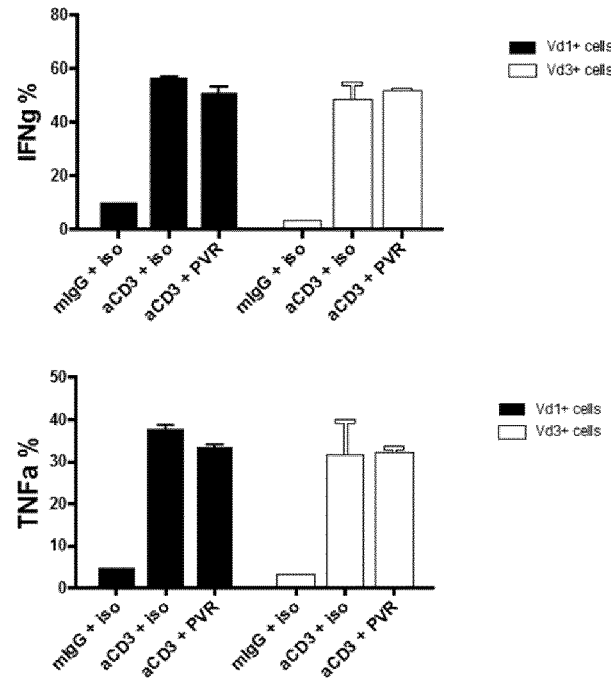
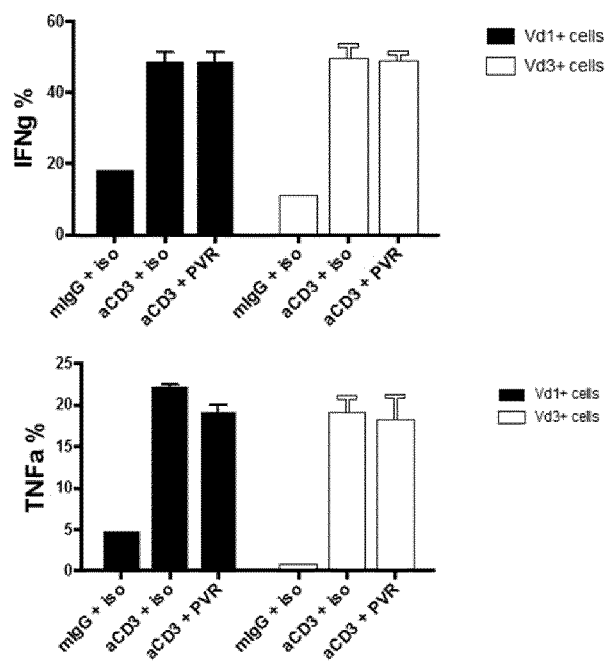


Figure 20

PVR inhibitory effect is lost on Vδ1+/Vδ3+ cells not expressing TIGIT

Donor 2
Culture method #2



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/065730

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K35/17 A61P35/00
ADD. C12N5/0783

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, EMBASE, BIOSIS

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Further documents are listed in the continuation of Box C.



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INTERNATIONAL SEARCH REPORT

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PCT/EP2018/065730

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