(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 April 2023 (27.04.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/069322 A9

(51) International Patent Classification:
A61K 35/17 (2015.01) *A61P 37/06* (2006.01)
A61P 31/00 (2006.01) *C12N 5/0783* (2010.01)
A61P 35/00 (2006.01)

(21) International Application Number:
PCT/US2022/046745

(22) International Filing Date:
14 October 2022 (14.10.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/257,805 20 October 2021 (20.10.2021) US

(71) Applicant: **UNIVERSITY OF PITTSBURGH – OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION** [US/US]; 1st Floor Gardner Steel Conference Center, 130 Thackeray Avenue, Pittsburgh, Pennsylvania 15260 (US).

(72) Inventors: **LOTZE, Michael T.**; 5134 Westminster Place, Pittsburgh, Pennsylvania 15232 (US). **MURTHY, Pranav**; 7070 Bennington Woods Drive, Pittsburgh, Pennsylvania 15237 (US).

(74) Agent: **FINN III, J. Patrick**; P.O Box 1022, Minneapolis, Minnesota 55440 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(48) Date of publication of this corrected version:
10 August 2023 (10.08.2023)

(15) Information about Correction:
see Notice of 10 August 2023 (10.08.2023)

(54) Title: METHODS AND MATERIALS FOR EXPANDING TUMOR INFILTRATING GAMMA-DELTA T CELLS

(57) Abstract: This document provides methods and materials for expanding tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells) in culture. For example, methods and materials for expanding large numbers of tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells that are predominantly $V\delta1^+$) from tissue obtained from a mammal having cancer (e.g., a tumor sample), an autoimmune condition, or an infection are provided. Populations of such tumor infiltrating γδ T cells and methods and materials for using such tumor infiltrating γδ T cells and/or such populations to treat cancer within a mammal (e.g., a human) also are provided.

WO 2023/069322 A9

METHODS AND MATERIALS FOR EXPANDING TUMOR INFILTRATING GAMMA-DELTA T CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 63/257,805, filed October 20, 2021. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

This document relates to methods and materials for expanding tumor infiltrating gamma-delta (γδ) T cells (e.g., tumor infiltrating γδ T cells) in culture. For example, this document provides methods and materials for expanding large numbers of tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells that are predominantly $V\delta1^+$) from tissue obtained from a mammal having cancer (e.g., a tumor sample). This document also provides populations of such tumor infiltrating γδ T cells and methods and materials for using such tumor infiltrating γδ T cells and/or such populations to treat cancer within a mammal (e.g., a human).

2. Background Information

Cancer immunotherapies including adoptive cell therapy (ACT) with tumor infiltrating lymphocytes (TIL) depend on T cell effector functions. These αβ T cell receptor (TCR) expressing cells target cancer cells through recognition of peptide or lipid antigens presented by major histocompatibility complex (MHC) Class I and II and MHC-like CD1 molecules. TIL therapies that include lymphodepletion, adoptive transfer of *ex vivo* expanded autologous TIL, and post infusion administration of high dose interleukin-2 (IL-2) has provided durable complete responses in patients with treatment refractory metastatic melanoma, cervical cancer, and other epithelial cancers. With current TIL therapy protocols providing objective clinical response and in particular, complete responses, in many treated patients, improvements in the understanding of the mechanisms of treatment response can help broaden the application of these treatments (Dafni *et al.*, *Ann. Oncol.*, 30:1902-1913 (2019)).

Clinical manifestation of cancer often occurs following years of cancer immune editing with the emergence of poorly immunogenic tumor cell variants, many of which have lost Class I MHC molecules (Schreiber *et al.*, *Science*, 331:1565-1570 (2011)). Despite efforts to reinvigorate immune responses with ACT, genomic instability of cancer cells promotes Darwinian selection processes associated with mutational downregulation or complete loss of immune reactive tumor associated peptide antigens that provide a means of immune escape (Dudley *et al.*, *J. Clin. Oncol.*, 23:2346-2357 (2005); Khong *et al.*, *Nat. Immunol.*, 3:999-1005 (2002); Zitvogel *et al.*, *Nat. Rev. Immunol.*, 6:715-727 (2006); and Orlando *et al.*, *Nat. Med.*, 24:1504-1506 (2018)).

As noted, immune evasion is also mediated by reduced expression or lack of MHC-Class 1 antigen presentation that is pervasive across several solid tumors and limits the efficacy of αβ T cell immunotherapy (Dhatchinamoorthy *et al.*, *Front. Immunol.*, 12:636568 (2021); Tran *et al.*, *N. Engl. J. Med.*, 375:2255-2262 (2016); and Chowell *et al.*, *Science*, 359:582-587 (2018)). More recently, T cell intrinsic factors, including functional exhaustion associated with lack of effective co-stimulation, inhibitory receptor expression and abrogation of stem cell like memory differentiation dictate persistence and response to immunotherapy (Ahmadzadeh *et al.*, *Blood*, 114:1537-1544 (2009); Baitsch *et al.*, *J. Clin. Invest.*, 121:2350-2360 (2011); Miller *et al.*, *Nat. Immunol.*, 20:326-336 (2019); Sade-Feldman *et al.*, *Cell*, 175:998-1013 e1020 (2018); Jansen *et al.*, *Nature*, 576:465-470 (2019); and Krishna *et al.*, *Science*, 370:1328-1334 (2020)). Therapeutic interventions that can overcome challenges inherent to tumor cell immune escape and suppression paradigms can further improve immunotherapy treatment outcomes.

γδ TCR expressing cells are an evolutionarily conserved lymphocytic subset whose MHC-unrestricted recognition of pathogen derived or host cell non-peptide metabolites and stress antigens provide compelling opportunities to discern their utility in immunosurveillance and cancer immunotherapy (Vantourout *et al.*, *Nat. Rev. Immunol.*, 13:88-100 (2013); Silva-Santos *et al.*, *Nat. Rev. Immunol.*, 15:683-691 (2015); Silva-Santos *et al.*, *Nat. Rev. Cancer*, 19:392-404 (2019); Sebestyen *et al.*, *Nat. Rev. Drug Discov.*, 19:169-184 (2020); and Ribot *et al.*, *Nat. Rev. Immunol.*, 21:221-232 (2021)). γδ T cells, especially $V\delta1^+$ cells, are predominantly tissue resident immune effectors that display diverse roles in mediating TCR- and natural cytotoxicity receptor (NCR)-dependent tumor surveillance. As such, they coordinate and mediate both innate and adaptive immune responses (Vantourout *et al.*, *Nat. Rev. Immunol.*, 13:88-100 (2013); Silva-Santos *et al.*, *Nat.*

Rev. Immunol., 15:683-691 (2015); Silva-Santos *et al.*, *Nat. Rev. Cancer*, 19:392-404 (2019); Sebestyen *et al.*, *Nat. Rev. Drug Discov.*, 19:169-184 (2020); Ribot *et al.*, *Nat. Rev. Immunol.*, 21:221-232 (2021); and Davey *et al.*, *Trends Immunol.*, 39:446-459 (2018)). The presence of these cells is associated with better outcomes in patients with many types of cancer. For example, patients with leukemia recovering an increased number of γδ T cells following bone marrow transplantation experienced greater long-term survival (Godder *et al.*, Bone Marrow Transplant., 39:751-757 (2007)). Furthermore, a meta-analysis of infiltrating immune cell gene expression signatures of 25 solid tumor types from the cancer genome atlas (TCGA) identified γδ T cells to be the most significant cell type associated with favorable prognosis (Gentles *et al.*, *Nat. Med.*, 21:938-945 (2015)). Early and ongoing efforts targeting phosphoantigen reactive, blood resident Vγ9Vδ2 cells have established the clinical feasibility and safety of γδ cancer cell therapy (Sebestyen *et al.*, *Nat. Rev. Drug Discov.*, 19:169-184 (2020)).

SUMMARY

This document provides methods and materials for expanding tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells) in culture. For example, this document provides methods and materials for expanding tumor infiltrating γδ T cells obtained from tissue (e.g., a tumor sample) to obtain large numbers (e.g., greater than $1x10^7$, greater than $1x10^8$, greater than $5x10^8$, or greater than $1x10^9$) of tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells that are predominantly $V\delta1^+$) within, for example, 25 to 30 days.

As described herein, γδ T cells obtained from tumor tissue (and/or healthy tissue that is within 30 mm of a tumor) can be expanded *in vitro* using a combination of cytokines (e.g., IL-2 plus IL-4 plus IL-15 (IL-2/IL-4/IL-15)) to produce populations of tumor infiltrating γδ T cells having desired percentages of cells having desired phenotypes. For example, this document provides methods and materials for expanding tumor infiltrating γδ T cells by culturing a first population containing tumor infiltrating γδ T cells in the presence of IL-2 for 5 to 15 days (e.g., 6 to 15 days, 7 to 15 days, 8 to 15 days, 9 to 15 days, 9 to 13 days, 10 to 12 days, or 7 to 10 days) to produce a second population of cells, and subsequently culturing the second population of cells in the presence of IL-2, IL-4, and IL-15 (and optionally PBMCs such as irradiated allogeneic PBMCs and optionally an anti-CD3 agonistic antibody) for 8 to 21 days (e.g., 10 to 21 days, 12 to 21 days, 14 to 21 days, 8 to 18 days, 8 to 16 days, 8 to 14 days, 10 to 20 days, 10 to 18 days, 12 to 18 days, 10 to 16 days, 12 to 16 days, or 13 to 15

days) to produce an expanded population of tumor infiltrating γδ T cells. In some cases, a population of expanded tumor infiltrating γδ T cells can be obtained by (a) obtaining a tissue sample containing a tumor and/or healthy tissue that was within 30 mm of a tumor, (b) obtaining a first cell population containing tumor infiltrating γδ T cells from that tissue, (c) optionally enriching that first cell population so that the resulting enriched population contains a higher ratio of tumor infiltrating γδ T cells to total $CD3^+$ cells, and (d) culturing the first cell population (or the optional enriched population) in the presence of IL-2, IL-4, IL-15, PBMCs (e.g., irradiated PBMCs), and an anti-CD3 antibody for 8 to 21 days (e.g., 10 to 21 days, 12 to 21 days, 14 to 21 days, 8 to 18 days, 8 to 16 days, 8 to 14 days, 10 to 20 days, 10 to 18 days, 12 to 18 days, 10 to 16 days, 12 to 16 days, or 13 to 15 days) to obtain a population of expanded tumor infiltrating γδ T cells.

In some cases, greater than 85 percent of the $CD3^+$ cells of an expanded population provided herein can be γδ TCR^+ cells, less than 10 percent of the $CD3^+$ cells of that population can be αβ TCR^+ cells, less than 10 percent of the $CD45^+$ cells of that population can be NK cells, greater than 30 percent of the γδ TCR^+ cells of that population can be $V\delta1^+$ cells, less than 60 percent of the γδ TCR^+ cells of that population can be $V\delta1^-V\delta2^-$ cells, less than 25 percent of the γδ TCR^+ cells of that population can be $V\delta2^+$ cells, greater than 70 percent of the γδ TCR^+ cells of that population can be T_{EM} cells, less than 25 percent of the γδ TCR^+ cells of that population can be T_{EMRA} cells, as high as 10 percent of the γδ TCR^+ cells of that population can be $CD69^+$ $CD103^+$ Tissue resident memory (T_{RM}) cells, as high as 50 percent of the γδ TCR^+ cells of that population can be $CD56^+$ cells, from 1 to 40 percent of the γδ TCR^+ cells of that population can be $CD137^+$ cells, less than 25 percent of the γδ TCR^+ cells of that population can be $PD\text{-}1^+$ cells, from 5 to 40 percent of the γδ TCR^+ cells of that population can be $BTLA^+$ cells, greater than 60 percent of the γδ TCR^+ cells of that population can be $NKG2D^+$ cells, and greater than 20 percent of the γδ TCR^+ cells of that population can be $NKp46^+$ cells.

As also described herein, the populations of tumor infiltrating γδ T cells provided herein can be administered to a mammal (e.g., human) having cancer to treat cancer within that mammal. For example, a population of tumor infiltrating γδ T cells provided herein can be administered (e.g., intravenously administered) to a mammal (e.g., a human) having cancer as an adoptive cellular therapy to treat that cancer either alone or in combination with (a) tumor infiltrating αβ T cells and/or (b) one or more therapeutic agents such as one or more checkpoint inhibitors (e.g., anti-PD-1 antibodies and/or anti-PD-L1 antibodies), IL-2, one or

more lymphodepleting chemotherapy agents (e.g., cyclophosphamide and/or fludarabine), one or more tumor infiltrating lymphocyte enhancement agents (e.g., CpG and/or oncolytic viruses such as vaccinia viruses), brachytherapy, or combinations thereof. In such cases, the administered tumor infiltrating γδ T cells can provide effective immune responses against cancer cells within the mammal, thereby reducing the number of cancer cells within the mammal.

In general, one aspect of this document features a method for producing a cell population comprising γδ T cells. The method comprises (or consists essentially of or consists of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing

the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

In another aspect, this document features an isolated cell population comprising (or consisting essentially of or consisting of) polyclonal γδ T cells, wherein the population comprises greater than 1 x 10^8 γδ T cells. Greater than 85 percent of the $CD3^+$ cells the cell

population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be PD-1^+ cells. From 5 to 40 percent of the γδ TCR^+ cells of the cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the cell population can be $NKp46^+$ cells. The cells of the cell population can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The cell population can be a cell population that was produced using a method for producing a cell population comprising γδ T cells as described in any statement or combination of statements from the following paragraph.

The method can comprise (or can consist essentially of or can consist of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2

and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$

$CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be PD-1^+ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

In another aspect, this document features a method for providing a mammal with γδ T cells. The method comprises (or consists essentially of or consists of) administering, to a mammal, a cell population produced as described in any statement or combination of statements from the preceding paragraph. The mammal can be a human. The mammal can be a mammal having cancer. The cells of the first cell population can be allogenic or autologous to the mammal administered the cell population. The method can comprise administering αβ T cells to the mammal.

In another aspect, this document features a method for providing a mammal with γδ T cells. The method comprises (or consists essentially of or consists of) administering a cell population (e.g., an isolated cell population) to a mammal. The mammal can be a human. The mammal can be a mammal having cancer, an autoimmune condition, or an infection. The cells of the cell population can be allogenic or autologous to the mammal. The method can comprise administering αβ T cells to the mammal. The cell population (e.g., isolated cell population) can comprise (or can consist essentially of or can consist of) polyclonal γδ T cells, wherein the population comprises greater than 1×10^8 γδ T cells. Greater than 85 percent of the $CD3^+$ cells the cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the cell population

can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be PD-1^+ cells. From 5 to 40 percent of the γδ TCR^+ cells of the cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the cell population can be $NKp46^+$ cells. The cells of the cell population can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The cell population can be a cell population that was produced using a method for producing a cell population comprising γδ T cells as described in any statement or combination of statements from the following paragraph.

The method can comprise (or can consist essentially of or can consist of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively

selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be PD-1^+ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

In another aspect, this document features a method for treating cancer. The method comprises (consists essentially of or consists of) administering, to a mammal having cancer, a cell population produced as described in any statement or combination of statements from the preceding paragraph. The mammal can be a human. The cells of the first cell population can be allogenic or autologous to the mammal having cancer. The method can comprise administering αβ T cells to the mammal.

In another aspect, this document features a method for treating cancer. The method comprises (consists essentially of or consists of) administering a cell population (e.g., an isolated cell population) to a mammal having cancer. The mammal can be a human. The cells of the cell population can be allogenic or autologous to the mammal having cancer. The method can comprise administering αβ T cells to the mammal. The cell population (e.g., isolated cell population) can comprise (or can consist essentially of or can consist of) polyclonal γδ T cells, wherein the population comprises greater than 1 x 10^8 γδ T cells. Greater than 85 percent of the $CD3^+$ cells the cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be PD-1^+ cells. From 5 to 40 percent of the γδ TCR^+ cells of the cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the cell population can be $NKp46^+$ cells. The cells of the cell population can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The cell population can be a cell population that was produced using a method for producing a cell population comprising γδ T cells as described in any statement or combination of statements from the following paragraph.

The method can comprise (or can consist essentially of or can consist of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at

least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

In another aspect, this document features a method for treating an autoimmune condition. The method comprises (consists essentially of or consists of) administering a cell population (e.g., an isolated cell population) to a mammal having an autoimmune condition. The mammal can be a human. The cells of the cell population can be allogenic or autologous to the mammal having the autoimmune condition. The method can comprise administering αβ T cells to the mammal. The cell population (e.g., isolated cell population) can comprise (or can consist essentially of or can consist of) polyclonal γδ T cells, wherein the population comprises greater than 1 x 10^8 γδ T cells. Greater than 85 percent of the $CD3^+$ cells the cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the cell

population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the cell population can be $NKp46^+$ cells. The cells of the cell population can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The cell population can be a cell population that was produced using a method for producing a cell population comprising γδ T cells as described in any statement or combination of statements from the following paragraph.

The method can comprise (or can consist essentially of or can consist of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the

absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second

cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

In another aspect, this document features a method for treating an infection. The method comprises (consists essentially of or consists of) administering a cell population (e.g., an isolated cell population) to a mammal having an infection. The mammal can be a human. The cells of the cell population can be allogenic or autologous to the mammal having the infection. The method can comprise administering αβ T cells to the mammal. The cell population (e.g., isolated cell population) can comprise (or can consist essentially of or can consist of) polyclonal γδ T cells, wherein the population comprises greater than 1×10^8 γδ T cells. Greater than 85 percent of the $CD3^+$ cells the cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the cell population can be $NKp46^+$ cells. The cells of the cell population can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The cell population can be a cell population that was produced using a method for producing a cell population comprising γδ T cells as described in any statement or combination of statements from the following paragraph.

The method can comprise (or can consist essentially of or can consist of) culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein the second cell population comprises at least 10 times more γδ T cells than the first cell population. The γδ T cells can be human cells. The γδ T cells can be tumor infiltrating γδ T cells. The first cell population can be (i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor, (ii) a population of γδ T cells obtained from healthy tissue, (iii) a population of γδ T cells obtained from infected tissue, or (iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells. The method can comprise obtaining the first cell population from the tissue comprising the tumor. The method can comprise obtaining the first cell population from the healthy tissue that was within 30 mm of the tumor. The first cell population can be a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was cultured in the presence of 100 international units/mL to 4000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to the culturing in the presence of IL-2, IL-4, and IL-15. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells. The first cell population can be a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells. The method can comprise removing at least some αβ T cells from a cell population to obtain the first cell population. The removing can comprise positively selecting αβ T cells and removing the positively selected αβ T cells. The method can comprise isolating at least some γδ T cells from a cell population to obtain the first cell population. The isolating can comprise positively selecting γδ T cells and isolating the positively selected γδ T cells. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for the 8 to 21 days can comprise culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for the 8 to 21 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 12 to 16 days. The culturing the first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 can be for 13 to 15 days. The second cell population can comprise at least 50 times more γδ T cells than the first cell population, at least 100 times more γδ T cells than the first cell population, at

least 200 times more γδ T cells than the first cell population, at least 300 times more γδ T cells than the first cell population, or at least 400 times more γδ T cells than the first cell population. The second cell population can comprise greater than 1 x 10^8 γδ T cells. The IL-2 can be a human IL-2. The IL-4 can be a human IL-4. The IL-15 can be a human IL-15. Greater than 85 percent of the $CD3^+$ cells the second cell population can be γδ TCR^+ cells. Less than 10 percent of the $CD3^+$ cells of the second cell population can be αβ TCR^+ cells. Less than 10 percent of the $CD45^+$ cells of the second cell population can be NK cells. Greater than 30 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^+$ cells. Less than 60 percent of the γδ TCR^+ cells of the second cell population can be $V\delta1^-V\delta2^-$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $V\delta2^+$ cells. Greater than 70 percent of the γδ TCR^+ cells of the second cell population can be T_{EM} cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be T_{EMRA} cells. Less than 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. From 1 to 10 percent of the γδ TCR^+ cells of the second cell population can be $CD69^+$ $CD103^+$ T_{RM} cells. Less than 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 50 percent of the γδ TCR^+ cells of the second cell population can be $CD56^+$ cells. From 1 to 40 percent of the γδ TCR^+ cells of the second cell population can be $CD137^+$ cells. Less than 25 percent of the γδ TCR^+ cells of the second cell population can be $PD\text{-}1^+$ cells. From 5 to 40 percent of the γδ TCR^+ cells of the second cell population can be $BTLA^+$ cells. Greater than 60 percent of the γδ TCR^+ cells of the second cell population can be $NKG2D^+$ cells. Greater than 20 percent of the γδ TCR^+ cells of the second cell population can be $NKp46^+$ cells.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

DESCRIPTION OF THE DRAWINGS

Figures 1A-B are photographs of representative pseudomyxoma peritonei (PMP) TIL histology. PMP histology from representative patient tumors are provided using hematoxylin & eosin staining showing focal lymphocytic infiltration restricted to tumor associated stroma. Mucin pools (white) are devoid of lymphocytes.

Figure 2 is a table providing clinical variables describing PMP patients, whose tumors were used for lymphocyte repertoire sequencing. Representative retrospective tumors were used for histologic analysis and repertoire sequencing of low grade PMP treated with cytoreductive surgery and heated intraperitoneal chemotherapy (CRS-HIPEC). MSS = microsatellite stable; PD-L1 = programmed death ligand-1 positivity (which was 0.9% for the selected positive patient).

Figures 3A-G. Low grade PMP displayed an elevated BCR IgE fraction associated with TCR Vδ. Following dimer avoidance multiplex polymerase chain reaction (DAM-PCR) and next generation sequencing of RNA isolated from resected formalin fixed paraffin embedded (FFPE) low grade PMP (n=10) tumor tissue, T and B cell receptor CDR3 sequences were constructed with the migec v1.2.9 MiXCR pipeline. Figure 3A provides representative tree maps of a patient PMP tumor repertoire, where each rounded rectangle represents a unique CDR3, with the size of the rectangle corresponding to the relative frequency of the CDR3 clones across the entire repertoire. Total cohort mean CDR3 expression (Figure 3B), mean CDR3 amino acid length (Figure 3C), true entropy repertoire diversity (Figure 3D), and BCR immunoglobin fraction (Figure 3E). Figure 3F provides an IgE fraction comparison between the PMP cohort, healthy donor PBMCs (HD PBMC; n=238), and high grade pancreatic cancer tumors (pancreatic ductal adenocarcinoma; PDAC; n=68). Figure 3G provides a correlation of TCR Vδ% with BCR IgE fraction in the PMP cohort.

Figures 4A-E provides data of PMP repertoire sharing. PMP T and B cell repertoires were compared with healthy donor PBMC repertoires (n=238), identifying shared CDR3s (Public) localized to the Vα, Igκ and Igλ chains (Figure 4A). Figure 4B provides the percent breakdown of the n=238 healthy donors with shared uCDR3s within the PMP cohort. Figure 4C shows the identification of shared CDR3s sequences within PMP cohort that are distinct from public CDR3s, localized to BCR chains. Figure 4D shows the generational probability of 10 identified IgH sequences (SEQ ID NOs:1-10 from left to right) shared within PMP cohort, with higher probabilities associated with random recombination vs. lower

probabilities associated with antigen directed convergent evolution. Figure 4E shows the BCR immunoglobin fraction of the identified shared 11 IgH PMP CDR3s, composed primarily of IgG, IgE, and IgA. BCR sequences, specifically those of the heavy chain come in 5 different subtypes – IgA, D, E, G, and M; which have different antigen specificities, structural homology, and function. This figure shows that there are public BCRs that are in the PMP repertoire that are shared with the general population (2-3% of IgK and IgL). There are also CDR3 sequences that are shared within the BCR repertoire restricted to patients with PMP. Quantification of the generational probability of these amino acid sequences suggests certain shared sequences are due to random recombination, while others are due to antigen driven recombination, and thus suggestive of convergent evolution of BCR clones against common antigens to PMP tumors. Figure 4D, does this calculation for the 11 IgH sequences shared within patients in the PMP cohort. Figure 4E details the immunoglobulin fraction (BCR identity) of the shared PMP IgH sequences, showing they are primary IgG and IgE.

Figures 5A-D. γδ TIL sparsely infiltrate peritoneal surface malignancies. Figure 5A depicts an overview of the study outline. Tumor specimens were harvested prospectively from patients (n=26) with peritoneal surface malignancies undergoing cytoreductive surgery heated intraperitoneal chemotherapy (CRS-HIPEC). Tumor infiltrating lymphocytes were liberated from spatially distinct tumor fragments (n=40 per patient) initially with high concentrations of IL-2 in culture (3,000 IU/mL). RPMI (10% Human AB serum) media and IL-2 were replenished every 3 days in a gas permeable culture flask (G-REX®). On day 11, following spectral cytometry phenotyping, γδ TIL were negatively selected with magnetic bead isolation and rapidly expanded ($1x10^6$ cells) with parallel native αβ TIL cultures with a combination of an anti-CD3 antibody (OKT-3 30 ng/mL), IL-2 (3,000 IU/mL), irradiated (30 Gy), allogenic healthy donor PBMCs (1:100; $1x10^8$ cells), and other γ chain cytokines. Spectral cytometry phenotyping of expanded γδ and αβ TIL was completed on day 25. At the time of tumor fragmentation, the remaining spatially representative tumor fragments were utilized for tumor digestion (Miltenyi Biotech GentleMACS system) and cryopreserved until autologous tumor reactivity assessment with co-culture of expanded TIL and single cell suspension of tumor digest. Figure 5B provides a comparison of total TIL harvested at day 11 of pre-expansion (pre-REP) culture by peritoneal tumor histology (high grade colon cancer vs low grade (grade 1) appendix cancer). Figure 5C provides spectral cytometry phenotyping data from day 11 on viable TIL populations ($CD56^+$ $CD3^-$ NK cells; $CD3^+$ γδ TCR^+ cells; $CD3^+$ αβ TCR^+ $CD4^+$ T cells; and $CD3^+$ αβ TCR^+ $CD8^+$ T cells). Figure 5D

provides the percentages of γδ Vδ chain subsets as determined on day 11 of pre-expansion (pre-REP) culture by spectral cytometry ($CD3^+$ γδ TCR^+ $V\delta1^+$, $V\delta2^+$, or $V\delta1^-V\delta2^-$ cells).

Figure 6 is a table providing prospective CRS-HIPEC patient characteristics. The clinical characteristics of patients with peritoneal surface malignancies undergoing CRS-HIPEC whose tumors were prospectively collected for TIL culture are provided.

Figures 7A-E. Peritoneal tumor fragmentation and pre-rapid expansion phenotypic assessment. Figure 7A provides four sequential photographs of mucinous peritoneal tumor dissection and fragmentation into spatially distinct 2-3 mm^3 tumor fragments. On day 11, γδ TCR^+ % and total viable cell counts were compared by histology (Figure 7B) or prior chemotherapy (Figures 7D and 7E). Figure 7C provides the spectral cytometry gating strategy. $CD45^+$ immune cells were selected from live single cells. NK cells ($CD3^-$ $CD56^+$) and T cells ($CD3^+$) were selected. T cells were segmented by TCR αβ or γδ positivity. CD4/CD8 or Vδ1/Vδ2 populations were identified from selected T cell subsets.

Figures 8A-H. γδ TIL display a tissue resident effector memory phenotype with reduced PD-1, but greater NKG2D and CD137 expression compared to αβ TIL. Day 11 peritoneal tumor infiltrating lymphocyte spectral phenotyping comparing αβ and γδ TIL CD8 expression ($CD8\alpha^+$, $CD8\beta^+$, or double positivity; Figure 8A), memory phenotype ($CD62L^+$ $CD45RO^-$ Naïve; $CD62L^+$, $CD45RO^+$ Central Memory; $CD62L^-$ $CD45RO^+$ Effector Memory; $CD62L^-$ $CD45RO^-$ Effector Memory RA^+; Figures 8B and 8C), tissue resident memory phenotype ($CD69^+$, $CD103^+$, or double positive; Figure 8D), activation status (CD2, CD25, CD27, CD56, CD137, or 4-1BB; Figure 8E), exhaustion status (PD-1, LAG-3, TIGIT, BTLA, or PD- L1; Figure 8F), and natural cytotoxicity receptor (NCR) expression (NKG2D or NKp46; Figure 8G). Figure 8H provides a summary mean expression heat map of CD8, Memory, Activation, Exhaustion, and Natural Cytotoxicity Receptors (NCRs) phenotypes.

Figures 9A-E are representative flow diagrams showing αβ and γδ TIL percent positive cells gated by fluorescence minus one (FMO) control and unstimulated PBMC (negative control) on CD8 (Figure 9A), activation status (CD56 and CD137 or 4-1BB; Figure 9B), tissue resident memory phenotype ($CD69^+$, $CD103^+$, or double positive; Figure 9C), exhaustion status (PD-1 and BTLA; Figure 9D), and natural cytotoxicity receptor expression (NKG2D; Figure 9E).

Figures 10A-C. Use of IL-4 and IL-15 for rapid expansion of γδ TIL. $1x10^6$ negatively selected γδ TILs or native αβ TILs were expanded for 14 days in culture with an anti-CD3 antibody (OKT-3, 30 ng/mL), irradiated (30 Gy) allogenic PBMC feeders cells

(1:100), IL-2 (3,000 IU/mL), RPM 1640 (5 % human serum), and the indicated other γ chain cytokines (IL-4 100 ng/mL; IL-7 20 ng/mL; IL-15 70 ng/mL; or combinations thereof) cultured in gas permeable flasks (G-REX®) with cytokines and media replaced every three days. Day 7, 10, and 14 rapid expansion protocol total viable cell counts under individual and varied cytokine combinations were measured (Figure 10A). The negatively selected γδ TIL population expanded for 14 days with IL-2/IL-4/IL-15 contained minimal NK ($CD3^-$ $CD56^+$) and αβ T ($CD3^+$ αβ TCR^+) cells and γδ T cells ($CD3^+$ γδ TCR^+) that were primarily of $V\delta1^+$ or $V\delta1^-\delta2^-$ cells as assessed by spectral cytometry (Figure 10B). Average change in absolute percent positive cells between day 11 phenotyping (i.e., pre-expansion) and day 14 post-expansion (i.e., total day 25) of αβ TIL (expanded using an anti-CD3 antibody (OKT-3, 30 ng/mL), irradiated (30 Gy) allogenic PBMC feeders cells (1:100), IL-2 (3,000 IU/mL), and RPM 1640 (5 % human serum) and γδ TIL (expanded using a combination of IL-2/IL-4/IL-15) are shown in Figure 10C. Statistics indicate significant change from day 11 to day 25 of culture as a result of the IL-2/IL-4/IL-15 expansion.

Figures 11A-D. Fold expansion and phenotyping of rapidly expanded γδ and αβ TIL. Figure 11A shows the fold expansion of negatively selected γδ TIL (e.g., negatively selected by means of αβ TCR depletion) and native αβ TIL following 14 days in culture with the indicated combinations of different cytokines. Figure 11B provides representative flow plots of post-expansion, negatively selected γδ TIL expanded in culture for 14 days in IL-2, IL-4, and IL-15. Figure 11C shows the phenotypes of the cells present in the post-IL-2 only expansion of native αβ TIL population. Figure 11D provides a summary mean expression heat map of memory markers, activation markers, exhaustion markers, and natural cytotoxicity receptors (NCRs) in IL-2 expanded, positively selected αβ TIL and in IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL following 14 days of expansion. Statistics indicate significant difference between γδ and αβ TIL at day 25 of culture as a result of the IL-2/IL-4/IL-15 expansion.

Figures 12A-D. MHC independent, γδ TCR mediated autologous tumor recognition. TIL were thawed and rested overnight in IL-2 (3,000 IU/mL) media prior to washing twice in PBS and co-culture. Autologous tumor reactivity was assessed by co-culturing $1x10^5$ of 14 day rapidly expanded αβ TIL (IL-2 expanded, native αβ TIL) or 14 day rapidly expanded γδ TIL (IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL) with $1x10^5$ tumor digest cells in a 96 well plate for 24 hours in cytokine free media. IFNγ production was assessed in culture supernatants by ELISA. Figure 12A shows IFNγ production of the expanded αβ or γδ TIL

following non-specific CD3/CD28 stimulation (Dynabeads, positive control), following co-culture with autologous PBMCs ($1x10^5$ cells, negative control), or following co-culture with tumor digest. MHC unrestricted TIL reactivity of expanded γδ TIL and αβ TIL was assessed with the K562 leukemia cancer cell line and a series of colon cancer cell lines (HCT116, RKO, SW480, and SW80) passaged twice prior to co-culture (Figure 12B). In a subset of patients, γδ TIL were cultured with autologous tumor digests in the presence of blocking antibodies (isotype control mouse IgG 10 μg/mL, anti-MHC-1 (W6/32 10 μg/mL), anti-γδ TCR (7A5 3 μg/mL), or anti-NKG2D (1D11, 10 μg/mL)) (Figure 12C). Figure 12D shows the correlation of IFNγ production with the percentage of Vδ1 γδ TIL post-expansion.

Figure 13 is a table showing cancer cell line NKG2D ligand expression. Tested cancer cell line natural killer receptor ligand mRNA Z-scores were queried from the Cancer Line Encyclopedia. Cell lines with stable or upregulation of MICA and MICB enable γδ TIL recognition.

Figure 14 is a table describing the clinical characteristics of patients with resected melanoma whose tumor specimens were utilized for tumor digestion and TIL expansion.

Figures 15A-E. High dose IL-2 expands the highest number of γδ TCR^+ TIL during pre-rapid expansion protocol (pre-REP). Cryopreserved melanoma tumor digests (n=15) were thawed and plated ($5x10^6$ cells/well) in G-REX® culture wells along with complete media and high dose IL-2 (3,000 IU/mL, n=15, black), γ-chain cytokine combination (IL-2 3,0000 IU/mL, IL-4 100 ng/mL, IL-15 70 ng/mL, n=15, red), or γ-chain cytokine combination plus anti-CD137 mAb (Urelumab, 10 μg/mL, n=10, black and white). Cultures were replenished with cytokine or antibody on Day 4 and Day 8, with 50% of the media replaced on day 8. On Day 11, expanded TIL were harvested through a 70-μm filter, counted (A, B), and analyzed by spectral cytometry for TIL populations (B-E).

Figures 16A-G. Vδ1 TIL are associated with a pan cancer survival benefit. Figure 16A provides the mean expression (Log transcripts per million (TPM)) of γδ TIL subsets (TRDV1, TRDV2, and TRDV3 genes) and αβ TIL (TRBC2 Beta Chain 2 Constant Region) in the 20 most common primary solid tumor types as analyzed by the GEPIA2 tool of the cancer genome atlas (TCGA) database of bulk RNA sequencing of primary tumors. Figures 16B-G provide Kaplan Meier survival analyses by normalized (ACTB beta actin) TRDV1 expression above (high) or below (low) the median for selected tumor types where autologous TIL therapy are tested (SKCM= Cutaneous Melanoma; HNSC=Head & Neck Squamous Cell Carcinoma; LUAD+LUSC= Lung Adenocarcinoma and Lung Squamous Cell

Carcinoma; BRCA=Breast Carcinoma; and CESC= Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma). Log rank P values are displayed along with 95 CI of survival estimates.

Figures 17A-G. Vδ1 survival benefit in additional primary cancers. Full cohort TRDV1 tumor (T) and normal (N) tissue expression are plotted for tumor types with the highest Vδ1 expression: Lung Adenocarcinoma (LUAD), Kidney Renal Cell Carcinoma (KIRC), Breast Carcinoma (BRCA), and Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC) (Figure 17A). Figures 17B-G provide Kaplan Meier survival analyses by normalized (ACTB beta actin) TRDV1 expression above (high) or below (low) the median for additional selected tumor types. Log rank P values are displayed along with 95 CI of survival estimates. TCGA = Full Cancer Genome Atlas Tumors; GBM = Glioblastoma; HNSC = Head & Neck Squamous Cell Carcinoma; SKCM = Cutaneous Melanoma; ESCA = Esophageal Carcinoma; LUAD = Lung Adenocarcinoma; LUSC = Lung Squamous cell carcinoma; BRCA = Breast Carcinoma; MESO = Mesothelioma; LIHC = Liver Hepatocellular Carcinoma; STAD = Stomach Adenocarcinoma; PAAD = Pancreatic Ductal Adenocarcinoma; KIRC = Kidney Renal Cell Carcinoma; BLCA = Bladder Urothelial Carcinoma; COAD = Colorectal Adenocarcinoma; READ = Rectal Adenocarcinoma; OV = Ovarian serous cystadenocarcinoma; UCEC = Uterine Corpus Endometrial Carcinoma; CESC = Cervical squamous cell carcinoma and endocervical adenocarcinoma; PRAD = Prostate adenocarcinoma; and SARC = Sarcoma.

Figures 18A-S. Correlation of TRDV1 and TRBC2 genes in the 20 most common primary solid tumor types as analyzed by GEPIA2 tool of the cancer genome atlas (TCGA) database of bulk RNA sequencing of primary tumors. Pearson correlation and p value were reported. TCGA abbreviations of Figure 17 were used. All correlations were significant.

Figures 19A-H. No Vδ1 survival benefit in certain primary cancers. Figures 19A-H provide Kaplan Meier survival analyses by normalized (ACTB beta actin) TRDV1 expression above (high) or below (low) the median for selected tumor types. Log rank P values were displayed along with 95 CI of survival estimates.

DETAILED DESCRIPTION

This document provides methods and materials for expanding tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells) in culture. For example, this document provides methods and materials for expanding tumor infiltrating γδ T cells obtained from tissue (e.g., a

tumor sample) to obtain large numbers (e.g., greater than $1x10^7$, greater than $1x10^8$, greater than $5x10^8$, or greater than $1x10^9$) of tumor infiltrating γδ T cells (e.g., tumor infiltrating γδ T cells that are predominantly $V\delta1^+$) than can be permissible for therapeutic use.

As described herein, tissue containing a tumor (or healthy tissue that is within 30 mm of a tumor, or healthy tissue that is within 20 mm of a tumor, or healthy tissue that is within 10 mm of a tumor) can contain tumor infiltrating γδ T cells and can be obtained from a mammal (e.g., a human cancer patient). In some cases, one or more lymph nodes adjacent to a tumor and/or one or more tumor draining lymph nodes can contain tumor infiltrating γδ T cells and can be obtained from a mammal (e.g., a human cancer patient). For example, lung tissue containing a lung tumor (or healthy lung tissue that is within 30 mm (e.g., within 20 mm or within 10 mm) of a lung tumor or a tumor draining lymph node of a lung tumor) can be obtained from a mammal (e.g., a human lung cancer patient) and used as a source of tumor infiltrating γδ T cells. In another example, skin tissue containing a skin tumor (or healthy skin tissue that is within 30 mm (e.g., within 20 mm or within 10 mm) of a skin tumor or a tumor draining lymph node of a skin tumor) can be obtained from a mammal (e.g., a human skin cancer patient) and used as a source of tumor infiltrating γδ T cells. Other examples of tissues that can be obtained and used as described herein include, without limitation, tissue containing a glioblastoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a glioblastoma), tissue containing a head & neck squamous cell carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a head & neck squamous cell carcinoma), tissue containing a cutaneous melanoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a cutaneous melanoma), tissue containing a lung adenocarcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a lung adenocarcinoma), tissue containing a lung squamous cell carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a lung squamous cell carcinoma), tissue containing a breast carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a breast carcinoma), tissue containing a mesothelioma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a mesothelioma), tissue containing a liver hepatocellular carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a liver hepatocellular carcinoma), tissue containing a pancreatic ductal adenocarcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a pancreatic ductal adenocarcinoma), tissue containing a kidney renal cell carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a kidney renal cell

carcinoma), tissue containing a bladder urothelial carcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a bladder urothelial carcinoma), tissue containing a cervical squamous cell carcinoma and endocervical adenocarcinoma (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a cervical squamous cell carcinoma and endocervical adenocarcinoma), tissue containing a lymph node metastases, tissue containing a peritoneum tumor (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a peritoneum tumor), tissue containing a bone tumor (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a bone tumor), tissue containing an endocrine gland tumor (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of an endocrine gland tumor), tissue containing a reproductive organ tumor (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a reproductive organ tumor), or tissue containing a brain (or healthy tissue within 30 mm (e.g., within 20 mm or within 10 mm) of a brain tumor).

Once a tissue is obtained, the tissue can be cultured in a manner that promotes the isolation of tumor infiltrating γδ T cells from the tissue. For example, one or more pieces (e.g., 2-3 mm^3 pieces) of the tissue can be cultured in the presence of IL-2 for 5 to 15 days (e.g., 6 to 15 days, 7 to 15 days, 8 to 15 days, 9 to 15 days, 9 to 13 days, 10 to 12 days, 7 to 10 days, or 8 to 14 days). In some cases, the one or more pieces (e.g., 2-3 mm^3 pieces) of the tissue can be cultured in a gas permeable rapid expansion flask. Any appropriate concentration of IL-2 can be used to promote the isolation of tumor infiltrating γδ T cells from the tissue. For example, from about 50 international units (IU) to about 6000 IU (e.g., from about 100 IU to about 6000 IU, from about 500 IU to about 6000 IU, from about 1000 IU to about 6000 IU, from about 1500 IU to about 6000 IU, from about 2000 IU to about 6000 IU, from about 2500 IU to about 6000 IU, from about 3000 IU to about 6000 IU, from about 3500 IU to about 6000 IU, from about 2500 IU to about 4000 IU, or from about 2500 IU to about 3500 IU) of IL-2 per mL of culture medium can be used.

In some cases, tissue (e.g., tumor tissue) can be mechanically and/or enzymatically digested, and a single cell tumor digest suspension can be cultured for a period of time or γδ T cells can be directly isolated at this time.

After culturing tissue containing tumor infiltrating γδ T cells with IL-2 for 5 to 15 days (e.g., 6 to 15 days, 7 to 15 days, 8 to 15 days, 9 to 15 days, 9 to 13 days, 10 to 12 days, 7 to 10 days, or 8 to 14 days), a cell population that exited the tissue can be harvested. In some cases, the harvested cell population can include tumor infiltrating αβ T cells and tumor infiltrating γδ T cells. In some cases, the harvested cell population can include a greater

number of tumor infiltrating αβ T cells than the number of tumor infiltrating γδ T cells. In some cases, an anti-αβ TCR antibody, an anti-CD28 antibody, an anti-4-1BBL antibody, an anti-GITR antibody, an anti-CD27 antibody, or a combination thereof can be used to promote a cell population that is enriched for γδ T cells from the harvested cell population.

Once the harvested cell population is obtained, an optional enrichment for γδ T cells can be performed. For example, magnetic beads containing an anti-αβ TCR antibody can be used in a negative selection process to remove αβ T cells from the harvested cell population to obtain a cell population enriched for γδ T cells. In some cases, an anti-TCR γδ antibody, an anti-Vδ1 antibody, an anti-NKG2D antibody, or a combination thereof can be used in a positive selection process to isolate γδ T cells from the harvested cell population to obtain a cell population enriched for γδ T cells.

Briefly, when using antibodies to remove non-γδ T cells (e.g., αβ T cells) from or to isolate γδ T cells from the harvested cell population to obtain a cell population enriched for γδ T cells, the antibodies can be biotinylated and can be attached to a magnetic substrate (e.g., a magnetic bead) via streptavidin. In some cases, flow activated cell sorting (FACS) can be used to remove non-γδ T cells (e.g., αβ T cells) from or to isolate γδ T cells from the harvested cell population to obtain a cell population enriched for γδ T cells.

In some cases, the harvested cell population (or a portion thereof) can be used for expanding the number of γδ T cells without the optional enrichment step.

Once the harvested cell population (with or without the optional enrichment for γδ T cells) is obtained, the harvested cell population (or a portion thereof) can be used to perform an expansion step that increases the number of γδ T cells present. In some cases, this expansion step can increase the starting number of γδ T cells present in the starting cell population to a number of γδ T cells present in the resulting cell population that is from 10 to 1000 fold greater (e.g., 10 to 600 fold, 20 to 600 fold, 30 to 600 fold, 40 to 600 fold, 50 to 600 fold, 75 to 600 fold, 100 to 600 fold, 200 to 1000 fold, 250 to 1000 fold, 300 to 1000 fold, 350 to 1000 fold, 400 to 1000 fold, 450 to 1000 fold, 500 to 1000 fold, 200 to 1000 fold, 250 to 1000 fold, 300 to 51000 00 fold, 350 to 1000 fold, 400 to 1000 fold, or 450 to 1000 fold) greater than that starting number. In some cases, this expansion step can increase the starting number of γδ T cells present in the starting cell population to a number of γδ T cells present in the resulting cell population that is more than 200 fold greater (e.g., more than 250 fold greater, more than 300 fold greater, more than 350 fold greater, more than 400 fold greater, or more than 450 fold greater) than that starting number. In some cases, this

expansion step can expand the starting number of γδ T cells present in the starting cell population to a number of γδ T cells present in the resulting cell population that is 200 to 600 fold (e.g., 200 to 600 fold, 250 to 600 fold, 300 to 600 fold, 350 to 600 fold, 400 to 600 fold, 450 to 600 fold, 500 to 600 fold, 200 to 550 fold, 250 to 550 fold, 300 to 550 fold, 350 to 550 fold, 400 to 550 fold, 450 to 550 fold, 500 to 550 fold, 200 to 500 fold, 250 to 500 fold, 300 to 500 fold, 350 to 500 fold, 400 to 500 fold, or 450 to 500 fold) greater than that starting number. In some cases, this expansion step can increase the starting number of γδ T cells present in the starting cell population to a number of γδ T cells present in the resulting cell population that is greater than 25 percent (e.g., greater than 50 percent, greater than 75 percent, or greater than 95 percent) enriched in γδ T cells.

Any appropriate method can be used to promote the expansion of γδ T cells of a harvested cell population (or a portion thereof) or a harvested, γδ T cell-enriched cell population (or a portion thereof). For example, a harvested cell population (with or without the optional enrichment for γδ T cells) or a portion thereof can be cultured in the presence of IL-2, IL-4, and IL-15 to promote the expansion of γδ T cells. The amount of IL-2 can be from about 50 IU to about 6000 IU (e.g., from about 100 IU to about 6000 IU, from about 500 IU to about 6000 IU, from about 1000 IU to about 6000 IU, from about 1500 IU to about 6000 IU, from about 2000 IU to about 6000 IU, from about 2500 IU to about 6000 IU, from about 3000 IU to about 6000 IU, from about 3500 IU to about 6000 IU, from about 2500 IU to about 4000 IU, or from about 2500 IU to about 3500 IU) of IL-2/mL of culture medium. The amount of IL-4 can be from about 10 ng to about 200 ng (e.g., from about 20 ng to about 200 ng, from about 50 ng to about 200 ng, from about 75 ng to about 200 ng, from about 10 ng to about 150 ng, from about 10 ng to about 100 ng, from about 50 ng to about 150 ng, or from about 90 ng to about 110 ng) of IL-4/mL of culture medium. The amount of IL-15 can be from about 10 ng to about 200 ng (e.g., from about 20 ng to about 200 ng, from about 50 ng to about 200 ng, from about 75 ng to about 200 ng, from about 10 ng to about 150 ng, from about 10 ng to about 100 ng, from about 50 ng to about 150 ng, from about 50 ng to about 90 ng,or from about 60 ng to about 90 ng) of IL-15/mL of culture medium.

In some cases, a harvested cell population (with or without the optional enrichment for γδ T cells) or a portion thereof can be cultured in the presence of IL-2, IL-4, and IL-15 with the optional inclusion of IL-7 and/or IL-21. When optionally including IL-7, amount of IL-7 can be from about 10 ng to about 200 ng (e.g., from about 20 ng to about 200 ng, from about 50 ng to about 200 ng, from about 75 ng to about 200 ng, from about 10 ng to about

150 ng, from about 10 ng to about 100 ng, from about 50 ng to about 150 ng, or from about 90 ng to about 110 ng) of IL-7/mL of culture medium. When optionally including IL-21, amount of IL-21 can be from about 10 ng to about 200 ng (e.g., from about 20 ng to about 200 ng, from about 50 ng to about 200 ng, from about 75 ng to about 200 ng, from about 10 ng to about 150 ng, from about 10 ng to about 100 ng, from about 50 ng to about 150 ng, or from about 90 ng to about 110 ng) of IL-21/mL of culture medium.

Any appropriate IL-2, IL-4, and IL-15 (and optionally included IL-7 and/or IL-21) can be used to expand γδ T cells as described herein. For example, when expanding human γδ T cells, then human IL-2, human IL-4, and human IL-15 can be used to expand the human γδ T cells. In another example, when expanding horse γδ T cells, then horse IL-2, horse IL-4, and horse IL-15 can be used to expand the horse γδ T cells. In another example, when expanding monkey γδ T cells, then monkey IL-2, monkey IL-4, and monkey IL-15 can be used to expand the monkey γδ T cells. In another example, when expanding dog γδ T cells, then dog IL-2, dog IL-4, and dog IL-15 can be used to expand the dog γδ T cells.

The harvested cell population (with or without the optional enrichment for γδ T cells) or a portion thereof can be cultured in the presence of IL-2, IL-4, and IL-15 for any appropriate length of time to promote the expansion of γδ T cells. For example, a harvested cell population (with or without the optional enrichment for γδ T cells) or a portion thereof can be culture in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days (e.g., 10 to 21 days, 12 to 21 days, 14 to 21 days, 8 to 18 days, 8 to 16 days, 8 to 14 days, 10 to 20 days, 10 to 18 days, 12 to 18 days, 10 to 16 days, 12 to 16 days, or 13 to 15 days). In some cases, the IL-2, IL-4, and IL-15 in the culture can be replenished every 3 days, every 4-6 days, or every 2-3 days.

In some cases, the culture containing IL-2, IL-4, and IL-15 and being used to expand the number of γδ T cells can contain one or more additional agents. For example, in addition to IL-2, IL-4, and IL-15, the culture can contain anti-CD3 antibodies (e.g., soluble and/or immobilized anti-CD3 antibodies), anti-CD28 antibodies (e.g., soluble and/or immobilized anti-CD28 antibodies), irradiated PBMCs (e.g., irradiated PBMCs that are autologous to the mammal to be treated with the expanded γδ T cells), agonistic anti-γδ TCR antibodies (e.g., soluble and/or immobilized anti-γδ TCR antibodies such as Vδ1 antibodies; about 1 μg/mL; see, e.g., Zhou *et al.*, *Cell Mol. Immunol.*, 9(1):34-44 (2012)), anti-4-1BB antibodies (e.g., soluble and/or immobilized anti-4-1BB antibodies such as Urelumab; about 10 μg/mL; see,

e.g., Sakellariou-Thompson *et al.*, *Clin. Cancer Res.*, 23(23):7263-7275 (2017)), anti-TIGIT antibodies (e.g., soluble and/or immobilized anti-TIGIT antibodies; 1 μg/mL; see, e.g., Chauvin *et al.*, *J. Clin. Invest.*, 125(5):2046-58 (2015)), high glucose (e.g., from 5 mM to 25 mM, from 8 mM to 20 mM, from 8 mM to 12 mM, or from 9 mM to 11 mM of glucose; see, e.g., Lopes *et al.*, *Nat. Immunol.*, 22:179-192 (2021)), irradiated artificial antigen presenting cells (e.g., cloned K562 cells transfected with 4-1BBL, CD86, IL-15/membrane bound IL-15; 1:100 ratio; see, e.g., Deniger *et al.*, *Clin. Cancer Res.*, 20(22):5708-5719 (2014)), PHA (about 1 μg/mL), irradiated EBV transfected B cell lines (1:100 ratio; see, e.g., Ma *et al.*, *J. Exp. Med.*, 208(3):491-503 (2011)), anti-OX40 antibodies (e.g., soluble and/or immobilized anti-OX40 antibodies), phosphoinositide 3-kinase (PI 3-kinase) inhibitors (e.g., Idelalisib, Copanlisib, Duvelisib, Alpelisib, or Umbralisib), CDK4/6 inhibitors (e.g., palbociclib, ribociclib, or abemaciclib; see, e.g., Lelliott *et al.*, *Cancer Discov.*, 11(10):2582-2601 (2021)), CBL-B inhibitors (e.g., NX-0255 or NX-1607; see, e.g., Rountree *et al.*, *Cancer Res.*, July 1 2021 (81)(13 Supplement):1595), STS1 inhibitors (see, e.g., Hwang *et al.*, *Exp. Mole. Med.*, 52:750-761 (2020)), CISH (see, e.g., Palmer *et al.*, *J. Exp. Med.*, 212(12):2095-2113 (2015)), TET2 (see, e.g., Fraietta *et al.*, *Nature*, 558(7709):307-312 (2018)), or combinations thereof. For example, in addition to IL-2, IL-4, and IL-15, the culture can contain anti-CD3 antibodies (e.g., soluble anti-CD3 antibodies) and irradiated PBMCs. The amount of anti-CD3 antibodies can be from about 0.1 μg to about 1 μg of anti-CD3 antibodies per mL of culture medium. The amount of anti-CD28 antibodies can be from about 500 ng to about 5 μg of anti-CD28 antibodies per mL of culture medium. The amount of irradiated PBMCs can be based on the number of input γδ T cells such that the ratio of γδ T cells:PBMCs is from about 1:25 to about 1:200 (e.g., 1:100).

After expanding the number of γδ T cells in the presence of IL-2, IL-4, and IL-15, the cells can be washed to remove any particular components of the culture medium. For example, after the expansion step is completed, the resulting cell population can be washed to remove any remaining IL-2, IL-4, IL-15, anti-CD3 antibodies, and/or anti-CD28 antibodies, and/or the expanded γδ T cells can be concentrated. In some cases, after the expansion step, the expanded γδ T cells can be cultured in the absence of IL-2, IL-4, and/or IL-15 for any appropriate length of time. For example, after the rapid expansion step, the population of expanded γδ T cells can be cultured in the absence of IL-2, IL-4, and/or IL-15 for 10 to 75 days (e.g., 10 to 60 days, 10 to 50 days, or 10 to 25 days). In some cases, expanded γδ T cells

can be obtained from multiple donors (e.g., multiple humans) and pooled to provide a population of γδ T cells for treating one or more patients (e.g., one or more humans).

As described herein, a cell population containing expanded γδ T cells that results from a γδ T cell expansion in the presence of IL-2, IL-4, and IL-15 as described herein can have a particularly desired make up of cells. For example, in some cases, greater than 85 percent (e.g., greater than 90 percent, greater than 91 percent, greater than 92 percent, greater than 93 percent, greater than 94 percent, greater than 95 percent, greater than 96 percent, greater than 97 percent, greater than 98 percent, or greater than 99 percent) of the $CD3^+$ cells of a population provided herein can be γδ TCR^+ cells. In some cases, less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD3^+$ cells of a population provided herein can be αβ TCR^+ cells. In some cases, less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD45^+$ cells of a population provided herein can be NK cells.

In some cases, a cell population containing expanded γδ T cells that results from a γδ T cell expansion in the presence of IL-2, IL-4, and IL-15 as described herein can vary and can include not only αβ T cells, but also phenotypic NKT, NK and B cells in various proportions.

In some cases, greater than 85 percent (e.g., greater than 90 percent, greater than 91 percent, greater than 92 percent, greater than 93 percent, greater than 94 percent, greater than 95 percent, greater than 96 percent, greater than 97 percent, greater than 98 percent, or greater than 99 percent) of the $CD3^+$ cells of a population provided herein can be γδ TCR^+ cells and less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD3^+$ cells of that population can be αβ TCR^+ cells.

In some cases, greater than 85 percent (e.g., greater than 90 percent, greater than 91 percent, greater than 92 percent, greater than 93 percent, greater than 94 percent, greater than 95 percent, greater than 96 percent, greater than 97 percent, greater than 98 percent, or greater than 99 percent) of the $CD3^+$ cells of a population provided herein can be γδ TCR^+ cells and less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7

percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD45^+$ cells of that population can be NK cells.

In some cases, greater than 85 percent (e.g., greater than 90 percent, greater than 91 percent, greater than 92 percent, greater than 93 percent, greater than 94 percent, greater than 95 percent, greater than 96 percent, greater than 97 percent, greater than 98 percent, or greater than 99 percent) of the $CD3^+$ cells of a population provided herein can be $\gamma\delta$ TCR^+ cells, less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD3^+$ cells of that population can be $\alpha\beta$ TCR^+ cells, and less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD45^+$ cells of that population can be NK cells.

In some cases, greater than 30 percent (e.g., greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the $\gamma\delta$ TCR^+ cells of a population provided herein can be $V\delta1^+$ cells.

In some cases, less than 60 percent (e.g., less than 55 percent, less than 50 percent, less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the $\gamma\delta$ TCR^+ cells of a population provided herein can be $VD\delta1^-$ $V\delta2^-$ cells.

In some cases, less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $\gamma\delta$ TCR^+ cells of a population provided herein can be $V\delta2^+$ cells.

In some cases, greater than 30 percent (e.g., greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the $\gamma\delta$ TCR^+ cells of a population provided herein can be $V\delta1^+$ cells and less than 60 percent (e.g., less than 55 percent, less than 50 percent, less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent,

less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the γδ TCR^+ cells of that population can be $VD\delta1^-V\delta2^-$ cells.

In some cases, greater than 30 percent (e.g., greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be $V\delta1^+$ cells and less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be $V\delta2^+$ cells.

In some cases, greater than 30 percent (e.g., greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be $V\delta1^+$ cells, less than 60 percent (e.g., less than 55 percent, less than 50 percent, less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the γδ TCR^+ cells of that population can be $VD\delta1^-V\delta2^-$ cells, and less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be $V\delta2^+$ cells.

In some cases, greater than 70 percent (e.g., greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be T_{EM} cells.

In some cases, less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of a population provided herein can be T_{EMRA} cells.

In some cases, greater than 70 percent (e.g., greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be T_{EM} cells and less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4

percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be T_{EMRA} cells.

In some cases, a population provided herein can have a higher percentage (e.g., a percentage that is 2 to 40 percentage points higher, 5 to 40 percentage points higher, 10 to 40 percentage points higher, 15 to 40 percentage points higher, 20 to 40 percentage points higher, 5 to 35 percentage points higher, 5 to 30 percentage points higher, 5 to 25 percentage points higher, 5 to 20 percentage points higher, 5 to 15 percentage points higher, or 5 to 10 percentage points higher) of γδ TCR^+ T_{EM} cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, a population provided herein can have a lower percentage (e.g., a percentage that is 2 to 30 percentage points lower, 5 to 30 percentage points lower, 10 to 30 percentage points lower, 15 to 30 percentage points lower, 20 to 30 percentage points lower, 5 to 25 percentage points lower, 5 to 20 percentage points lower, 5 to 15 percentage points lower, or 5 to 10 percentage points lower) of γδ TCR^+ T_{EMRA} cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, a population provided herein can have a higher percentage (e.g., a percentage that is 2 to 40 percentage points higher, 5 to 40 percentage points higher, 10 to 40 percentage points higher, 15 to 40 percentage points higher, 20 to 40 percentage points higher, 5 to 35 percentage points higher, 5 to 30 percentage points higher, 5 to 25 percentage points higher, 5 to 20 percentage points higher, 5 to 15 percentage points higher, or 5 to 10 percentage points higher) of γδ TCR^+ T_{EM} cells and a lower percentage (e.g., a percentage that is 2 to 30 percentage points lower, 5 to 30 percentage points lower, 10 to 30 percentage points lower, 15 to 30 percentage points lower, 20 to 30 percentage points lower, 5 to 25 percentage points lower, 5 to 20 percentage points lower, 5 to 15 percentage points lower, or 5 to 10 percentage points lower) of γδ TCR^+ T_{EMRA} cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of a population provided herein can be $CD69^+$ $CD103^+$ T_{RM}. In some cases, from 1 to 10 percent (e.g., from 1 to 9

percent, from 1 to 8 percent, from 1 to 7 percent, from 1 to 6 percent, from 1 to 5 percent, from 1 to 4 percent, from 2 to 10 percent, from 3 to 10 percent, from 4 to 10 percent, from 5 to 10 percent, from 6 to 10 percent, from 2 to 8 percent, from 2 to 6 percent, from 4 to 8 percent, or from 4 to 6 percent) of the γδ TCR^+ cells of a population provided herein can be $CD69^+$ $CD103^+$ T_{RM} cells.

In some cases, less than 50 percent (e.g., less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the γδ TCR^+ cells of a population provided herein can be $CD56^+$ cells. In some cases, from 1 to 50 percent (e.g., from 1 to 45 percent, from 1 to 40 percent, from 1 to 35 percent, from 1 to 30 percent, from 1 to 25 percent, from 1 to 20 percent, from 5 to 50 percent, from 10 to 50 percent, from 15 to 50 percent, from 20 to 50 percent, from 10 to 40 percent, from 15 to 35 percent, or from 20 to 30 percent) of the γδ TCR^+ cells of a population provided herein can be $CD56^+$ cells.

In some cases, from 1 to 40 percent (e.g., from 1 to 35 percent, from 1 to 30 percent, from 1 to 25 percent, from 1 to 20 percent, from 1 to 15 percent, from 1 to 10 percent, from 5 to 40 percent, from 10 to 40 percent, from 15 to 40 percent, from 20 to 40 percent, from 5 to 35 percent, from 10 to 30 percent, or from 15 to 25 percent) of the γδ TCR^+ cells of a population provided herein can be $CD137^+$ cells.

In some cases, a population provided herein can have a higher percentage (e.g., a percentage that is 2 to 50 percentage points higher, 5 to 50 percentage points higher, 2 to 40 percentage points higher, 5 to 40 percentage points higher, 10 to 40 percentage points higher, 15 to 40 percentage points higher, 20 to 40 percentage points higher, 5 to 35 percentage points higher, 5 to 30 percentage points higher, 5 to 25 percentage points higher, 5 to 20 percentage points higher, 5 to 15 percentage points higher, or 5 to 10 percentage points higher) of $CD137^+$ γδ TCR^+ cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of a population provided herein can be $PD\text{-}1^+$ cells.

In some cases, a population provided herein can have a lower percentage (e.g., a percentage that is 5 to 90 percentage points lower, 5 to 80 percentage points lower, 5 to 75

percentage points lower, 5 to 70 percentage points lower, 5 to 75 percentage points lower, 5 to 70 percentage points lower, 5 to 65 percentage points lower, 5 to 60 percentage points lower, 5 to 55 percentage points lower, 5 to 50 percentage points lower, 5 to 45 percentage points lower, 5 to 40 percentage points lower, 5 to 35 percentage points lower, 5 to 30 percentage points lower, 5 to 25 percentage points lower, 5 to 20 percentage points lower, 5 to 15 percentage points lower, 5 to 10 percentage points lower, 10 to 90 percentage points lower, 10 to 80 percentage points lower, 10 to 75 percentage points lower, 10 to 70 percentage points lower, 10 to 75 percentage points lower, 10 to 70 percentage points lower, 10 to 65 percentage points lower, 10 to 60 percentage points lower, 10 to 55 percentage points lower, 10 to 50 percentage points lower, 10 to 45 percentage points lower, 10 to 40 percentage points lower, 10 to 35 percentage points lower, 10 to 30 percentage points lower, 10 to 25 percentage points lower, 10 to 20 percentage points lower, 10 to 15 percentage points lower, 25 to 90 percentage points lower, 25 to 80 percentage points lower, 25 to 75 percentage points lower, 25 to 70 percentage points lower, 25 to 75 percentage points lower, 25 to 70 percentage points lower, 25 to 65 percentage points lower, 25 to 60 percentage points lower, 25 to 55 percentage points lower, 25 to 50 percentage points lower, 25 to 45 percentage points lower, 25 to 40 percentage points lower, 25 to 35 percentage points lower, or 25 to 30 percentage points lower) of PD-1$^+$ γδ TCR$^+$ cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, from 5 to 40 percent (e.g., from 5 to 35 percent, from 5 to 30 percent, from 5 to 25 percent, from 5 to 20 percent, from 5 to 15 percent, from 10 to 40 percent, from 10 to 35 percent, from 10 to 30 percent, from 10 to 25 percent, from 10 to 20 percent, or from 15 to 25 percent) of the γδ TCR$^+$ cells of a population provided herein can be BTLA$^+$ cells.

In some cases, from 5 to 40 percent (e.g., from 5 to 35 percent, from 5 to 30 percent, from 5 to 25 percent, from 5 to 20 percent, from 5 to 15 percent, from 10 to 40 percent, from 10 to 35 percent, from 10 to 30 percent, from 10 to 25 percent, from 10 to 20 percent, or from 15 to 25 percent) of the αβ TCR$^+$ cells of a population provided herein can be BTLA$^+$ cells.

In some cases, greater than 60 percent (e.g., greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR$^+$ cells of a population provided herein can be NKG2D$^+$ cells.

In some cases, greater than 20 percent (e.g., greater than 25 percent, greater than 30 percent, greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be $NKp46^+$ cells.

In some cases, a population provided herein can have a higher percentage (e.g., a percentage that is 5 to 90 percentage points higher, 5 to 85 percentage points higher, 5 to 80 percentage points higher, 5 to 75 percentage points higher, 5 to 70 percentage points higher, 5 to 65 percentage points higher, 5 to 60 percentage points higher, 5 to 55 percentage points higher, 5 to 50 percentage points higher, 5 to 45 percentage points higher, 5 to 40 percentage points higher, 5 to 35 percentage points higher, 5 to 30 percentage points higher, 5 to 25 percentage points higher, 10 to 90 percentage points higher, 10 to 85 percentage points higher, 10 to 80 percentage points higher, 10 to 75 percentage points higher, 10 to 70 percentage points higher, 10 to 65 percentage points higher, 10 to 60 percentage points higher, 10 to 55 percentage points higher, 10 to 50 percentage points higher, 10 to 45 percentage points higher, 10 to 40 percentage points higher, 10 to 35 percentage points higher, 10 to 30 percentage points higher, 10 to 25 percentage points higher, 15 to 90 percentage points higher, 15 to 85 percentage points higher, 15 to 80 percentage points higher, 15 to 75 percentage points higher, 15 to 70 percentage points higher, 15 to 65 percentage points higher, 15 to 60 percentage points higher, 15 to 55 percentage points higher, 15 to 50 percentage points higher, 15 to 45 percentage points higher, 15 to 40 percentage points higher, 15 to 45 percentage points higher, 15 to 30 percentage points higher, 15 to 25 percentage points higher, or 20 to 40 percentage points higher) of $NKp46^+$ cells following cell expansion in the presence of IL-2, IL-4, and IL-15 than the starting population had before cell expansion in the presence of IL-2, IL-4, and IL-15.

In some cases, (a) greater than 85 percent (e.g., greater than 90 percent, greater than 91 percent, greater than 92 percent, greater than 93 percent, greater than 94 percent, greater than 95 percent, greater than 96 percent, greater than 97 percent, greater than 98 percent, or greater than 99 percent) of the $CD3^+$ cells of a population provided herein can be γδ TCR^+ cells, (b) less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD3^+$ cells of that population can be αβ TCR^+

cells, (c) less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the $CD45^+$ cells of that population can be NK cells, (d) greater than 30 percent (e.g., greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be $V\delta1^+$ cells, (e) less than 60 percent (e.g., less than 55 percent, less than 50 percent, less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the γδ TCR^+ cells of that population can be $VD\delta1^-V\delta2^-$ cells, (f) less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be $V\delta2^+$ cells, (g) greater than 70 percent (e.g., greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of a population provided herein can be T_{EM} cells, (h) less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be T_{EMRA} cells, (i) less than 10 percent (e.g., less than 9 percent, less than 8 percent, less than 7 percent, less than 6 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be $CD69^+$ $CD103^+$ T_{RM} or from 1 to 10 percent (e.g., from 1 to 9 percent, from 1 to 8 percent, from 1 to 7 percent, from 1 to 6 percent, from 1 to 5 percent, from 1 to 4 percent, from 2 to 10 percent, from 3 to 10 percent, from 4 to 10 percent, from 5 to 10 percent, from 6 to 10 percent, from 2 to 8 percent, from 2 to 6 percent, from 4 to 8 percent, or from 4 to 6 percent) of the γδ TCR^+ cells of that population can be $CD69^+$ $CD103^+$ T_{RM} cells, (j) less than 50 percent (e.g., less than 45 percent, less than 40 percent, less than 35 percent, less than 30 percent, less than 25 percent, less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, or less than 2 percent) of the γδ TCR^+ cells of that population can be $CD56^+$ cells or from 1 to 50 percent (e.g., from 1 to 45 percent, from 1 to 40 percent, from 1 to 35 percent, from 1 to 30 percent, from 1 to 25 percent, from 1 to 20 percent, from 5 to 50 percent, from 10 to 50 percent, from 15 to 50 percent, from 20 to 50 percent, from 10 to

40 percent, from 15 to 35 percent, or from 20 to 30 percent) of the γδ TCR^+ cells of that population can be $CD56^+$ cells, (k) from 1 to 40 percent (e.g., from 1 to 35 percent, from 1 to 30 percent, from 1 to 25 percent, from 1 to 20 percent, from 1 to 15 percent, from 1 to 10 percent, from 5 to 40 percent, from 10 to 40 percent, from 15 to 40 percent, from 20 to 40 percent, from 5 to 35 percent, from 10 to 30 percent, or from 15 to 25 percent) of the γδ TCR^+ cells of that population can be $CD137^+$ cells, (l) less than 25 percent (e.g., less than 20 percent, less than 15 percent, less than 10 percent, less than 5 percent, less than 4 percent, less than 3 percent, less than 2 percent, or less than 1 percent) of the γδ TCR^+ cells of that population can be PD-1^+ cells, (m) from 5 to 40 percent (e.g., from 5 to 35 percent, from 5 to 30 percent, from 5 to 25 percent, from 5 to 20 percent, from 5 to 15 percent, from 10 to 40 percent, from 10 to 35 percent, from 10 to 30 percent, from 10 to 25 percent, from 10 to 20 percent, or from 15 to 25 percent) of the γδ TCR^+ cells of that population can be $BTLA^+$ cells, (n) greater than 60 percent (e.g., greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of that population can be $NKG2D^+$ cells, and (o) greater than 20 percent (e.g., greater than 25 percent, greater than 30 percent, greater than 35 percent, greater than 40 percent, greater than 45 percent, greater than 50 percent, greater than 55 percent, greater than 60 percent, greater than 65 percent, greater than 70 percent, greater than 75 percent, greater than 80 percent, greater than 85 percent, greater than 90 percent, or greater than 95 percent) of the γδ TCR^+ cells of that population can be $NKp46^+$ cells.

In addition to providing the cell populations described herein and the methods for producing those cell populations as described herein, this document provides methods for using the cell populations described herein to treat any appropriate disease, disorder, or condition. For example, the cell populations described herein can be used to treat autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus, and scleroderma, infections such as HIV infections, malaria, tuberculosis, hepatitis B, and SARS-CoV-2 infections, and/or cancer. For example, a cell population described herein can be administered to a mammal for use in, for example, adoptive cellular therapies to treat cancer. Any appropriate mammal can be treated with a cell population described herein. For example, humans, horses, cattle, pigs, dogs, cats, mice, and rats can be treated with a population of expanded tumor infiltrating γδ T cells described herein. Any appropriate number of cells can be within the cell population described herein that is administered to a

mammal (e.g., a human) to treat cancer. For example, a cell population described herein can have from about $1x10^7$ to about $1x10^{12}$ cells (e.g., from $5x10^7$ to $1x10^{11}$ cells, from $1x10^8$ to $1x10^{11}$ cells, from $5x10^8$ to $1x10^{11}$ cells, from $1x10^9$ to $1x10^{11}$ cells, or from $1x10^{10}$ to $1x10^{12}$ cells) and can be administered to a mammal (e.g., a human) to treat cancer. In some cases, a cell population described herein can be administered to a mammal (e.g., a human) to treat cancer such that from about $1x10^7$ to about $1x10^{12}$ (e.g., from $5x10^7$ to $1x10^{11}$, from $1x10^8$ to $1x10^{11}$, from $5x10^8$ to $1x10^{11}$, from $1x10^9$ to $1x10^{11}$, or from $1x10^{10}$ to $1x10^{12}$) of γδ T cells are delivered to the mammal.

Any appropriate cancer can be treated using a cell population described herein. For example, glioblastomas, head & neck squamous cell carcinomas, cutaneous melanomas, lung adenocarcinomas, lung squamous cell carcinomas, breast carcinomas, mesotheliomas, liver hepatocellular carcinomas, pancreatic ductal adenocarcinomas, kidney renal cell carcinomas, bladder urothelial carcinomas, cervical squamous cell carcinomas and endocervical adenocarcinomas, esophageal carcinomas, stomach adenocarcinomas, colorectal adenocarcinomas, rectal adenocarcinomas, ovarian serous cystadenocarcinomas, uterine corpus endometrial carcinomas, prostate adenocarcinomas, and sarcomas can be treated using a cell population described herein.

Any appropriate route of administration can be used to administer a cell population described herein to a mammal. For example, a cell population described herein can be administered intravenously, intraperitoneally, or intratumorally.

When treating a mammal having a condition (e.g., an autoimmune condition), a disease, or an infection (e.g., an HIV infection, malaria, tuberculosis, hepatitis B, and SARS-CoV-2 infection) other than cancer, any appropriate tissue source can be used to obtained γδ T cells. For example, when expanding γδ T cells to treat an autoimmune condition, tissue involved in the autoimmune condition containing γδ T cells or uninvolved tissue containing γδ T cells (e.g., involved or uninvolved skin, liver, kidney, esophagus, small bowel, and/or colon tissue) can be used as a tissue source to obtain γδ T cells as described herein. When expanding γδ T cells to treat an infection, tissue involved in the infection containing γδ T cells or uninvolved tissue containing γδ T cells (e.g., involved or uninvolved skin, liver, kidney, esophagus, small bowel, and/or colon tissue) can be used as a tissue source to obtain γδ T cells as described herein.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Expanded Tumor Infiltrating γδ T cells Demonstrate Potential Utility for Cancer Adoptive Cell Therapy

Clinical Cohorts and Sample Collection

A retrospective series of n=10 low grade (AJCC 8th edition Grade 1) PMP tumors specimens were identified from the University of Pittsburgh Medical Center Digestive Diseases Tissue Repository for immune repertoire sequencing following pathologic review for evidence of lymphocytic infiltrate (Figures 1A, 1B, and 2). All selected FFPE tumor specimens were obtained from patients with pathology confirmed grade 1 PMP who underwent CRS-HIPEC with no prior therapies. 5 FFPE tissue scrolls cut at a depth of 6 μm were placed in RNase/DNase free Eppendorf tubes, stored at 4°C until further processing.

Prospective TIL expansion was completed on n=26 consenting patients with peritoneal surface malignancies (PMP or colon cancer) undergoing standard of care CRS-HIPEC at the University of Pittsburgh Medical Center as part of a non-interventional tumor registry and tissue procurement clinical protocol (Figure 6). Peritoneal tumor tissue was stored at 4°C until further processing. Cryopreserved patient buffy coats were retrieved from the UPMC Digestive Diseases Tissue Repository at the time of TIL autologous tumor reactivity testing.

The pre-rapid expansion protocol (pre-REP) modulation of γδ TIL was assessed on n=15 tumor digests from patients undergoing resection for melanoma as part of a non-interventional tumor banking clinical protocol (Figure 14).

PMP Immune Repertoire Sequencing and Analysis

Total RNA was extracted from FFPE tissue scrolls using the Covaris M220 focused ultasonicator and truXTRAC FFPE total NA magnetic bead Ultra Kit according to the manufacturer's protocol. The iR-RepSeq-plus 7-Chain DAM-PCR amplification sequence kit (iRepertoire Inc) was used to generate next generation sequencing libraries covering the human TCR-Vα, -Vβ, -Vγ, and -Vδ, and BCR IgH, Igκ, and Igλ chains. 1000 ng of extracted RNA was amplified in a single assay incorporating unique molecular identifiers (UMIs) during the reverse transcription (RT) step by the Biomek-i5 workstation (Beckman Coulter). Amplified libraries were multiplexed and pooled for sequencing on the Illumina NovaSeq platform with a 500-cycle kit. Each sample was allotted 5 million total sequencing reads.

Raw data was demultiplexed and UMI guided assembly was performed using migec v1.2.9, and the resulting consensus fastqs were aligned and assembled into clonotypes using mixcr v3.0.14. The output T cell receptor sequence covers FR2 to FR4, as well as the beginning of the constant region.

Raw data were analyzed using the iRmap program (iRepertoire Inc.). Total reads were normalized to generate UMIs, and unique CDR3s (uCDR3s), mean CDR3 length, and sample Shannon true entropy scores were compared across all seven chains. The IgH chain immunoglobulin fraction was assembled with the TRUST algorithm and correlated with TCR and BCR repertoire metrics. Corollary immune repertoire analysis was completed on n=68 pancreatic tumor specimens from patients receiving neoadjuvant chemotherapy and curative resection and n=238 healthy donor PBMCs (iRepertoire). The publicity of PMP TCRs and BCRs was determined by the percent sharing with the n=238 health donor PBMCs. The generational probability of shared PMP specific BCR IgH clonotypes was calculated with the OLGA algorithm.

TIL Expansion

Mucinous peritoneal tumors were dissected to remove necrotic or fatty tissue and n=40 spatially distinct 2-3 mm^3 tumor fragments (Figure 7A) were placed in a gas permeable G-REX™ 100 Flask with complete media (RPMI 1640 (Cytiva HyClone™) supplemented with 10% human AB serum (Gemini Bio), 1% GlutaMAX (Gibco), 5% Penstrep, 1.25 μg/mL Amphotericin B (Gibco), 0.05 μMolar Mercaptoethanol (βME)), and 3,000 IU/mL IL-2 (aldesleukin, Clinigen Therapeutics) as described elsewhere (Jin *et al.*, *J. Immunother.*, 35:283-292 (2012)). For experiments expanding TIL from tumor digest, cryopreserved single cell suspensions were thawed, washed twice in PBS, filtered through a 70 μM cell strainer, and counted with a Cellometer K2 Fluorescent Cell Viability Counter (Nexcelom Bioscience). $5x10^6$ cells were plated in a G-REX™ 6 Well Flask with complete media and respective γ-chain cytokines (IL-4, 100 ng/mL; IL-15, 70 ng/mL; Miltenyi Biotec) or CD137 antibody (Urelumab, 10 μg/mL, Creative Biolabs). G-REX flasks were incubated in a humidified incubator at 37°C in 5% CO_2, and 5 days after culture initiation, half the media was removed and replaced with fresh media and IL-2. After day 5, half the media and IL-2 was replaced every 2 days. On day 11 of culture, TIL were filtered through a 70 μM cell strainer, and counted. $2x10^6$ harvested TIL were saved for spectral cytometry phenotyping and αβ rapid expansion protocol (REP) and the remaining TIL (maximum of $200x10^6$) were

used for negative γδ selection with the αβ TCR^+ magnetic bead isolation kit (Miltenyi Biotec) according to the manufacturer's protocol. A minimum of $0.3x10^6$ αβ TCR^+ depleted TIL were assessed by spectral cytometry for γδ TIL purity. Paired $1x10^6$ γδ and αβ TIL were expanded under the REP protocol with complete media supplemented with 5% human AB serum and 50% CTS AIMV (Gibco) media, mitogenic OKT-3 (30 ng/mL, Miltenyi Biotec), 1:100 allogenic irradiated feeder cells (2 pooled CMV negative healthy donors, San Diego Blood Bank), IL-2 (3,000 IU/mL) and γ chain cytokines (IL-4, 100 ng/mL; IL-7, 20 ng/mL; IL-15, 70 ng/mL; all Miltenyi Biotec) where indicated. 7 days following the initiation of REP, cultures were counted, resuspended and split 50% into new G-REX flasks and supplemented with fresh CTS AIMV media and cytokines. Cells were counted again on the 10^{th} day of the REP, and half the media was removed and replaced with fresh CTS AIMV and cytokines. On the 14^{th} day of the REP (25^{th} day of culture), TIL were pooled and counted. $1x10^6$ γδ and αβ TIL were saved for spectral cytometry phenotyping, and the remaining expanded TIL were cryopreserved in 10% DMSO in Fetal Bovine Serum with a CoolCell® (Corning) freezing system in -80°C and transferred to a liquid nitrogen freezer within 24 hours for long term storage.

Tumor Digestion

Following randomization and selection of n=40 spatially distinct 2-3 mm^3 tumor fragments, the remaining tumor fragments (if available) were enzymatically and mechanically digested into a single cell suspension with the human tumor dissociation kit (Miltenyi Biotec) and OctoMACS with Heaters Disassociater (Miltenyi Biotec) according to the manufacturer's protocol. Digested single cell suspensions were filtered with a 70 μm strainer, treated with 10 mL ACK lysis buffer (Gibco) for 5 minutes, washed twice with PBS, counted, and cryopreserved as described above.

Whole Blood PBMC Isolation

Whole blood was collected in BD Vacutainer® EDTA tubes, diluted 1:1 with PBS and centrifuged atop 15 mL Lymphoprep™ density gradient media (Stemcell Technologies) in a SepMate™ 50 Tube at 1200 G, 20 minutes. Plasma and mononuclear cells were removed, washed with PBS, treated with 10 mL ACK lysis buffer (Gibco) for 5 minutes, washed twice with PBS, counted, and cryopreserved as described above.

Spectral Cytometry

Day 11 bulk TIL cultures, αβ TCR^+ depleted cells, and post-REP Day 25 TIL cultures were utilized for spectral cytometry to assess the phenotypic expression of T cell memory, activation, exhaustion, and NCRs. Cells were strained with a 30 μm filter, washed with cytometry buffer (2% FBS in 4°C PBS), incubated 5 minutes with Human TruStain FcX™ block (Biolegend), washed, and stained with a master mix of fluorescent conjugated antibodies and Brilliant Stain Buffer (BD) for 25 minutes at 4°C protected from light. Samples were washed and resuspended in 200 μL of cytometry buffer and analyzed on the 5 laser Cytek® Aurora Spectral Cytometer. Single color spectral signatures were measured with UltraComp eBeads™ (Invitrogen) and spectrally resolved along with TIL autofluorescence spectral signature using the SpectroFlo® software. Following gating of single cell, live, $CD45^+$ immune cell, $CD56^+$ NK cells, $CD3^+$ cells, αβ TCR^+ $CD4^+$ and $CD8^+$ T cells, and γδ TCR^+ $V\delta1^+$, $V\delta2^+$, and $V\delta1^-V\delta2^-$ T cells (Figure 7C), expression of phenotypic markers was assessed based on fluoresce minus one (FMO) controls on FlowJo v10.7 software. Expression of phenotypic markers within αβ and γδ TCR^+ TIL was assessed on day 11 and day 25 of culture, and the change in the expression of the markers between the two time points was assessed within αβ and γδ TCR^+ TIL populations.

TIL-Tumor Reactivity

To assess the autologous tumor reactivity of expanded αβ (IL-2 only) and γδ (IL-2, IL-4, and IL-15) TIL, cryopreserved TIL were thawed and rested overnight in IL-2 (3,000 IU/mL) media, washed twice with PBS, counted and plated ($1x10^5$ cells) in a 96 well round bottom plate in IL-2 free complete media alone, with CD3-CD28 stimulation (Dynabeads, 2.5 μL/well, Invitrogen), $1x10^5$ autologous PBMC, or $1x10^5$ autologous tumor digest single cell suspension with culture volume normalized to 200 μL for 24 hours in a humidified incubator at 37°C in 5% CO_2 as described elsewhere (Dudley *et al.*, *J. Immunother.*, 26:332-342 (2003)). 50 μL of supernatant was harvested from duplicate co-cultures, diluted 1:2, and assessed for production of IFNγ with the Human IFNγ ELISA Kit (Invitrogen) according to the manufacturer's instructions. TIL-autologous tumor digest reactivity was compared with co-culture with autologous PBMC and between paired γδ and αβ TIL. In certain cases, γδ TIL or autologous tumor digest were also incubated with blocking antibodies (TIL: isotype control mouse IgG (Invitrogen, 10 μg/mL), anti-γδ TCR (Novus Biologicals, clone 7A5, 3 μg/mL), or anti-NKG2D (BD, clone 1D11, 10 μg/mL) or tumor digest: isotype control mouse

IgG (10 μg/mL) or anti-MHC-1 (Invitrogen, W6/32 10 μg/mL) for 2 hours prior to co-culture.

To assess the MHC unrestricted recognition of TIL, γδ and αβ TIL were similarly co-cultured with $1x10^5$ cancer cell lines (K562, HCT116, RKO, SW480, or SW48; all from ATCC, authenticated and mycoplasma negative (eMyco™ plus PCR kit)) following a minimum of two passages of culture in complete media in a humidified incubator at 37°C in 5% CO_2.

Cancer Cell Encyclopedia (CCLE) Analysis

To evaluate the expression of NKG2D ligands in the tested cancer cell lines, mRNA Z-scores of MICA, MICA, ULBP1, ULBP2, and ULBP3 were queried for the K562, HCT116, RKO, SW480, and SW48 cells from the Cancer Cell Line Encyclopedia using the cBioPortal for cancer genomics.

TCGA Analysis

The tumor specific Vδ1 infiltration and prognostic ability was assessed in the 20 most common primary solid tumors (NCI) of bulk RNA sequencing data in The Cancer Genome Atlas (TCGA) with the Gene Expression Profiling Interactive Analysis Server 2 (GEPIA2). Mean expression (log transcripts per million) of γδ TIL subsets (TRDV1, TRDV2, and TRDV3) and αβ TIL (TRBC2 Beta Chain 2 Constant Region) were calculated. Kaplan Meier survival analysis by normalized (ACTB beta actin) TRDV1 expression above (high) or below (low) the median for selected tumor types was completed with calculation of Log rank p value and 95% confidence interval of survival estimates. TRDV1 expression was directly correlated with TRBC2 expression across selected tumors, and corresponding Pearson correlation coefficient and P values were calculated.

Statistical Analysis

Data were expressed as mean ± standard deviation. Graphical visualization and statistical analysis were performed using Microsoft Excel and GraphPad Prism 9. Descriptive statistics, Two-tailed non-parametric test, Mann-Whitney U tests (unpaired), and Wilcoxon signed-rank (paired, for all comparisons of αβ and γδ TIL) tests were used. Correlations were calculated with the Pearson correlation coefficient and plotted with nonlinear regression and 95% confidence bands. P values < 0.05 were considered

statistically significant, and significance levels were set to * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Results

Low Grade Pseudomyxoma Peritonei (PMP) Display Elevated B Cell Receptor (BCR) IgE Fraction Associated with TCR Vδ

Following pathologic analysis of previously resected peritoneal tumor specimens (Figures 1A and 1B), a representative cohort (n=10) of treatment naïve patients with low grade (G1) PMP who were treated with standard of care cytoreductive surgery and heated intraperitoneal chemotherapy (CRS-HIPEC, Figure 2) were identified. All patients displayed microsatellite stable tumors, limited programmed death ligand-1 (PD-L1) positivity (n=1), with 7 patients requiring at least one follow-up CRS-HIPEC for tumor recurrence. H&E staining identified infiltrating lymphocyte populations restricted to the tumor associated stroma that were notably absent from mucin pools (Figures 1A and 1B).

With limited prior understanding of the adaptive immune response to this understudied tumor type, full TCR and BCR sequencing of the resected FFPE tumor specimen from the first operative resection (Figures 3A-3G) was completed. Following dimer avoidance multiplex PCR (DAM- PCR) of the bulk tumor RNA, cDNA library preparation, and NGS, complete unique CDR3 (uCDR3) sequences were constructed with the migec v1.2.9 MixCR pipeline for adaptive immune repertoire analysis (Han *et al.*, *Cancer Treat. Res.*, 180:111-147 (2020); Han *et al.*, *Methods Mol.* Biol., 2055:369-397 (2020); and Bolotin *et al.*, *Nat. Methods*, 12:380-381 (2015)). Representative tree maps of a single patient's TCR Vα, Vβ, Vδ, and BCR IgH, Igκ, Igλ repertoire are shown in Figure 3A. Notably, the seven-chain repertoire was predominantly made up of BCR transcripts with only 2.06% of total average reads accounting for TCR clones that were primarily derived from αβ T cells (Figure 3B). With the Vδ chain only being 0.09% of reads on average, Vγ reads were predictably not verifiably detected. As previously reported, the CDR3 length of the Vδ chain (18.8 ± 2.0 amino acids) was greater and more variable than the Vα (13.5 ± 0.2, $p<0.001$) and Vβ (14.1 ± 0.3, $p=0.005$) chains (Figure 3C) (Rock *et al.*, *J. Exp. Med.*, 179:323-328 (1994)). The Vδ chain was similar in length to IgH (17.6 ± 0.5), which was greater than the Igκ (11.1 ± 0.03, $p<0.0001$) and Igλ (12.5 ± 0.09, $p<0.0001$) chains. Calculation of the true Shannon entropy of the repertoires exhibited similar diversity across chains, although the Vδ was

markedly decreased compared to Vαβ and BCR repertoires (Figure 3D) (Bortone *et al.*, *Cancer Immunol. Res.*, 9:103-112 (2021)).

Comparison with a cohort of healthy donor PBMC repertoires (n=238) revealed that this low grade PMP cohort displayed a highly private repertoire with only Vα (0.06% of chain), Igκ (3.7%), and Igλ (1.9%), demonstrating shared public CDR3s (Figures 4A-B). Analysis of shared CDR3s within the patient group identified shared putative convergent disease specific BCR, but not TCR CDR3s (Figure 4C). Given that shared disease associated CDR3s can arise from both random recombination and convergent evolution of antigen driven recombination, the generational probability of these shared BCR CDR3s were calculated, revealing a spectrum of antigen driven IgH CDR3s, that were primarily IgG or IgE (Figures 4D-E) (Murugan *et al.*, *Proc. Natl. Acad. Sci. USA*, 109:16161-16166 (2012); and Sethna *et al.*, *Bioinformatics*, 35:2974-2981 (2019)).

Given the unexpected abundance of BCR transcripts, the immunoglobin fraction of the total low grade PMP IgH repertoire was further analyzed, which revealed an expected distribution dominated by IgG (53.4 ± 12.0%) and IgA (21.7 ± 9.9%) (Figure 3E). However, an unusually elevated IgE fraction (12.2 ± 2.1%) that was substantially greater than that observed in healthy donor PBMC (n=238, 0.9 ± 0.6%, $p<0.0001$) and high-grade pancreatic cancer tumor (n=68, 5.6 ± 3.2%, $p<0.0001$) repertoires was observed (Figure 3F). When correlating the BCR IgE fraction with other repertoire features, a strong positive correlation with Vδ expression ($r = +0.81$, $p=0.013$), but not with Vα or Vβ chains, was observed (Figure 3G). The IgE expression levels and association with γδ TIL was intriguing as intraepithelial γδ T cells were previously shown to be required for tumor protective IgE class switching in response to epithelial DNA damage (Crawford *et al.*, *Nat. Immunol.*, 19:859-870 (2018)).

γδ TIL Sparsely Infiltrate Peritoneal Surface Malignancies

With the understanding that peritoneal γδ TIL display a diverse polyclonal and private repertoire, the following was performed to prospectively assess γδ TIL. Tumor specimens were collected from consenting patients with peritoneal surface malignancies undergoing CRS-HIPEC (n=26) (Figure 5A). 30.7% of patients were female with an average of age of 59.3 ± 12.2 and BMI of 27.5 ± 8.4 (Figure 6). Patients in this prospective cohort had peritoneal tumors of low grade appendiceal (n=14, 54%) and high grade colorectal (n=12, 46%) cancer. Three patients (11.5%) previously underwent CRS-HIPEC with 14 patients (54%) previously receiving systemic chemotherapy.

Mucinous peritoneal tumors were dissected into spatially distinct 2-3 mm^3 fragments (Figure 7A) and cultured in gas permeable rapid expansion flasks (G-REX™). Peritoneal TIL were liberated with high dose IL-2 (3,000 IU/mL) and harvested following 11 days of culture. While there was no difference in the number of total viable TIL between treatment naïve patients and those receiving preoperative chemotherapy (Figure 7B), low grade appendix cancers ($4.9x10^7 \pm 6.1x10^7$ cells) had greater total viable TIL compared to high grade colon cancers ($3.9x10^7 \pm 10.3x10^7$ cells, p=0.028, Figure 5B).

Multispectral flow cytometry was utilized to define the composition and phenotype of the bulk proliferating peritoneal TIL populations. IL-2 reactive $CD56^+CD3^-$ Natural Killer (NK) cells and $CD3^+$ T cells were the major constituents of the $CD45^+$ TIL population (Figure 5C). NK cells represented on average 20.2% of all $CD45^+$ cells, with certain individual patients having less than 4% and others having greater than 40% NK cells (Figure 5C). γδ TCR^+ cells (3.4 ± 4.4%) represented a small fraction of all $CD3^+$ T cells, which were primarily $CD4^+$ (57.3 ± 21.7) or $CD8^+$ (36.1 ± 19.3) αβ TCR^+ cells. $CD3^+$ γδ TCR^+ cells were primarily $V\delta1^+$ (49.0±31.5%) or $V\delta1^-V\delta2^-$ (27.7±25.1%), with $V\delta2^+$ (21.0 ± 29.5%) cells being less prevalent on average, despite accounting for greater than 60% of γδ TCR^+ cells in five patients (Figure 5D). Despite considerable heterogeneity of γδ TIL populations, no substantive differences in γδ TCR^+ cell phenotypes were observed between patients with appendiceal or colon tumors or with prior treatment (Figures 7D-E).

γδ TIL Display a Tissue Resident Effector Memory Phenotype with reduced PD-1, but Greater NKG2D and CD137 Expression Compared to αβ TIL

To better understand the phenotype of the γδ and αβ TIL populations, markers of T cell memory, differentiation, and activation, inhibitory receptors associated with T cell exhaustion, and expression of natural cytotoxicity receptors (NCRs) were assessed (Figures 8A-H and 9A-E). Compared to $CD8^-$ γδ T cells, intraepithelial $CD8\alpha\beta^+$ γδ T cells typically display a heightened T helper type 1 (Th1) phenotype associated with gut homeostasis and mucosal healing (Mikulak *et al.*, *JCI Insight*, 4(24):e125884 (2019); and Kadivar *et al.*, *J. Immunol.*, 197:4584-4592 (2016)). In this subset of expanded peritoneal TIL, $CD8\alpha^+$ (14.6 ± 36.1%), $CD8\beta^+$ (11.0 ± 17.4%), or $CD8\alpha\beta^+$ (6.69 ± 15.3%) γδ TIL represented a small fraction of cells, much lower than corresponding αβ TIL in individual cultures (Figure 8A).

The majority of γδ TIL displayed an effector memory phenotype (T_{EM}: $CD45RO^+$ $CD62L^-$, 75.5 ± 15.8%) that was comparable to that observed in αβ TIL (71.2 ± 20.8%,

Figures 8B-C). At 11 days of culture following initial resection, αβ TIL had a greater proportion of central memory cells (T_{CM}: $CD45RO^+$ $CD62L^+$, 22.4 ± 21.7% vs 9.1 ± 12.0%, $p<0.0001$) compared to γδ TIL. γδ TIL also had a relative greater proportion of terminally differentiated effector memory RA cells (T_{EMRA}: $CD45RO^-$, $CD62L^-$, 14.9 ± 12.9% vs 4.4 ± 4.6%, $p<0.0001$). Tissue resident memory T cells (T_{RM}) expressing the tissue retention markers CD69 and CD103 display long term protective immunity and are associated with improved outcomes following immunotherapy (Okla *et al.*, *J. Exp. Med.*, 218(4):e20201605 (2021)). γδ TIL displayed higher amounts of $CD69^+$ (69.9 ± 30.5% vs 56.6 ± 31.8%, $p=0.003$, Figure 8D), $CD103^+$ (25.8 ± 24.1% vs 16.6 ± 19.4%, $p=0.016$), and double positive T_{RM} cells (20.8 ± 16.2 vs 12.3 ± 13.0, $p=0.020$) compared to αβ TIL.

Given that the composition of *ex vivo* expanded TIL populations is highly dependent on spatial heterogeneity and culture conditions promoting the proliferation of tumor dominant and minority populations associated with differential tumor reactivity, expression of activation and exhaustion molecules (Poschke *et al.*, *Clin. Cancer Res.*, 26:4289-4301 (2020)) were compared. Expanded γδ (92.2%) and αβ (97.4%) TIL displayed high levels of CD2 (Figure 8E), a costimulatory molecule whose signaling enables immunologic synapse formation, the so-called CD2 corolla, and buffers PD-1 mediated exhaustion (McKinney *et al.*, *Nature*, 523:612-616 (2015); and Demetriou *et al.*, *Nat. Immunol.*, 21:1232-1243 (2020)). The IL-2 receptor α chain (CD25) was moderately expressed on γδ (28%) and αβ (32.4%) TIL. The costimulatory tumor necrosis receptor family member CD27 has been implicated as a thymic regulator of interferon γ (IFNγ) expression over IL-17 producing γδ T cells (Ribot *et al.*, *Nat. Immunol.*, 10:427- 436 (2009); and Ribot *et al.*, *Cell. Mol. Life Sci.*, 68:2345-2355 (2011)). Increased $CD27^+$ T cells have also been associated with objective clinical response in a prior trial of predominantly αβ TIL therapy (Rosenberg *et al.*, *Clin. Cancer Res.*, 17:4550- 4557 (2011)). γδ TIL showed a range of expression of CD27 that on average (40.2%) was similar to that of αβ (39.5%) TIL. Besides identifying NK cells, the neural cell adhesion molecule, CD56, is a marker of enhanced T cells Th1 cytokine production and cytolytic capability and was expressed to a substantially greater degree in γδ (19.2 ± 14.1%) than αβ TIL (4.5 ± 5.4%, $p<0.0001$) (Kelly-Rogers *et al.*, *Hum. Immunol.*, 67:863-873 (2006); Cohavy *et al.*, *J. Immunol.*, 178:5524-5532 (2007); and Almehmadi *et al.*, *Immunology*, 142:258-268 (2014)). Upregulation of CD137 (4-1BB) has been identified as a marker of tumor reactive T cells with enhanced clonal expansion and proliferation (Cooper *et al.*, *Eur. J. Immunol.*, 32:521-529 (2002); and Ye *et al.*, *Clin. Cancer Res.*, 20:44-55 (2014)).

CD137 expression under these conditions was low across all cells, but notably higher on γδ (8.0 ± 10.5%) when compared to αβ (1.8 ± 2.3%, p=0.0002) TIL.

Inhibitory immune receptor expression are simultaneous markers of tumor reactivity, immune exhaustion, and potential for suppression (Ahmadzadeh *et al.*, *Blood*, 114:1537-1544 (2009); Baitsch *et al.*, *J. Clin. Invest.*, 121:2350-2360 (2011); Miller *et al.*, *Nat. Immunol.*, 20:326-336 (2019); and Gros *et al.*, *J. Clin. Invest.*, 124:2246-2259 (2014)). With the exception of PD-L1, γδ TIL displayed more variable expression of PD-1, LAG-3, TIGIT, and BTLA compared to αβ TIL (Figure 8F). PD-1 was lower on γδ (39.4 ± 27.4%) compared to αβ (57.7 ± 16.9%, p=0.004) TIL. Expression of LAG3 (12.2% and 14.8%) and TIGIT (25.2% and 31.5%) were generally expressed at lower levels than PD-1 for both αβ and γδ TIL subsets. BTLA, a dual regulator of T cell co-stimulation and suppression of TCR signaling, is a marker of enhanced T cell survival and TIL therapy response that exhibited somewhat higher expression on γδ (39.5 ± 25.3%) compared to αβ (26.6 ± 18.0%, p=0.032) TIL (Radvanyi *et al.*, *Clin. Cancer Res.*, 18:6758-6770 (2012); Haymaker *et al.*, *Oncoimmunology*, 4:e1014246 (2015); and Ritthipichai *et al.*, *Clin. Cancer Res.*, 23:6151-6164 (2017)). In addition to being expressed on tumor cells, suppressive myeloid populations, and T regulatory cells, PD-L1 expression on effector T cells promotes self-tolerance and accelerated tumorigenesis in murine models (Daley *et al.*, *Cell*, 166:1485-1499 e1415 (2016); and Diskin *et al.*, *Nat. Immunol.*, 21:442-454 (2020)). PD-L1 expression was low for both expanded γδ (3.4%) and αβ (1.8%) TIL.

The innate-like NK cell properties of γδ T cells, including expression of the NCRs NKG2D and NKp46 confer additional reactivity to stress antigens and antitumor potential (Silva-Santos *et al.*, *Nat. Rev. Cancer*, 19:392-404 (2019); Wu *et al.*, *Sci. Transl. Med.*, 11(513):aax9364 (2019); Mikulak *et al.*, *JCI Insight*, 4(24):e125884 (2019); and Foord *et al.*, *Sci. Transl. Med.*, 13(577):abb0192 (2021)). While expression of NKG2D was uniformly high on γδ TIL (72.8 ± 7.9%) and higher than αβ TIL (38.0 ± 19.8%, p=0.007), NKp46 expression was more heterogenous (17.4 ± 22.4%) and did not differ from αβ TIL (23.6 ± 30.1%) (Figure 8G). A summary heatmap of the mean expression of all evaluated phenotypic markers on γδ and αβ TIL are included in Figure 8H.

Expansion of γδ TIL

To consider the adoptive transfer of γδ TIL displaying a favorable tissue resident effector memory phenotype with limited exhaustion and enhanced expression of CD137 and

NKG2D, an expansion protocol was designed to generate a clinically feasible number of γδ TIL. γδ TIL were negatively selected with depletion of αβ TCR$^+$ cells. Then, 1x10^6 γδ TIL (or bulk αβ TIL for comparison) were expanded for 14 days with mitogenic CD3 stimulation (OKT-3, 30 ng/mL), high concentrations of IL-2 (3,000 IU/mL), and irradiated allogenic healthy donor PBMCs (Figure 10A). This IL-2-dependent expansion protocol was insufficient to expand γδ TIL (5.5 fold expansion, Figure 11A) and may explain the limited number of γδ TIL observed in prior TIL therapies (Donia *et al.*, *Oncoimmunology*, 1:1297-1304 (2012)).

Different combinations of cytokines were evaluated (in combination with anti-CD3 and irradiated PBMCs) to determine if a population of γδ TIL having a desired phenotype can be obtained in appropriate numbers and percentages. While addition of IL-15 (25.6 fold expansion) or IL-7 (164.3 fold expansion) increased expansion of selected γδ TIL, a combination of IL-2, IL-4, and IL-15 (453.8 ± 100.8 fold expansion) demonstrated considerably enhanced γδ TIL expansion (p=0.0008) that was largely comparable to that observed for the IL-2 only expansion of native αβ TIL (725.5 ± 153 fold expansion) (Figure 11A).

Spectral cytometric phenotyping of the negatively selected γδ TIL that were IL-2/IL-4/IL-15 expanded (Figure 11B) displayed a high purity of γδ TCR$^+$ cells (95.3 ± 3.1% of CD3$^+$ cells, Figure 10B) with minimal NK cells (2.3 ± 2.5% of CD45$^+$ cells) or αβ TCR$^+$ cells (3.87 ± 3.3% of CD3$^+$ cells). The negatively selected γδ TIL that were IL-2/IL-4/IL-15 expanded were predominantly Vδ1$^+$ (63.2 ± 28.3% of γδ TCR$^+$) or VDδ1$^-$Vδ2$^-$ (29.8 ± 24.2%) cells with a minor proportion of Vδ2$^+$ cells (8.5 ± 10.4%) (Figure 10B). In comparison, the native αβ TIL that were IL-2 expanded were primarily αβ TCR$^+$ (90.8% ± 6.5% of CD3$^+$ cells; which were CD8$^+$ (57.3 ± 23.1%) or CD4$^+$ cells (39.0 ± 22.8%)), with minimal NK (1.27 ± 2.1%) or γδ TCR$^+$ (2.5 ± 3.5%) cells (Figure 11C).

The IL-2/IL-4/IL-15 expansion of the negatively selected γδ TIL resulted in increased proliferation of T_{EM} γδ TIL (87.1 ± 7.2% vs 75.5 ± 15.8%, p=0.034), with reduced T_{EMRA} (7.0 ± 6.3% vs 14.9 ± 12.9%, p=0.031) compared to the negatively selected γδ TIL preparation before IL-2/IL-4/IL-15 expansion (Figure 10C). An increased number of infused T_{EM} and reduced number of T_{EMRA} populations are associated with clinical response to TIL therapy (Goff *et al.*, *J. Clin. Oncol.*, 34:2389-2397 (2016)).

Following IL-2/IL-4/IL-15 expansion of negatively selected γδ TIL (5.3 ± 2.7% vs 20.8 ± 16.2%, p<0.0001) and IL-2 only expansion of native αβ TIL (1.7 ± 1.5% vs 12.3 ±

13.0%, p=0.004), the number of $CD69^+$ $CD103^+$ T_{RM} cells were reduced compared to the pre-expansion TIL, but higher in the γδ TIL population (p=0.004, Figure 11D). Expression of CD2, CD25, and CD27 were generally stable following both IL-2/IL-4/IL-15 expansion of negatively selected γδ TIL and IL-2 only expansion of native αβ TIL. CD56 expression was increased in the IL-2 only expanded, native αβ TIL (30.3 ± 23.3% vs 4.5 ± 5.3%, p=0.0007), but not increased in the IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL (21.6 ± 25.8% vs 19.2 ± 14.1%) following the expansion as both populations exhibited similar levels of expression. CD137 exhibited increased expression in the IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL following expansion (18.2 ± 13.7% vs 8.0 ± 10.5%, p=0.006) and that level remained higher than the level observed in the IL-2 only expanded, native αβ TIL (6.18 ± 8.9%, p=0.036). PD-1 expression was reduced in both the IL-2 only expanded, native αβ TIL (36.2 ± 22.5% vs 57.7 ± 16.9%, p=0.030) and the IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL (9.7 ± 7.3% vs 39.4 ± 27.4%, p=0.0006) as compared to pre-expansion, but the level remained lower in the post-expansion γδ TIL compared to the post-expansion αβ TIL (p=0.002). While expression of LAG3 and TIGIT was stable for both populations following expansion, BTLA expression slightly increased in the IL-2 only expanded, native αβ TIL (38.7% ± 14.1% vs 26.6 ± 18.0%, p=0.129) and slightly decreased in the IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL (20.8 ± 9.8% vs 39.5 ± 25.3%, p=0.154) and was higher in the post-expansion αβ TIL compared to the post-expansion γδ TIL (p=0.030). While αβ TIL did not exhibit altered NCR expression of NKG2D and NKp46 following the IL-2 only expansion, the IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL maintained high expression of NKG2D (77.9 ± 14.2%) and had an increased number of $NKp46^+$ expressing cells (56.1 ± 32.8% vs 17.4 ± 22.4%, p=0.011; post-expansion compared to pre-expansion) that was greater than that observed in the IL-2 only expanded, native αβ TIL (15.7 ± 22.3%, p=0.029).

MHC Independent, γδ TCR mediated Autologous Tumor Recognition

Completed and ongoing trials of TIL therapy in patients with metastatic epithelial cancer have identified *in vitro* TIL reactivity to autologous patient tumor as a key determinant of objective clinical response (Tran *et al.*, *Science*, 344:641-645 (2014); Stevanovic *et al.*, *J. Clin. Oncol.* 33:1543-1550 (2015); Stevanovic *et al.*, *Clin. Cancer Res.*, 25:1486-1493 (2019); Chandran *et al.*, *Lancet Oncol.*, 18:792-802 (2017); and Zacharakis *et al.*, *Nat. Med.*, 24:724-730 (2018)). To measure the tumor reactivity of the expanded peritoneal TIL, in

patients with available specimens (n=11), IFNγ production was assessed following 24-hour co-culture of a 1:1 ratio of autologous tumor digest cryopreserved at the time of resection and either IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL or IL-2 only expanded, native αβ TIL (Figure 12A). Following non-specific stimulation with beads coated with anti-CD3/anti-CD28 mAbs, both αβ (1556 ± 849 pg/mL) and γδ (1638 ± 1023 pg/mL) TIL produced similar levels of IFNγ. Both αβ (135.8 ± 103.5 vs 27.4 ± 18.4 pg/mL, p=0.002) and γδ TIL (380.7 ± 207.6 vs 25.2 ± 12.1 pg/mL, p=0.001) produced significantly greater amounts of IFNγ during co-culture with autologous tumor digest compared TIL co-cultured with autologous PBMC. The γδ TIL displayed greater autologous tumor reactivity when compared with paired αβ TIL (p=0.009). Notably, 6 of 11 (55%) αβ and 10 of 11 (91%) γδ TIL populations produced greater than 100 pg/mL of IFNγ following co-culture with tumor digest, a hypothesized threshold for screening TIL reactivity associated with clinical tumor regression (Chandran *et al.*, *Lancet Oncol.*, 18:792-802 (2017)).

Given that γδ TIL possess MHC unrestricted TCRs, their reactivity against a series of HLA unmatched cancer cell lines also was evaluated (Figure 12B). Compared to αβ TIL incapable of recognizing such unmatched cell lines, γδ TIL produced significantly higher amounts of IFNγ when cultured with the K562 leukemia cell line and a series of colon cancer cell lines (HCT116, RKO, and SW480). The reactivity of γδ TIL against the SW48 colon cancer cell line was markedly lower than against other cancer cell lines and no different than that observed from the αβ TIL/SW48 co-culture. The γδ TIL's lack of reactivity towards the SW48 line was hypothesized to be caused by reduced production or expression of γδ TCR or NKG2D antigens. Analysis of the mRNA expression of known ligands of the NKG2D receptor in the evaluated cell lines within the Cancer Cell Encyclopedia (CCLE) identified stable or increased expression of MICA and MICB in K562, HCT116, RKO, and SW480, but reduced expression of MICA (-0.35 Z score) and MICB (-1.01 Z score) in the SW48 line (Figure 13)(Barretina *et al.*, *Nature*, 483:603-607 (2012)).

Given the established role of γδ T cell NCR mediated recognition of target cells and uniformly high expression of NKG2D within this cohort of expanded peritoneal γδ TIL, the following was performed to identify its role, along with the γδ TCR, in mediating autologous tumor reactivity (Silva-Santos *et al.*, *Nat. Rev. Immunol.*, 15:683-691 (2015); and Silva-Santos *et al.*, *Nat. Rev. Cancer*, 19:392-404 (2019)). Following co-culture of IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL with autologous tumor digests (n=7), combinations of anti-MHC-1 (W6/32), anti-NKG2D (1D11), anti-γδ TCR (7A5), or isotype control (mouse

IgG) mAb were utilized to block the corresponding receptor binding and signaling (Figure 12C). While addition of anti-MHC-1 mAb showed no difference in γδ TIL IFNγ production and confirmed MHC independent recognition, addition of anti-γδ TCR mAb significantly reduced IFNγ production compared to blocking with the isotype control. Addition of the anti-NKG2D antibody showed minimal effect on IFNγ production and was not further reduced when blocked in combination with the γδ TCR, suggesting the involvement of the γδ TCR in mediating autologous tumor reactivity.

To identify additional factors associated with γδ TIL autologous tumor reactivity, the production of IFNγ following autologous tumor digest co-culture with IL-2/IL-4/IL-15 expanded, negatively selected γδ TIL phenotypic characteristics were compared. The percent composition of Vδ1 positively correlated ($r = +0.719$, $p=0.012$) with IFNγ production, supporting earlier reports of the enhanced anti-tumor potential of Vδ1 cells over that observed with other γδ subsets (Figure 12D) (Deniger *et al.*, *Clin. Cancer Res.*, 20:5708-5719 (2014); Fisher *et al.*, *Clin. Cancer Res.*, 20:5720-5732 (2014); and Cordova *et al.*, *PLoS One*, 7:e49878 (2012)).

Pre-Rapid Expansion Protocol Modulation of γ Chain Cytokines and CD137 Engagement Does Not Improve γδ TIL Expansion

Given the enhanced autologous tumor reactivity of γδ TIL in comparison to αβ TIL, methods for the specific expansion of γδ TIL during the pre-REP culture period, which determines the input number of γδ TIL available for REP, were investigated. With the increased number of γδ TIL following isolation and culture with IL-2, IL-4, and IL-15 during the REP, this γ chain combination was evaluated in a retrospective cohort of cryopreserved tumor digests (n=15, Figure 14) obtained from consenting patients undergoing resection following initial diagnosis or neoadjuvant therapy for melanoma. In addition to the γ chain combination, a humanized agonistic monoclonal antibody targeting the CD137 receptor (Urelumab, 10 μg/mL) was evaluated given the higher expression of CD137 on γδ TIL and prior reports of enhanced TIL expansion with CD137 engagement (Hall *et al.*, *J. Immunother. Cancer*, 4:61 (2016); Sakellariou-Thompson *et al.*, *Clin. Cancer Res.*, 23:7263-7275 (2017); Poch *et al.*, *Oncoimmunology*, 7:e1476816 (2018); Tavera *et al.*, *J. Immunother.*, 41:399-405 (2018)).

While the γ chain combination increased the total number of viable expanded TIL following 11 days of culture, due to increased $CD3^+$ αβ TCR^+ CD4 and CD8 TIL, no

differences in the number of γδ TCR^+ or $V\delta1^+$ cells were observed with or without CD137 stimulation compared to IL-2 alone (Figures 15A-E). Although no improvements in the expansion of γδ TIL were identified with use of the γ chain combination or CD137 engagement, these results support the continued utilization of high dose IL-2 or in combination with other γ chain cytokines during the pre-REP process to expand γδ TIL.

Tumor Specific Vδ1 Infiltration and Survival Benefit

With multiple clinical studies of TIL therapy identifying infusion of increased number of tumor reactive T cells associated with objective clinical response, and the aforementioned sparse infiltration of γδ TIL, the following was performed to identify target indications with increased γδ TIL and determine their impact on long term survival (Radvanyi *et al.*, *Clin. Cancer Res.*, 18:6758-6770 (2012); Goff *et al.*, *J. Clin. Oncol.*, 34:2389-2397 (2016) and Chandran *et al.*, *Lancet Oncol.*, 18:792-802 (2017)). Using bulk RNA sequencing data of the 20 most prevalent solid tumors from The Cancer Genome Atlas (TCGA), the expression of γδ and αβ TIL was identified with the Gene Expression Profiling Interactive Analysis 2 (GEPIA 2) tool (Figure 16A)(Tang *et al.*, *Nucleic Acids Res.*, 47:W556-W560 (2019); and Siegel *et al.*, *CA Cancer J. Clin.*, 71:7-33 (2021)). The infiltration of the primary γδ T cell subsets ($V\delta1^+$, $V\delta2^+$, and $V\delta3^+$cells) were identified with the corresponding Vδ gene (TRDV1, TRDV2, and TRDV3), while αβ TIL were identified with the Vβ 2 constant region of the TCR (TRBC2). This method enabled clear identification of γδ TIL populations as previously utilized RNA gene signatures of γδ T cells have been shown to incorrectly include other immune effector subsets during classification of γδ T cells (Gentles *et al.*, *Nat. Med.*, 21:938-945 (2015); and Tosolini *et al.*, *Oncoimmunology*, 6:e1284723 (2017)). Across most tumor types, the expression of TRDV1 trended to be higher than TRDV2 or TRDV3. Ovarian serous cystadenocarcinoma (OV) was the only indication to demonstrate notable expression of TRDV3. TRDV1 was differentially expressed across tumor types and was greatest in lung adenocarcinoma (LUAD, 0.5 median Log transcripts per million (TPM)), kidney renal cell carcinoma (KIRC, 0.5 Log TPM), breast carcinoma (BRCA, 0.4 Log TPM), and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, 0.4 Log TPM). Full cohort TRDV1 expression for these selected tumors is shown in Figure 17A. Glioblastoma (GBM), liver hepatocellular carcinoma (LIHC), bladder urothelial carcinoma (BLCA), uterine corpus endometrial carcinoma (UCEC), and prostate adenocarcinoma (PRAD) displayed the

lowest expression of TRDV1, (median TRDV1 Log TPM = 0). Across all tumor types, TRDV1 expression was positively correlated with expression of TRBC2 (Figures 18A-S).

Given the predominant infiltration of the Vδ1 subset across tumors compared to the other γδ T cell subsets and association with autologous tumor reactivity, the prognostic impact of TRDV1 expression on overall survival in the selected tumors was evaluated. Following normalization of TRDV1 expression with beta actin (ACTB), the cohorts were split into high and low expression groups based on the median expression level of TRDV1 of individual tumor types. When including all TCGA tumors available for analysis on the GEPIA 2 server, high expression of TRDV1 was associated with considerable survival benefit ($p<0.00001$, Figure 16B). High expression of TRDV1 was similarly associated with significant survival benefit in 12 of the 20 profiled solid tumors, including skin cutaneous melanoma (SKCM, $p=0.0006$), head and neck squamous cell carcinoma (HNSC, $p=0.002$), lung adenocarcinoma and lung squamous cell carcinoma (LUSC, $p=0.0004$), breast cancer BRCA ($p=0.007$), esophageal cancer CESC ($p=0.014$), and pancreatic ductal adenocarcinoma (PDAC, $p=0.077$), which are current indications utilizing TIL therapy (Figures 16C-G and 17B-G). Increased TRDV1 was also associated with survival benefit in patients with GBM, mesothelioma (MESO), LIHC, KIRC, and BLCA. In the remaining eight tumors, high expression of TRDV1 was not associated with substantive improvements in survival (Figures 19A-H).

Taken together, the results provided herein demonstrate that tumor infiltrating γδ T cells, displaying diverse, patient specific repertoires, tissue resident effector memory phenotypes, and superior autologous tumor reactivity, can be successfully expanded by themselves or in parallel with αβ TIL to unleash the full TCR repertoire against cancer.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for producing a cell population comprising γδ T cells, wherein said method comprises culturing a first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for 8 to 21 days to obtain a second cell population, wherein said second cell population comprises at least 10 times more γδ T cells than said first cell population.

2. The method of claim 1, wherein said γδ T cells are human cells.

3. The method of any one of claims 1-2, wherein said γδ T cells are tumor infiltrating γδ T cells.

4. The method of any one of claims 1-3, wherein said first cell population is:

(i) a population of tumor infiltrating γδ T cells obtained from (a) tissue comprising a tumor or (b) healthy tissue that was within 30 mm of a tumor,

(ii) a population of γδ T cells obtained from healthy tissue,

(iii) a population of γδ T cells obtained from infected tissue, or

(iv) a population of γδ T cells obtained from tissue harboring autoimmune T cells.

5. The method of claim 4, wherein said method comprises obtaining said first cell population from said tissue comprising said tumor.

6. The method of claim 4, wherein said method comprises obtaining said first cell population from said healthy tissue that was within 30 mm of said tumor.

7. The method of any one of claims 1-6, wherein said first cell population is a cell population that was cultured in the presence of 50 international units/mL to 6000 international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 3 to 15 days prior to said culturing in the presence of IL-2, IL-4, and IL-15.

8. The method of any one of claims 1-6, wherein said first cell population is a cell population that was cultured in the presence of 100 international units/mL to 4000

international units/mL of IL-2 and in the absence of IL-4 and IL-15 for 8 to 15 days prior to said culturing in the presence of IL-2, IL-4, and IL-15.

9. The method of any one of claims 1-8, wherein said first cell population is a cell population that was enriched for tumor infiltrating γδ T cells.

10. The method of any one of claims 1-8, wherein said first cell population is a cell population that was enriched for tumor infiltrating γδ T cells via (a) the removal of at least some αβ T cells or (b) the isolation of at least some γδ T cells.

11. The method of any one of claims 9-10, wherein said method comprises removing at least some αβ T cells from a cell population to obtain said first cell population.

12. The method of claim 11, wherein said removing comprises positively selecting αβ T cells and removing the positively selected αβ T cells.

13. The method of any one of claims 9-10, wherein said method comprises isolating at least some γδ T cells from a cell population to obtain said first cell population.

14. The method of claim 13, wherein said isolating comprises positively selecting γδ T cells and isolating the positively selected γδ T cells.

15. The method of any one of claims 1-14, wherein said culturing said first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 for said 8 to 21 days comprises culturing said first cell population comprising γδ T cells in the presence of IL-2, IL-4, IL-15, irradiated PBMCs, and an anti-CD3 antibody for said 8 to 21 days.

16. The method of any one of claims 1-15, wherein said culturing said first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 is for 12 to 16 days.

17. The method of any one of claims 1-15, wherein said culturing said first cell population comprising γδ T cells in the presence of IL-2, IL-4, and IL-15 is for 13 to 15 days.

18. The method of any one of claims 1-17, wherein said second cell population comprises:

at least 50 times more γδ T cells than said first cell population,

at least 100 times more γδ T cells than said first cell population,

at least 200 times more γδ T cells than said first cell population,

at least 300 times more γδ T cells than said first cell population, or

at least 400 times more γδ T cells than said first cell population.

19. The method of any one of claims 1-18, wherein said second cell population comprises greater than 1×10^8 γδ T cells.

20. The method of any one of claims 1-19, wherein said IL-2 is a human IL-2, wherein said IL-4 is a human IL-4, and wherein said IL-15 is a human IL-15.

21. The method of any one of claims 1-20, wherein greater than 85 percent of the $CD3^+$ cells of said second cell population are γδ TCR^+ cells.

22. The method of any one of claims 1-21, wherein less than 10 percent of the $CD3^+$ cells of said second cell population are αβ TCR^+ cells.

23. The method of any one of claims 1-22, wherein less than 10 percent of the $CD45^+$ cells of said second cell population are NK cells.

24. The method of any one of claims 1-23, wherein greater than 30 percent of the γδ TCR^+ cells of said second cell population are $V\delta1^+$ cells.

25. The method of any one of claims 1-24, wherein less than 60 percent of the γδ TCR^+ cells of said second cell population are $V\delta1^-V\delta2^-$ cells.

26. The method of any one of claims 1-25, wherein less than 25 percent of the γδ TCR^+ cells of said second cell population are $V\delta2^+$ cells.

27. The method of any one of claims 1-26, wherein greater than 70 percent of the γδ TCR$^+$ cells of said second cell population are T_{EM} cells.

28. The method of any one of claims 1-27, wherein less than 25 percent of the γδ TCR$^+$ cells of said second cell population are T_{EMRA} cells.

29. The method of any one of claims 1-28, wherein less than 10 percent of the γδ TCR$^+$ cells of said second cell population are CD69$^+$ CD103$^+$ T_{RM} cells.

30. The method of any one of claims 1-29, wherein from 1 to 10 percent of the γδ TCR$^+$ cells of said second cell population are CD69$^+$ CD103$^+$ T_{RM} cells.

31. The method of any one of claims 1-30, wherein less than 50 percent of the γδ TCR$^+$ cells of said second cell population are CD56$^+$ cells.

32. The method of any one of claims 1-31, wherein from 1 to 50 percent of the γδ TCR$^+$ cells of said second cell population are CD56$^+$ cells.

33. The method of any one of claims 1-32, wherein from 1 to 40 percent of the γδ TCR$^+$ cells of said second cell population are CD137$^+$ cells.

34. The method of any one of claims 1-33, wherein less than 25 percent of the γδ TCR$^+$ cells of said second cell population are PD-1$^+$ cells.

35. The method of any one of claims 1-34, wherein from 5 to 40 percent of the γδ TCR$^+$ cells of said second cell population are BTLA$^+$ cells.

36. The method of any one of claims 1-35, wherein greater than 60 percent of the γδ TCR$^+$ cells of said second cell population are NKG2D$^+$ cells.

37. The method of any one of claims 1-36, wherein greater than 20 percent of the γδ TCR$^+$ cells of said second cell population are NKp46$^+$ cells.

38. An isolated cell population comprising polyclonal γδ T cells, wherein said population comprises greater than 1 x 10^8 γδ T cells.

39. The cell population of claim 38, wherein greater than 85 percent of the $CD3^+$ cells of said cell population are γδ TCR^+ cells.

40. The cell population of any one of claims 38-39, wherein less than 10 percent of the $CD3^+$ cells of said cell population are αβ TCR^+ cells.

41. The cell population of any one of claims 39-40, wherein less than 10 percent of the $CD45^+$ cells of said cell population are NK cells.

42. The cell population of any one of claims 39-41, wherein greater than 30 percent of the γδ TCR^+ cells of said cell population are $V\delta1^+$ cells.

43. The cell population of any one of claims 39-42, wherein less than 60 percent of the γδ TCR^+ cells of said cell population are $V\delta1^-V\delta2^-$ cells.

44. The cell population of any one of claims 39-43, wherein less than 25 percent of the γδ TCR^+ cells of said cell population are $V\delta2^+$ cells.

45. The cell population of any one of claims 39-44, wherein greater than 70 percent of the γδ TCR^+ cells of said cell population are T_{EM} cells.

46. The cell population of any one of claims 39-45, wherein less than 25 percent of the γδ TCR^+ cells of said cell population are T_{EMRA} cells.

47. The cell population of any one of claims 39-46, wherein less than 10 percent of the γδ TCR^+ cells of said cell population are $CD69^+$ $CD103^+$ T_{RM} cells.

48. The cell population of any one of claims 39-47, wherein from 1 to 10 percent of the γδ TCR^+ cells of said cell population are $CD69^+$ $CD103^+$ T_{RM} cells.

49. The cell population of any one of claims 39-48, wherein less than 50 percent of the γδ TCR^+ cells of said cell population are $CD56^+$ cells.

50. The cell population of any one of claims 39-49, wherein from 1 to 50 percent of the γδ TCR^+ cells of said cell population are $CD56^+$ cells.

51. The cell population of any one of claims 39-50, wherein from 1 to 40 percent of the γδ TCR^+ cells of said cell population are $CD137^+$ cells.

52. The cell population of any one of claims 39-51, wherein less than 25 percent of the γδ TCR^+ cells of said cell population are PD-1^+ cells.

53. The cell population of any one of claims 39-52, wherein from 5 to 40 percent of the γδ TCR^+ cells of said cell population are $BTLA^+$ cells.

54. The cell population of any one of claims 39-53, wherein greater than 60 percent of the γδ TCR^+ cells of said cell population are $NKG2D^+$ cells.

55. The cell population of any one of claims 39-54, wherein greater than 20 percent of the γδ TCR^+ cells of said cell population are $NKp46^+$ cells.

56. The cell population of any one of claims 39-55, wherein the cells of said cell population are human cells.

57. The cell population of any one of claims 39-56, wherein said γδ T cells are tumor infiltrating γδ T cells.

58. The cell population of any one of claims 39-57, wherein cell population was produced using the method of any one of claims 1-37.

59. A method for providing a mammal with γδ T cells, wherein said method comprises administering a cell population produced as set forth in any one of claims 1-37 to a mammal.

60. The method of claim 59, wherein said mammal is a human.

61. The method of any one of claims 59-60, wherein said mammal has cancer.

62. The method of any one of claims 59-61, wherein the cells of said first cell population are allogenic or autologous to said mammal administered said cell population.

63. A method for providing a mammal with γδ T cells, wherein said method comprises administering said cell population of any one of claims 38-58 to a mammal.

64. The method of claim 63, wherein said mammal is a human.

65. The method of any one of claims 63-64, wherein said mammal has cancer, an autoimmune condition, or an infection.

66. The method of any one of claims 59-65, wherein the cells of said cell population are allogenic or autologous to said mammal.

67. A method for treating cancer, wherein said method comprises administering a cell population produced as set forth in any one of claims 1-37 to a mammal having cancer.

68. The method of claim 67, wherein said mammal is a human.

69. The method of any one of claims 67-68, wherein the cells of said first cell population are allogenic or autologous to said mammal having cancer.

70. A method for treating cancer, wherein said method comprises administering said cell population of any one of claims 38-58 to a mammal having cancer.

71. The method of claim 70, wherein said mammal is a human.

72. The method of any one of claims 70-71, wherein the cells of said cell population are allogenic or autologous to said mammal having cancer.

73. The method of any one of claims 59-72, wherein said method comprises administering αβ T cells to said mammal.

74. A method for treating an autoimmune condition, wherein said method comprises administering said cell population of any one of claims 38-58 to a mammal having an autoimmune condition.

75. The method of claim 74, wherein said mammal is a human.

76. The method of any one of claims 74-75, wherein the cells of said cell population are allogenic or autologous to said mammal having said autoimmune condition.

77. The method of any one of claims 74-76, wherein said method comprises administering αβ T cells to said mammal.

78. A method for treating an infection, wherein said method comprises administering said cell population of any one of claims 38-58 to a mammal having an infection.

79. The method of claim 78, wherein said mammal is a human.

80. The method of any one of claims 78-79, wherein the cells of said cell population are allogenic or autologous to said mammal having said infection.

81. The method of any one of claims 78-80, wherein said method comprises administering αβ T cells to said mammal.

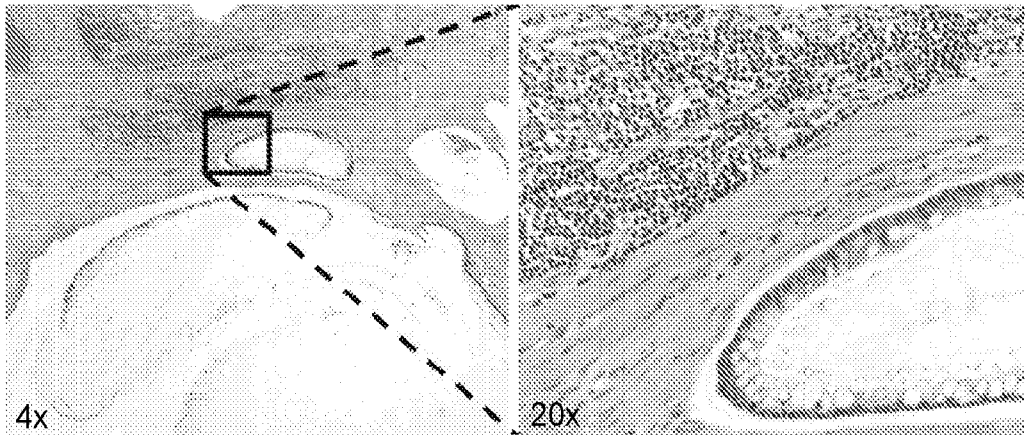

FIG. 1A

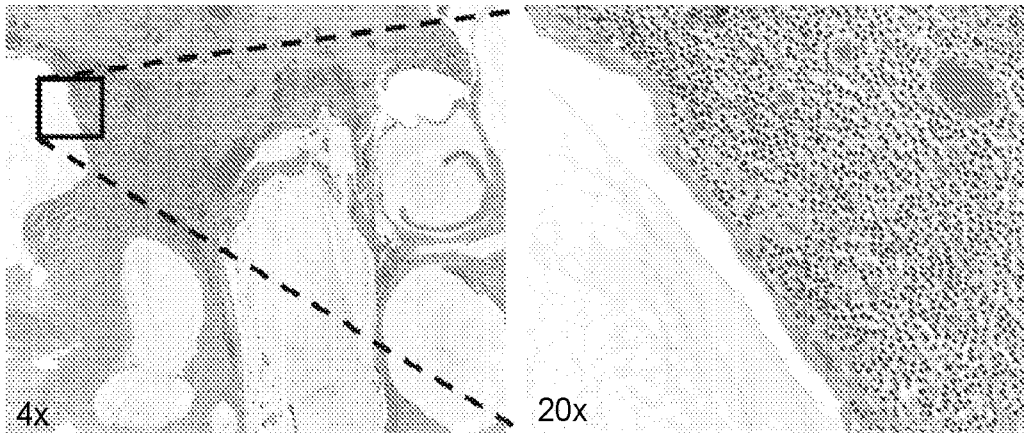

FIG. 1B

Low Grade (G1) Cohort	N=10
Age at 1st HIPEC	50.4±12.2
Female Gender	5 (50%)
BMI	26.0±4.4
1st HIPEC PCI Score	25±10.1
1st HIPEC CC Score = 0	5 (50%)
Prior Chemotherapy	0 (0%)
BRAF or SMAD4 Mutation	0 (0%)
MSS Stable	10 (100%)
PD-L1 Positivity	1 (10%)
2 CRS-HIPECs	6 (60%)
3 CRS-HIPECs	1 (10%)

FIG. 2

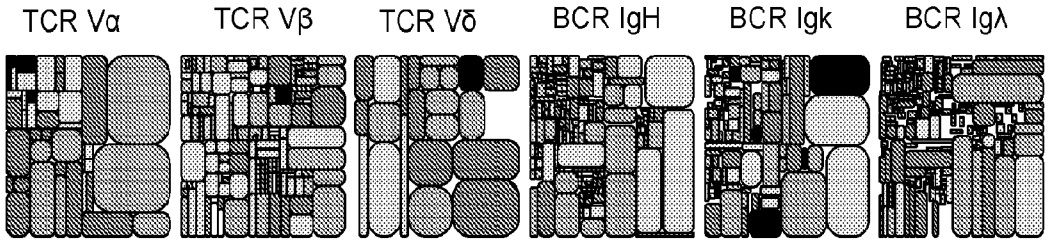
TCR Vα
TCR Vβ
TCR Vδ
BCR IgH
BCR Igk
BCR Igλ

FIG. 3A

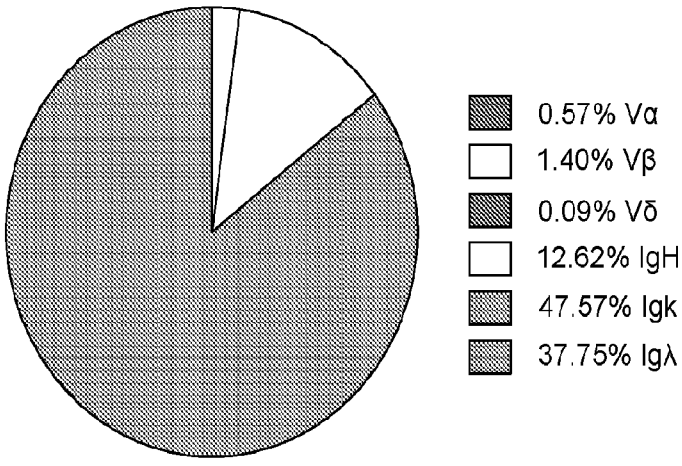
0.57% Vα
1.40% Vβ
0.09% Vδ
12.62% IgH
47.57% Igk
37.75% Igλ

FIG. 3B

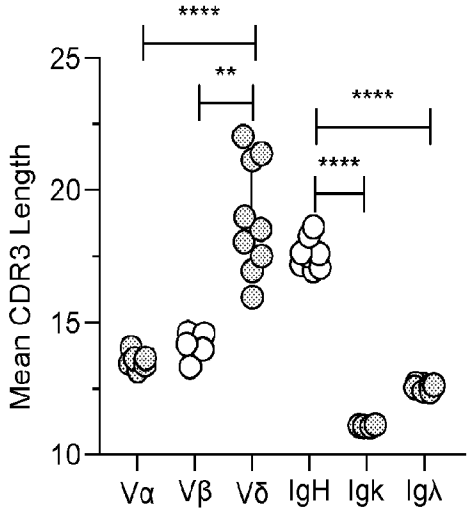
****
**
****
****
25
20
15
10
Mean CDR3 Length
Vα
Vβ
Vδ
IgH
Igk
Igλ

FIG. 3C

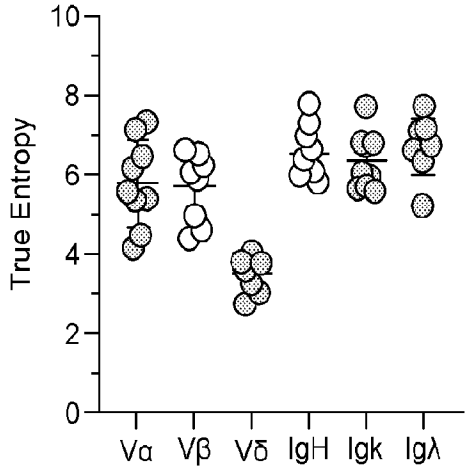
10
8
6
4
2
0
True Entropy
Vα
Vβ
Vδ
IgH
Igk
Igλ

FIG. 3D

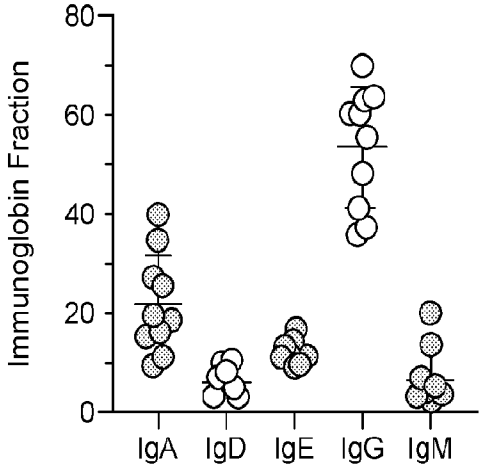
Immunoglobin Fraction
80
60
40
20
0
IgA
IgD
IgE
IgG
IgM

FIG. 3E

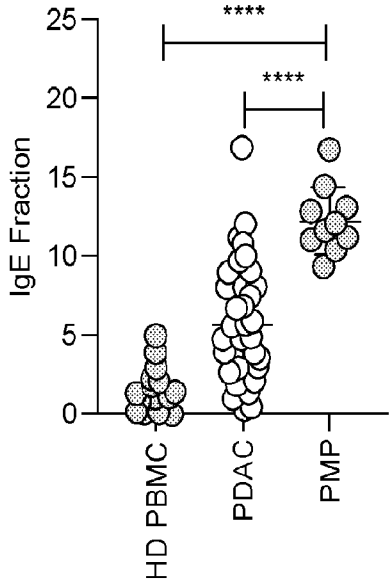
IgE Fraction
25
20
15
10
5
0
****
****
HD PBMC
PDAC
PMP

FIG. 3F

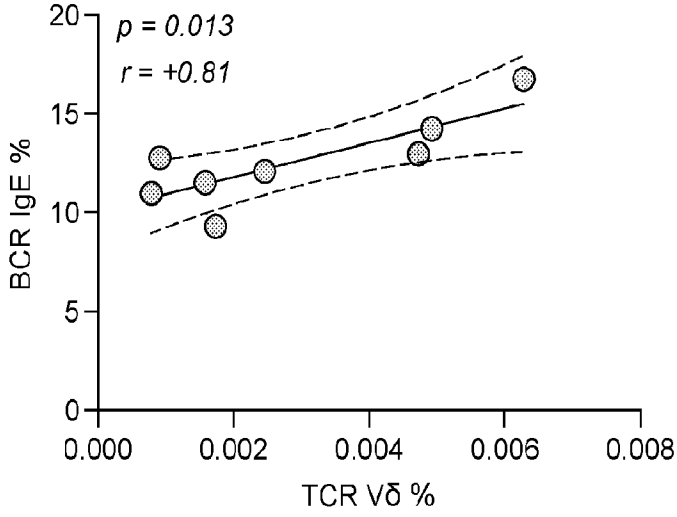

FIG. 3G

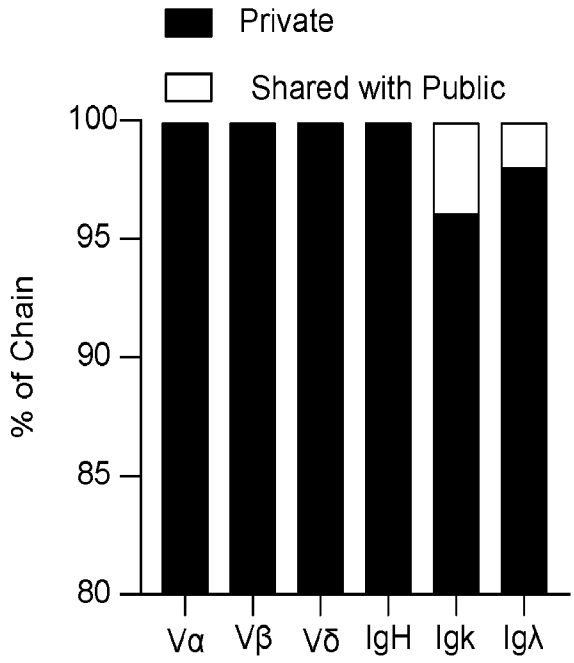
Private
Shared with Public
100
95
90
85
80
% of Chain
Vα
Vβ
Vδ
IgH
Igk
Igλ

FIG. 4A

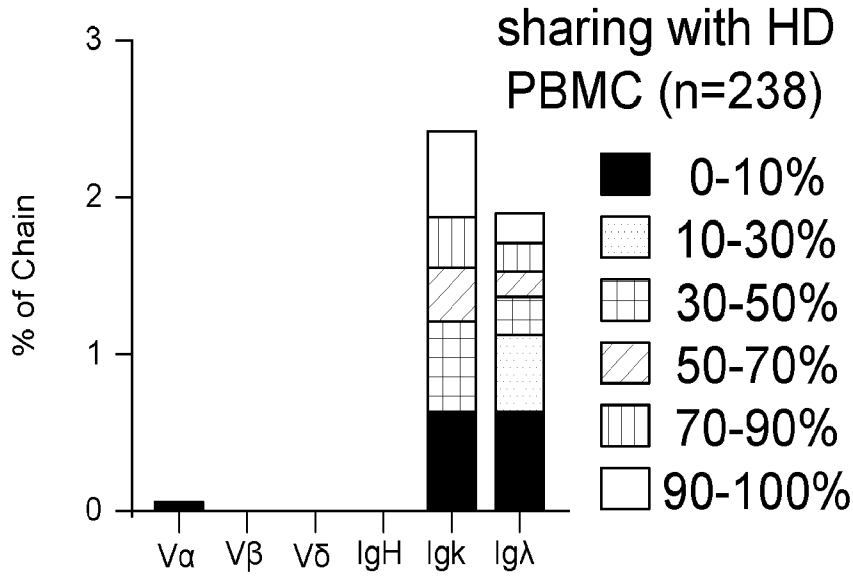
sharing with HD
PBMC (n=238)
0-10%
10-30%
30-50%
50-70%
70-90%
90-100%
3
2
1
0
% of Chain
Vα
Vβ
Vδ
IgH
Igk
Igλ

FIG. 4B

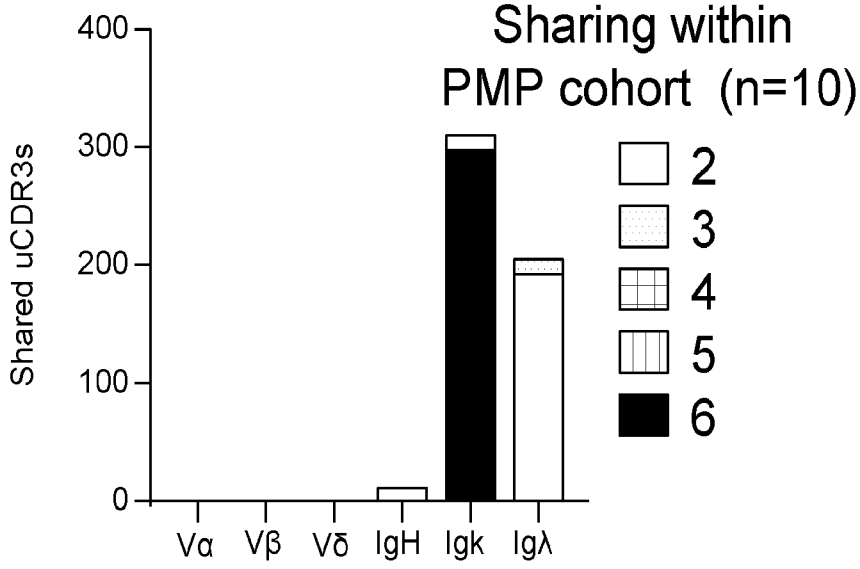
Sharing within
PMP cohort (n=10)
Shared uCDR3s
400
300
200
100
0
Vα
Vβ
Vδ
IgH
Igκ
Igλ
2
3
4
5
6

FIG. 4C

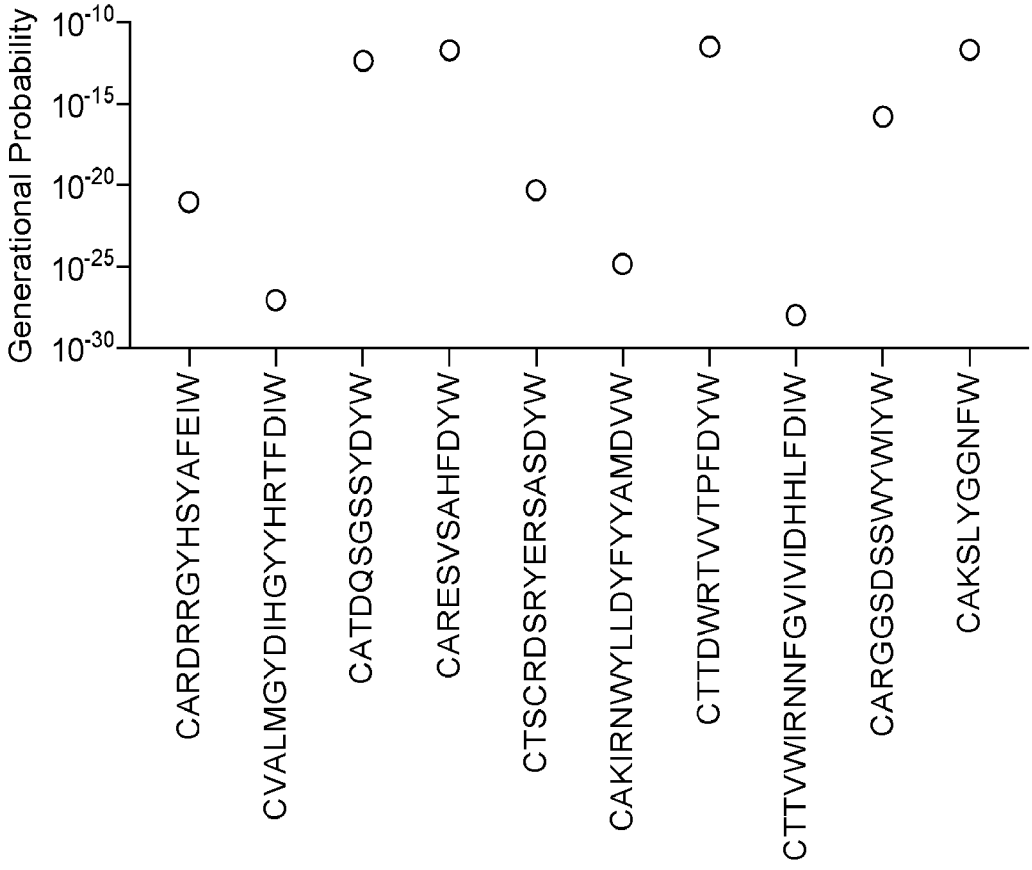
Generational Probability
10-10
10-15
10-20
10-25
10-30
CARDRRGYHSYAFEIW
CVALMGYDIHGYYHRTFDIW
CATDQSGSSYDYW
CARESVSAHFDYW
CTSCRDSRYERSASDYW
CAKIRNWYLLDYFYYAMDVW
CTTDWRTVVTPFDYW
CTTVWIRNNFGVIVIDHHLFDIW
CARGGSDSSWYWIYW
CAKSLYGGNFW

FIG. 4D

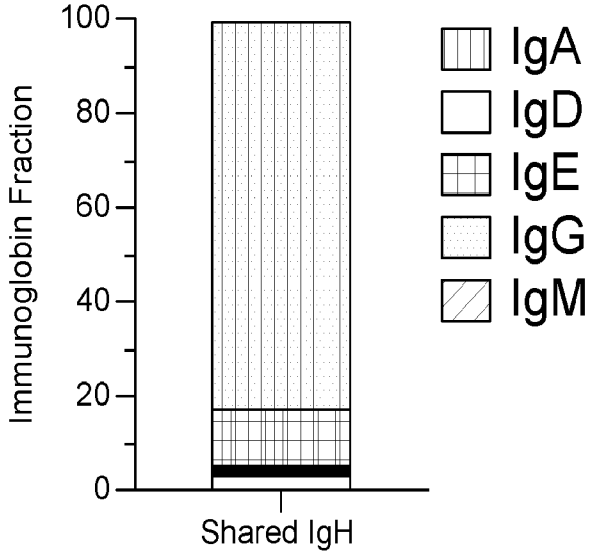
100
80
60
40
20
0
Immunoglobin Fraction
Shared IgH
IgA
IgD
IgE
IgG
IgM

FIG. 4E

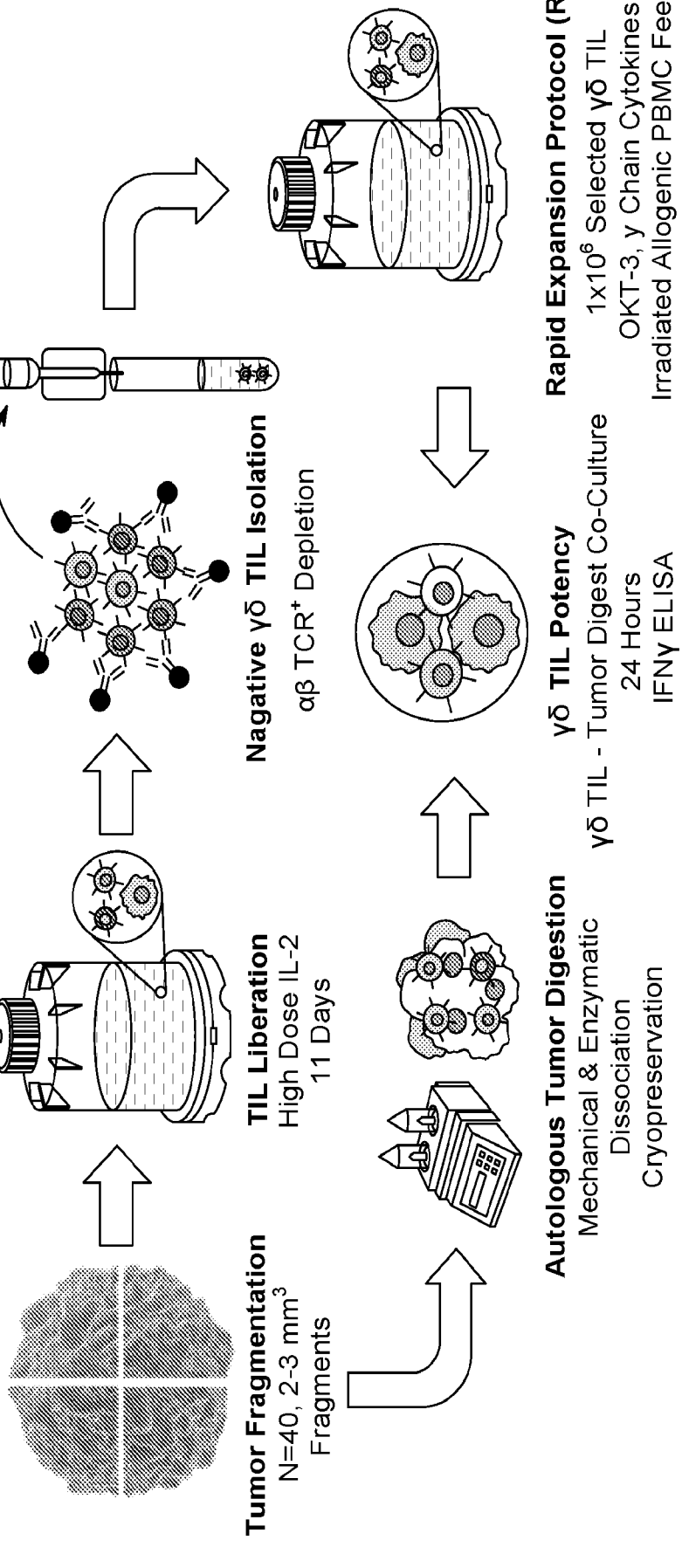
Tumor Fragmentation
N=40, 2-3 mm3
Fragments
TIL Liberation
High Dose IL-2
11 Days
Nagative γδ TIL Isolation
αβ TCR+ Depletion
Autologous Tumor Digestion
Mechanical & Enzymatic
Dissociation
Cryopreservation
γδ TIL Potency
γδ TIL - Tumor Digest Co-Culture
24 Hours
IFNγ ELISA
Rapid Expansion Protocol (REP)
1x10^6 Selected γδ TIL
OKT-3, γ Chain Cytokines
Irradiated Allogenic PBMC Feeders
14 Days

FIG. 5A

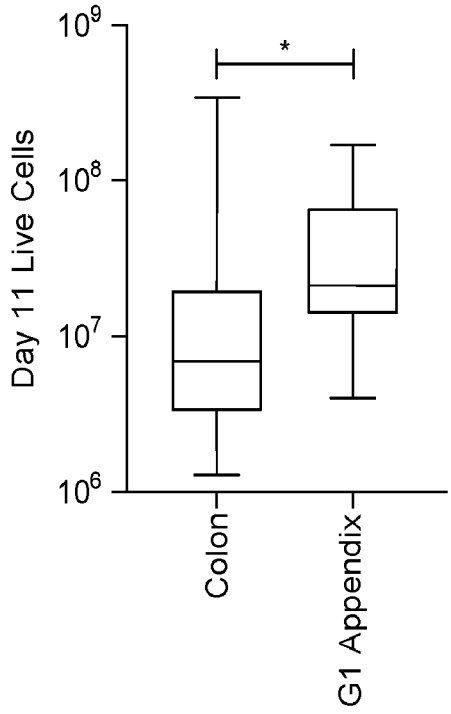
10^9
10^8
10^7
10^6
Day 11 Live Cells
*
Colon
G1 Appendix

FIG.5B

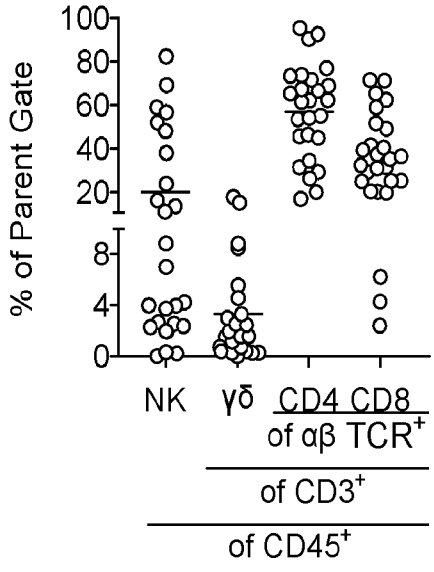
100
80
60
40
20
8
4
0
% of Parent Gate
NK
γδ
CD4 CD8
of αβ TCR+
of CD3+
of CD45+

FIG.5C

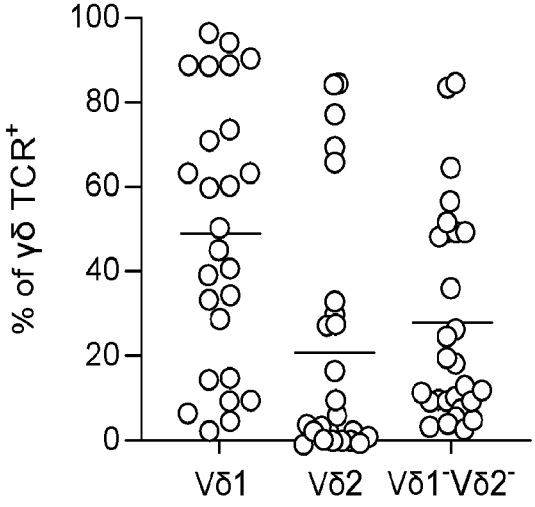

FIG.5D

Peritoneal Tumor Cohort	N=26
Age at Surgery	59.3±12.2
Female Gender	8 (30.7%)
BMI	27.5±8.4
Multiple CRS-HIPECs	3 (11.5%)
Appendiceal (G1)	14 (54%)
Colorectal	12 (46%)
Moderate	7 (58%)
Poor	5 (42%)
Prior Chemotherapy	14 (54%)
FOLFOX	7 (50%)
FOLFIRE	4 (29%)
Other	3 (21%)

FIG. 6

FIG. 7A

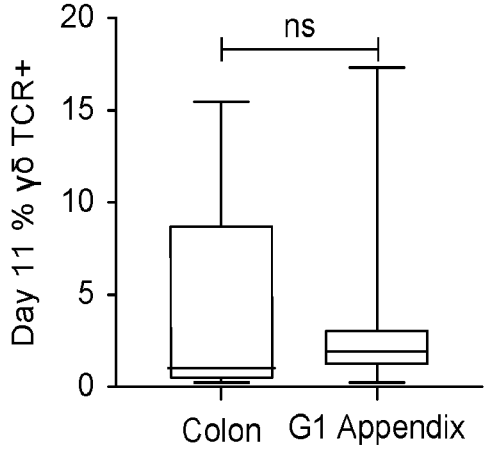

FIG. 7B

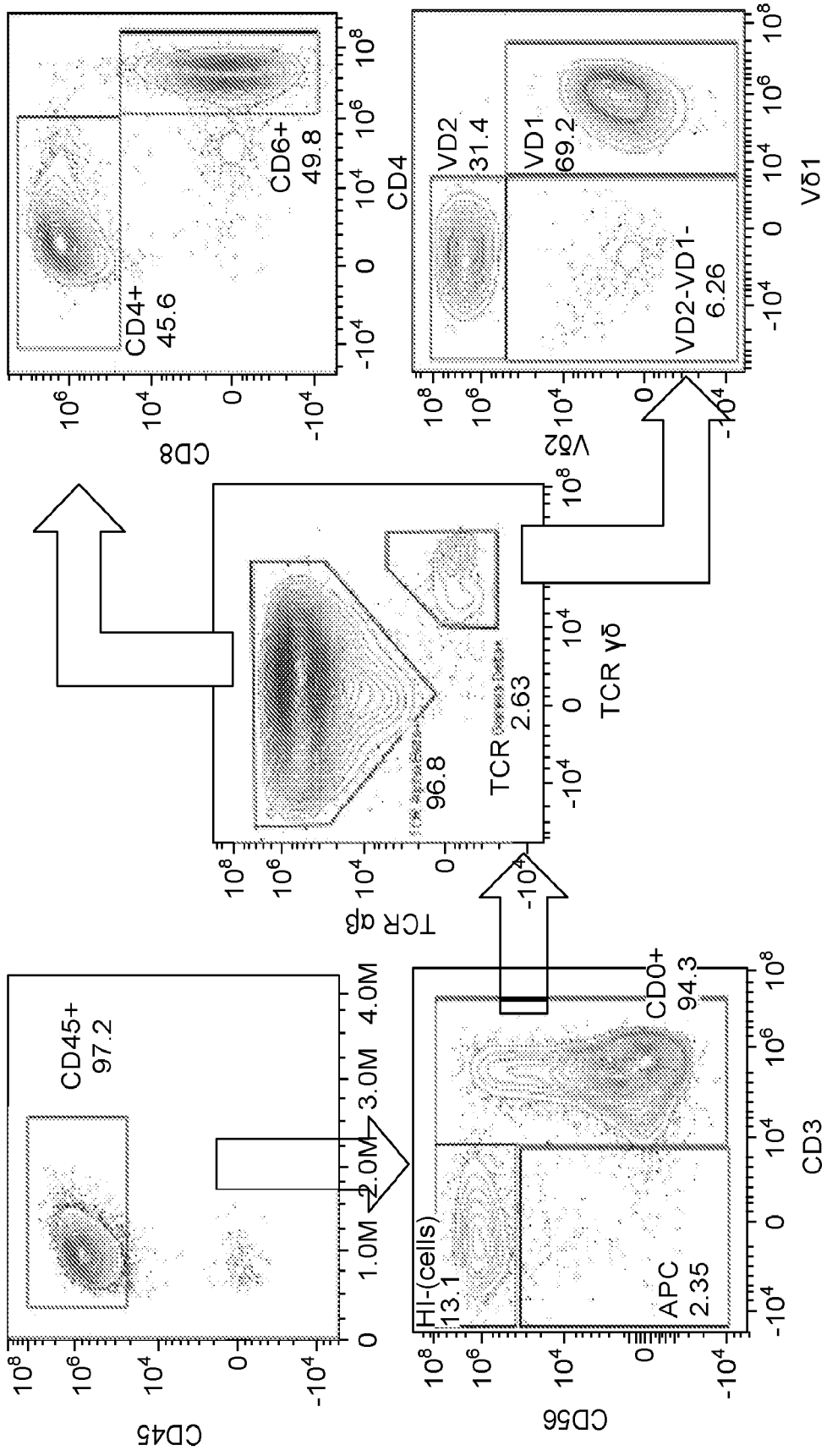
CD45
CD45+
97.2
CD56
HI-(cells)
13.1
APC
2.35
CD0+
94.3
CD3
TCR αβ
96.8
TCR
2.63
TCR γδ
CD8
CD4+
45.6
CD6+
49.8
CD4
Vδ2
VD2
31.4
VD1
69.2
VD2-VD1-
6.26
Vδ1

FIG. 7C

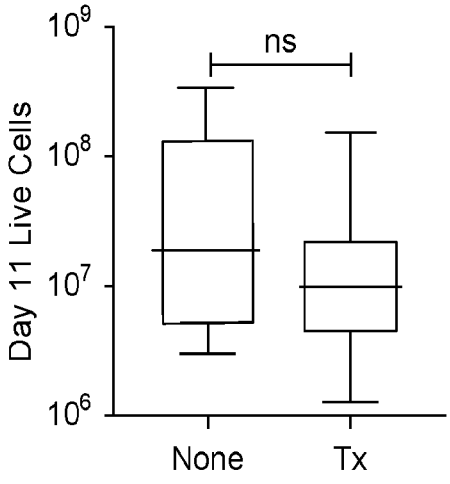
10^9
10^8
10^7
10^6
Day 11 Live Cells
ns
None
Tx

FIG. 7D

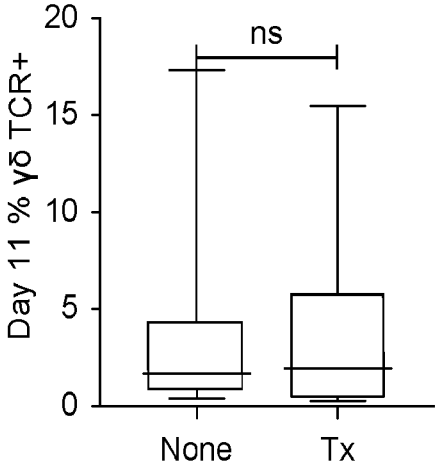
20
15
10
5
0
Day 11 % γδ TCR+
ns
None
Tx

FIG. 7E

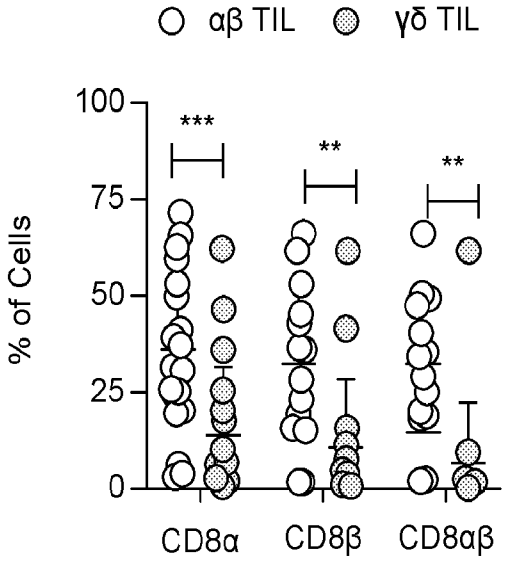
αβ TIL
γδ TIL
100
75
50
25
0
% of Cells
***
**
**
CD8α
CD8β
CD8αβ

FIG. 8A

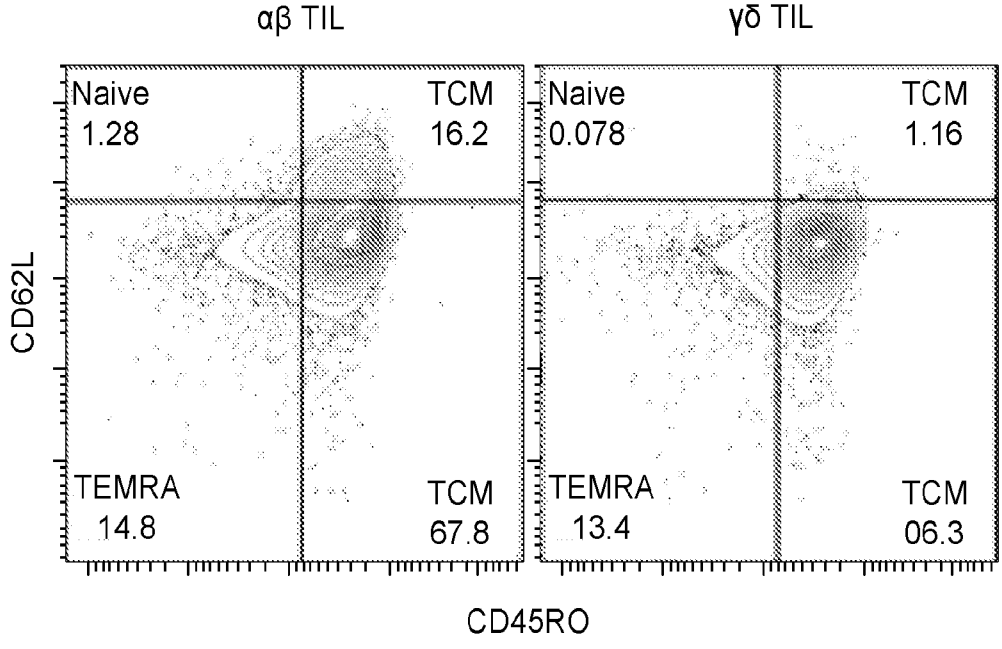
αβ TIL
γδ TIL
Naive
1.28
TCM
16.2
Naive
0.078
TCM
1.16
TEMRA
14.8
TCM
67.8
TEMRA
13.4
TCM
06.3
CD62L
CD45RO

FIG. 8B

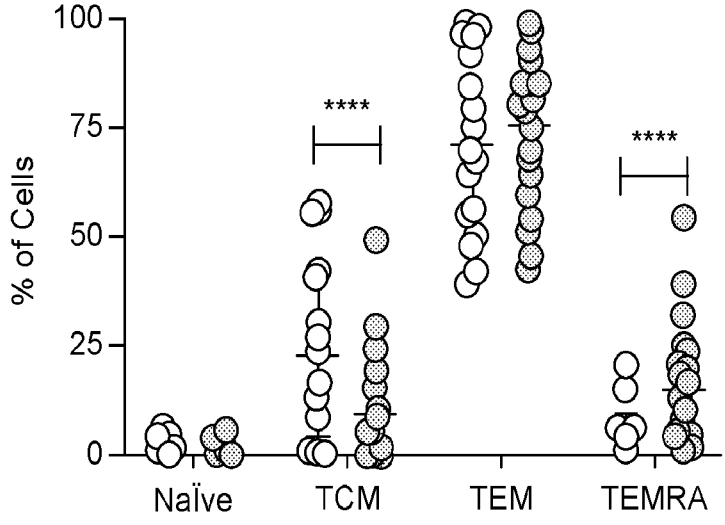
100
75
50
25
0
% of Cells
****
****
Naïve
TCM
TEM
TEMRA

FIG. 8C

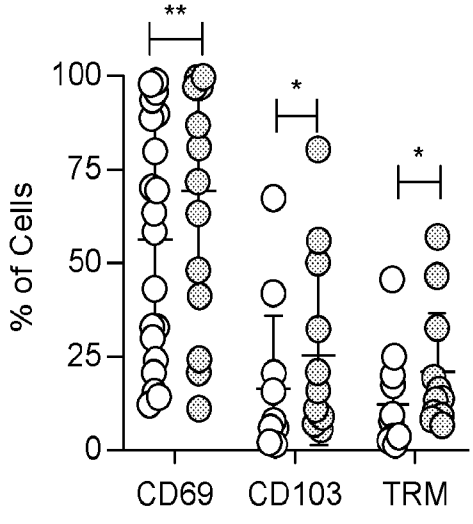
**
*
*
100
75
50
25
0
% of Cells
CD69
CD103
TRM

FIG. 8D

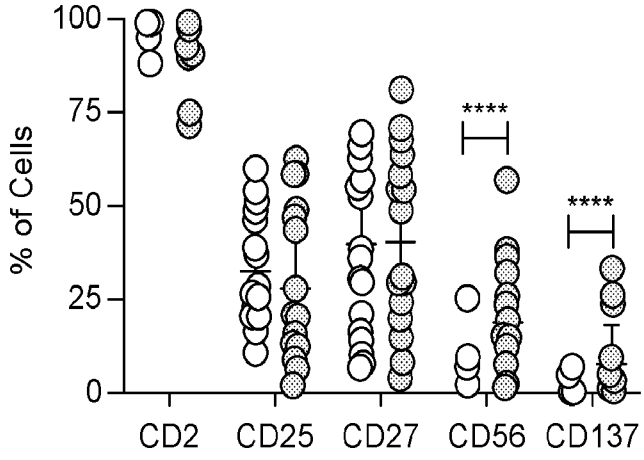
100
75
50
25
0
% of Cells
****
****
CD2
CD25
CD27
CD56
CD137

FIG. 8E

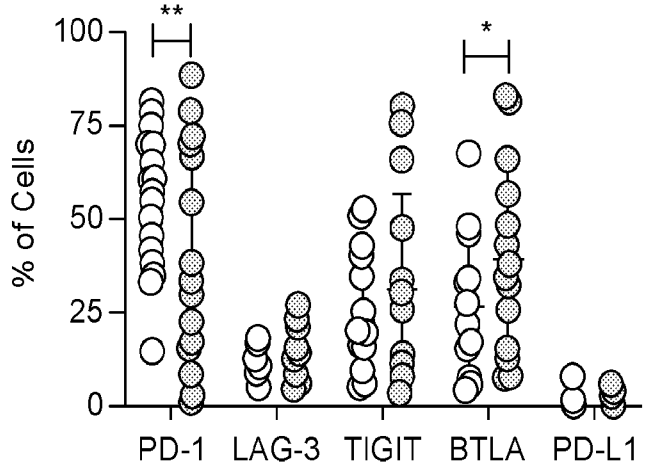
**
*
100
75
50
25
0
% of Cells
PD-1
LAG-3
TIGIT
BTLA
PD-L1

FIG. 8F

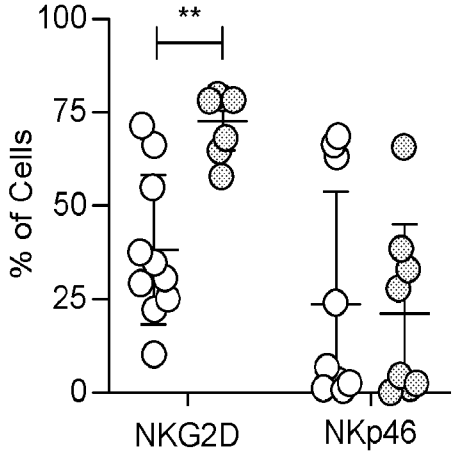
100
75
50
25
0
% of Cells
**
NKG2D
NKp46

FIG. 8G

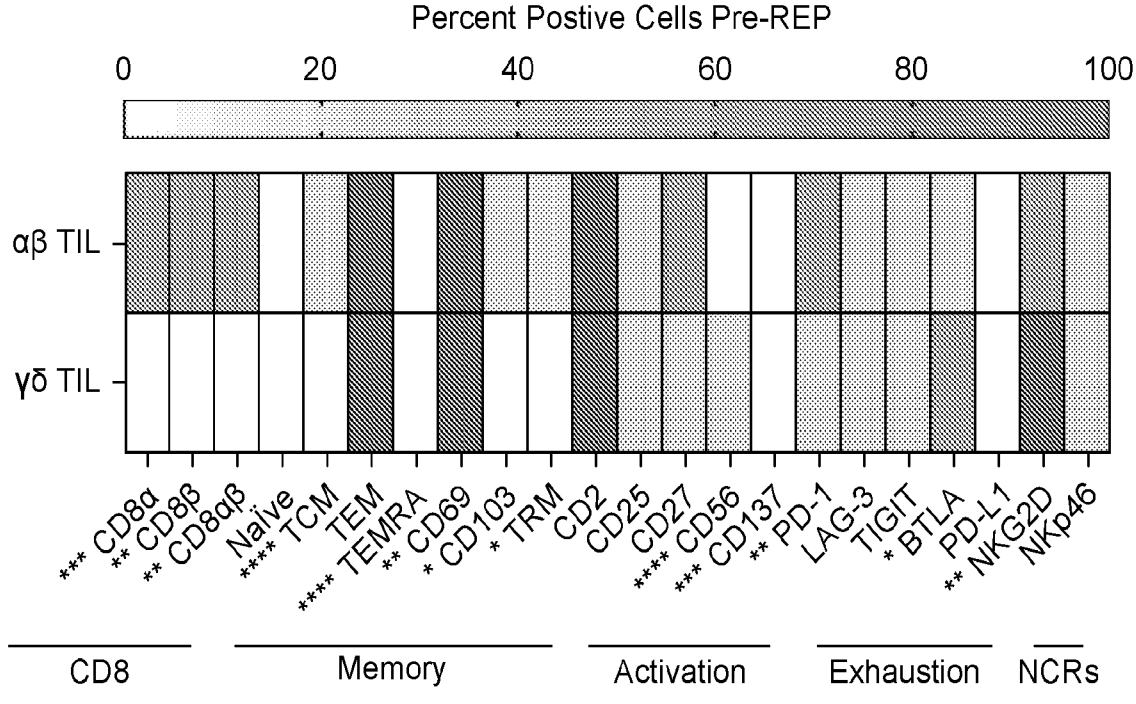
Percent Postive Cells Pre-REP
0
20
40
60
80
100
αβ TIL
γδ TIL
*** CD8α
** CD8β
** CD8αβ
Naïve
**** TCM
TEM
**** TEMRA
** CD69
* CD103
* TRM
CD2
CD25
CD27
**** CD56
*** CD137
** PD-1
LAG-3
TIGIT
* BTLA
PD-L1
** NKG2D
NKp46
CD8
Memory
Activation
Exhaustion
NCRs

FIG. 8H

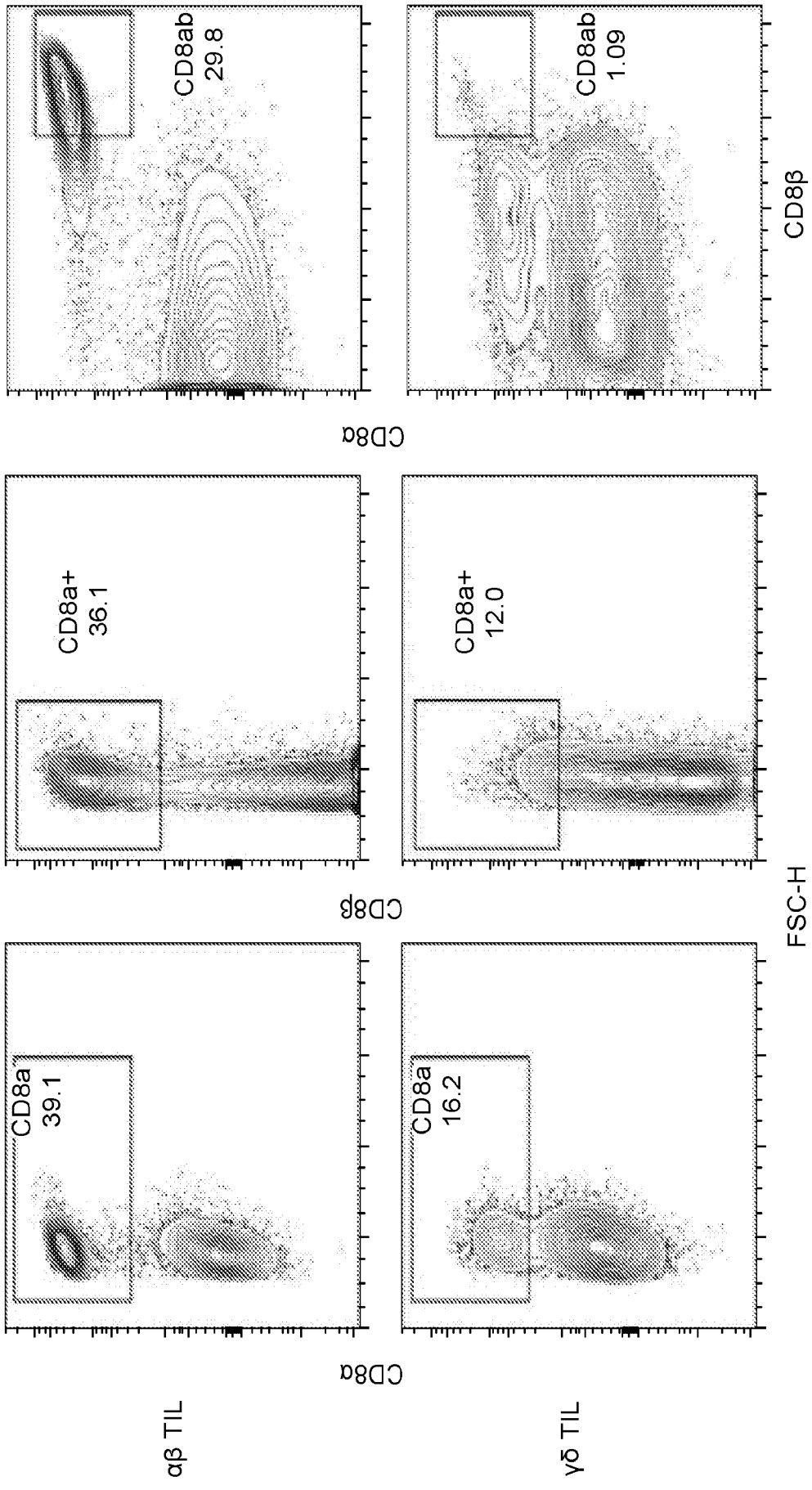
αβ TIL
γδ TIL
CD8α
CD8β
FSC-H
CD8a
39.1
CD8a
16.2
CD8a+
36.1
CD8a+
12.0
CD8ab
29.8
CD8ab
1.09

FIG. 9A

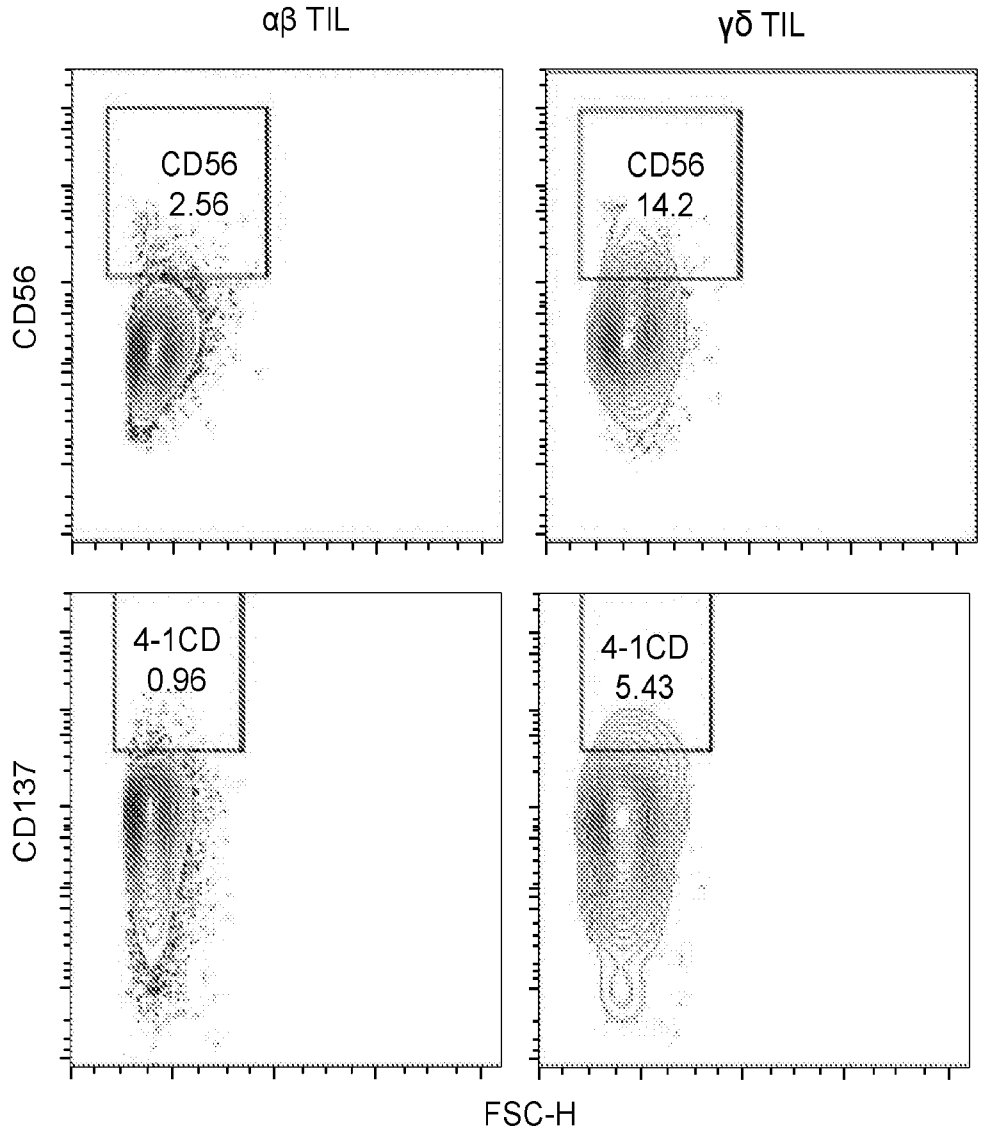
αβ TIL
γδ TIL
CD56
2.56
CD56
14.2
CD56
CD137
4-1CD
0.96
4-1CD
5.43
FSC-H

FIG. 9B

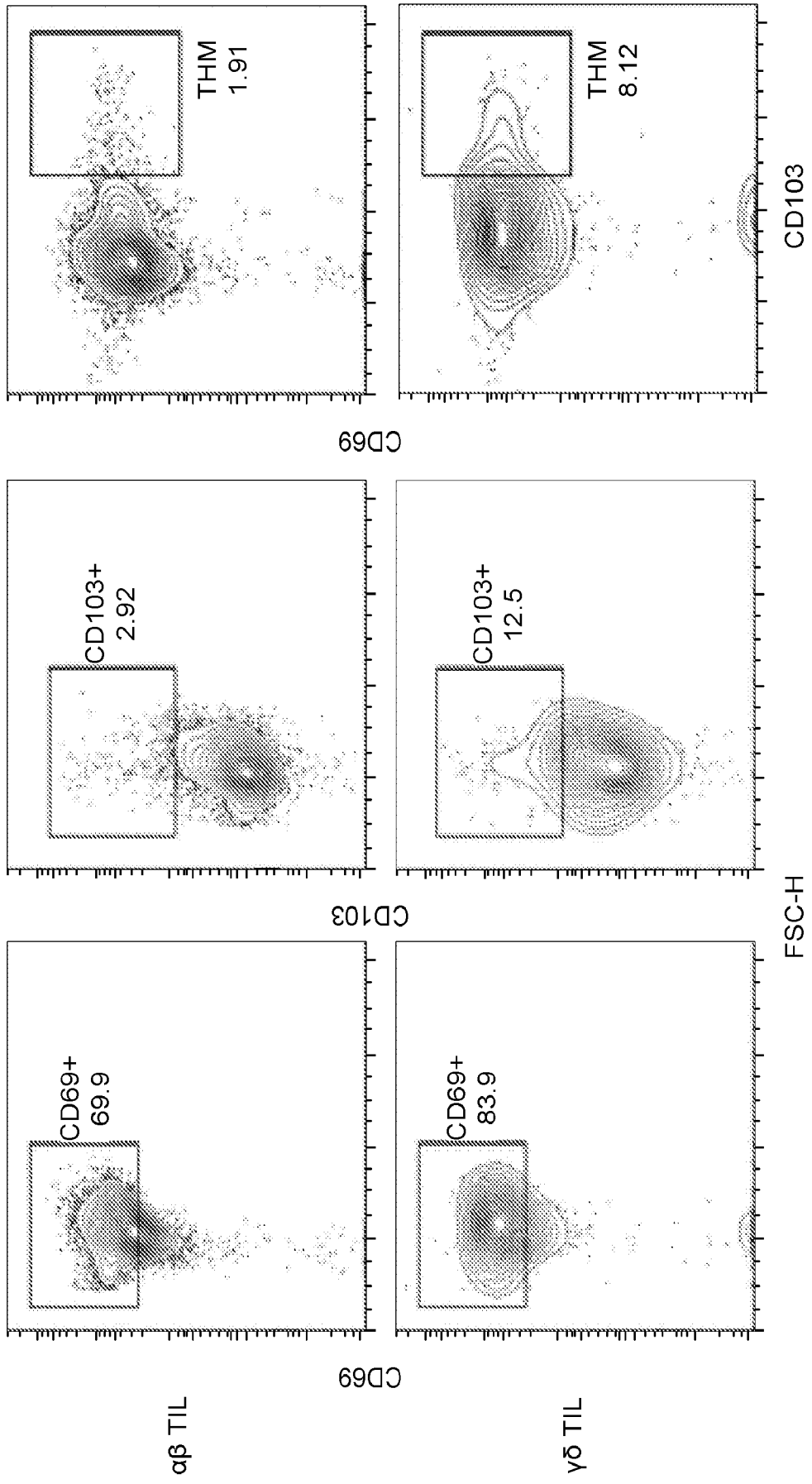
αβ TIL
γδ TIL
CD69
CD103
FSC-H
CD69+
69.9
CD69+
83.9
CD103+
2.92
CD103+
12.5
THM
1.91
THM
8.12
CD69
CD103

FIG. 9C

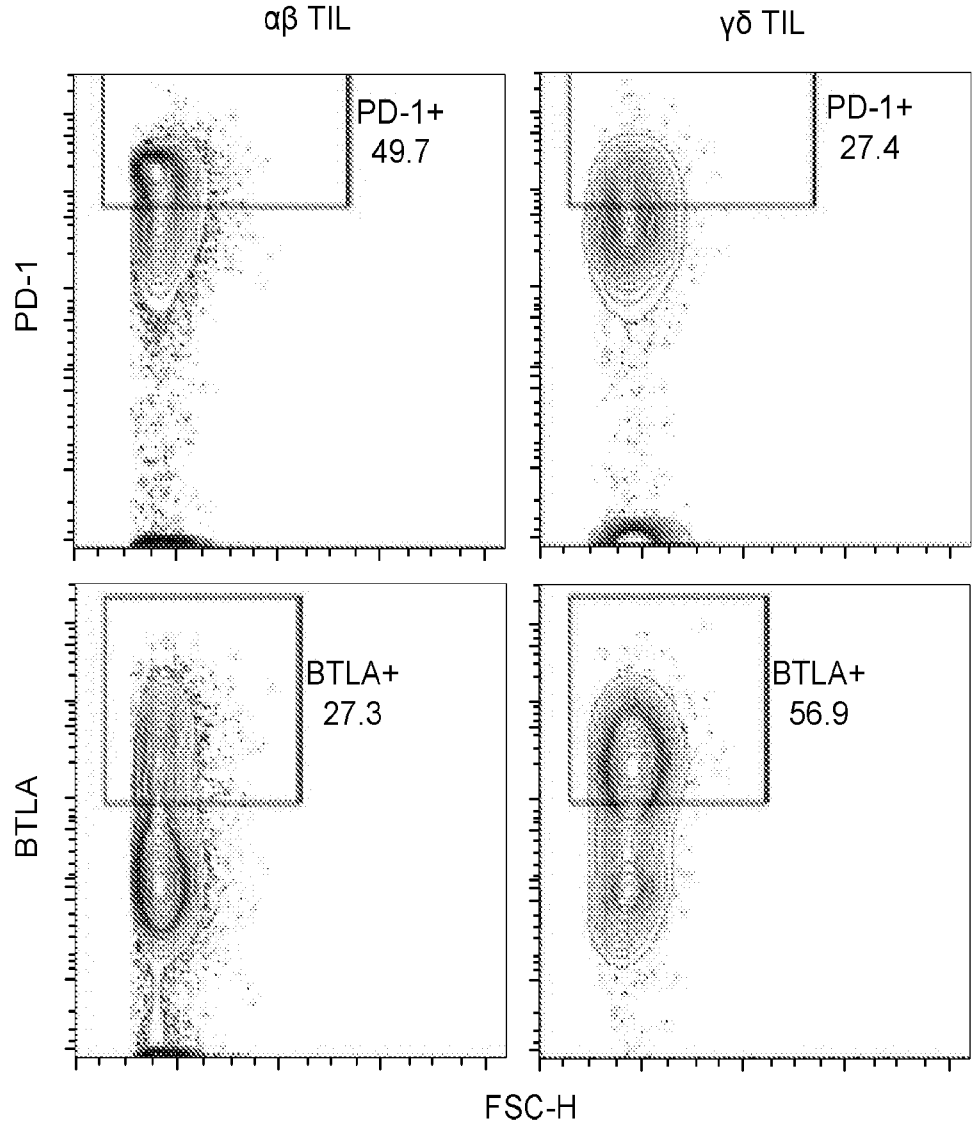

FIG. 9D

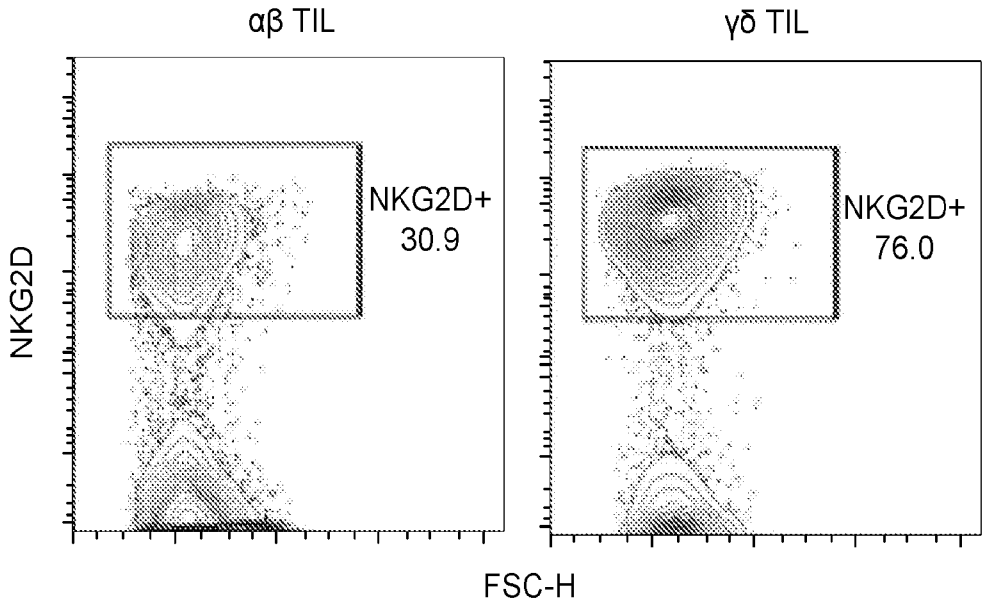

FIG. 9E

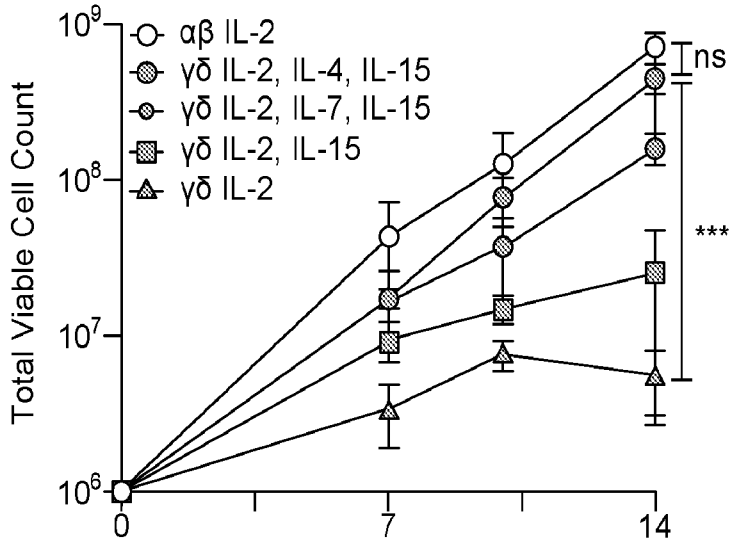

FIG. 10A

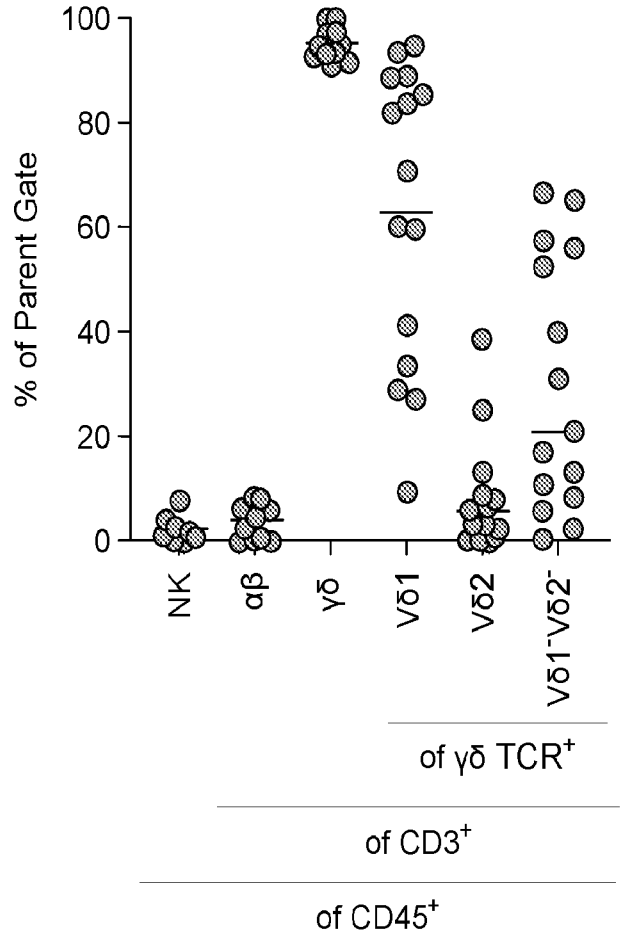

FIG. 10B

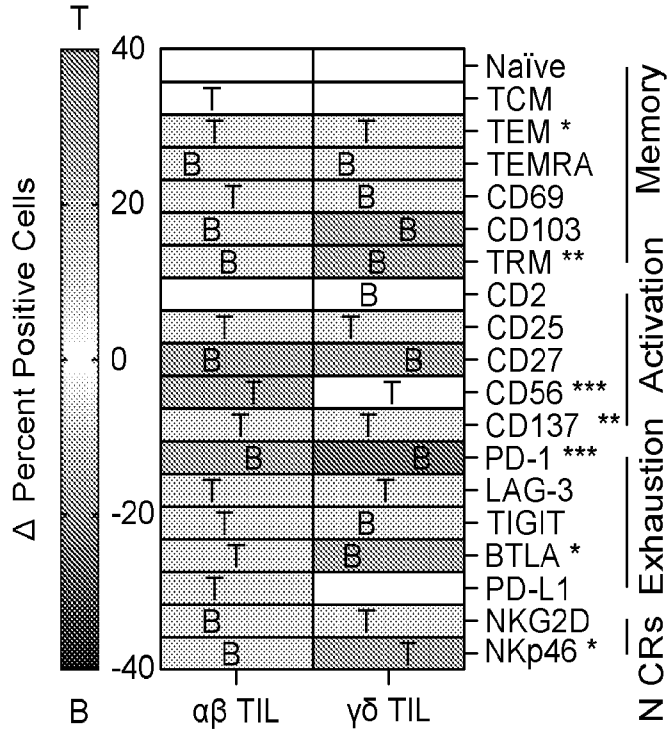

FIG. 10C

Condition	n	Mean Fold Expansion	SD
αβ IL-2	14	725.2	153
γδ IL-2, IL-4,IL-15	14	453.8	100.8
γδ IL-2, IL-7,IL-15	6	164.3	35.6
γδ IL-2, IL-15	6	25.6	22.9
γδ IL-2	10	5.5	2.4

FIG. 11A

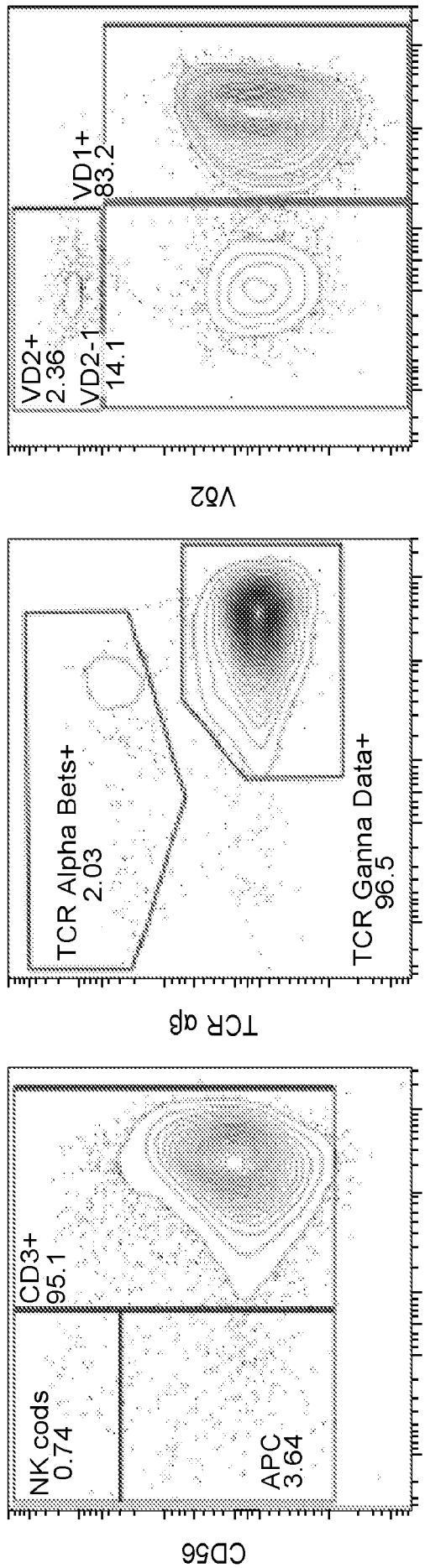
CD56
CD3
NK cods
0.74
APC
3.64
CD3+
95.1
TCR αβ
TCR γδ
TCR Alpha Bets+
2.03
TCR Ganna Data+
96.5
Vδ2
Vδ1
VD2+
2.36
VD2-1
14.1
VD1+
83.2

FIG. 11B

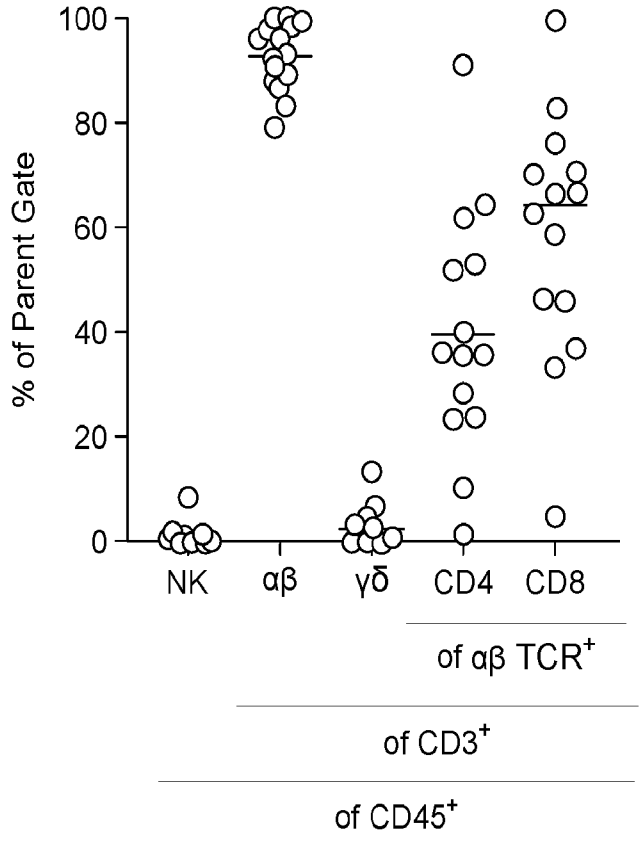

FIG. 11C

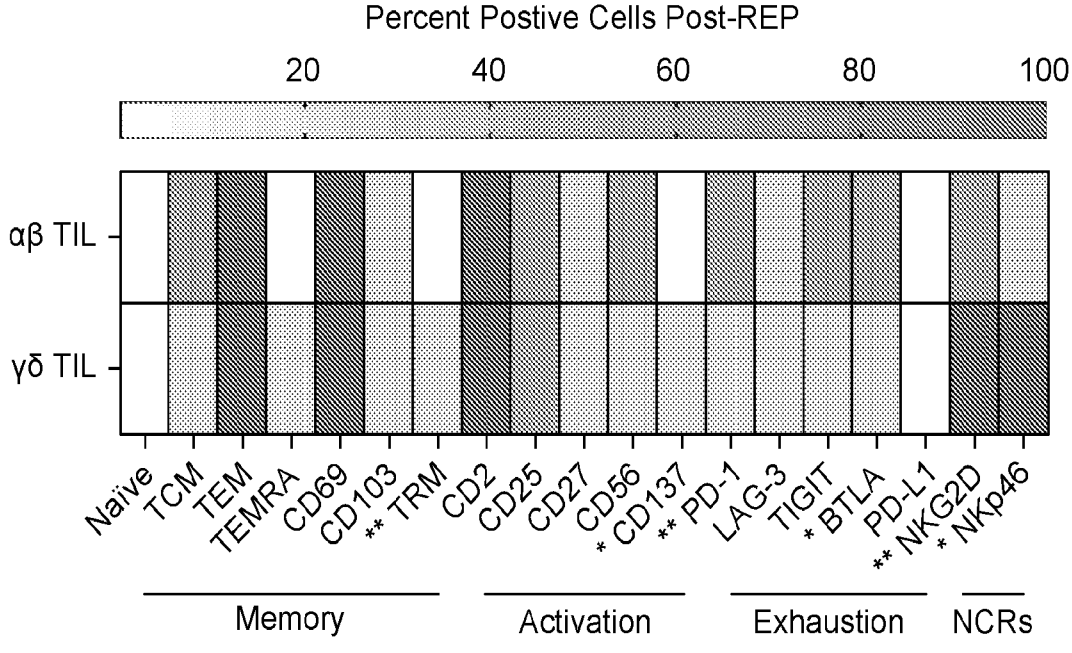

FIG. 11D

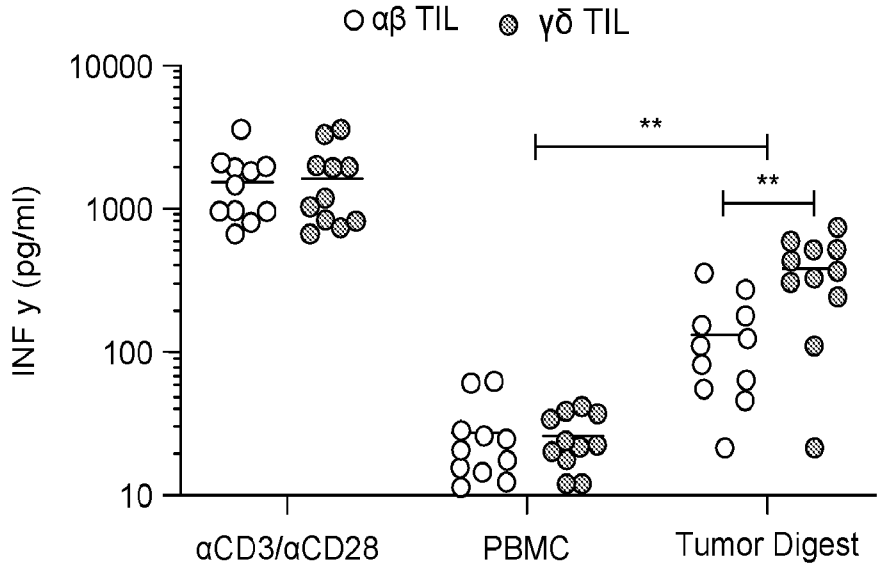

FIG. 12A

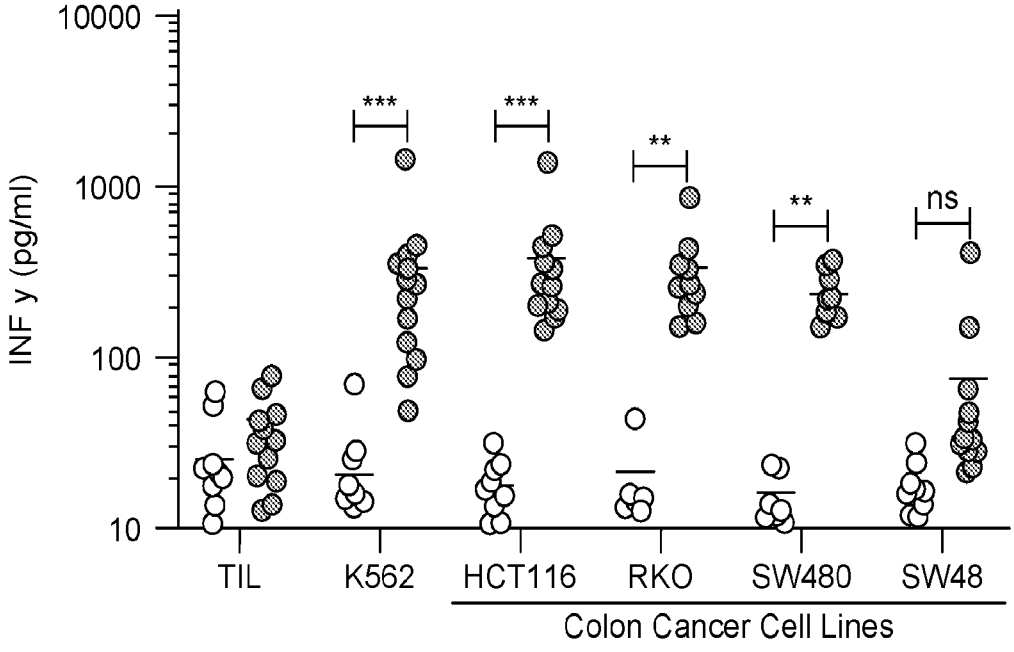

FIG. 12B

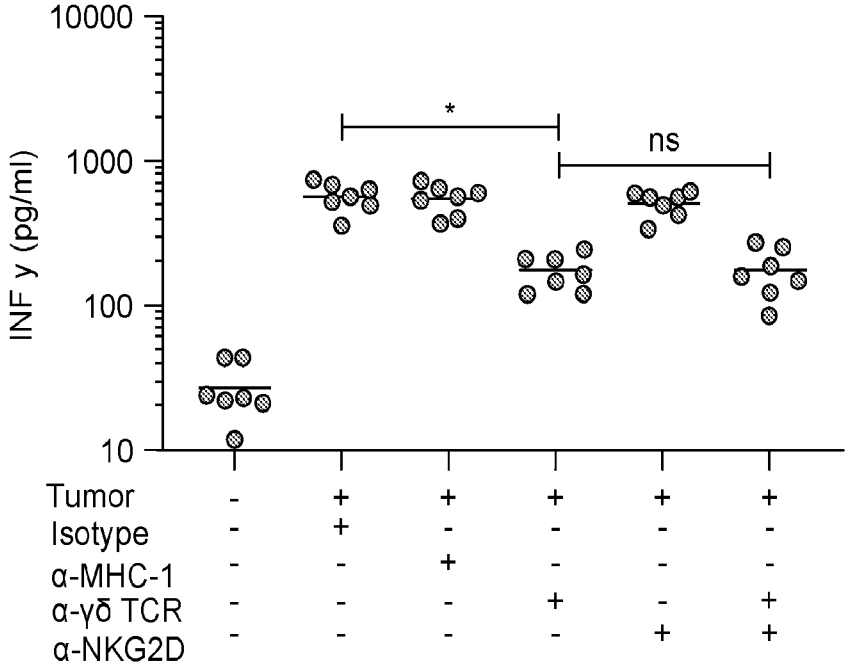

FIG. 12C

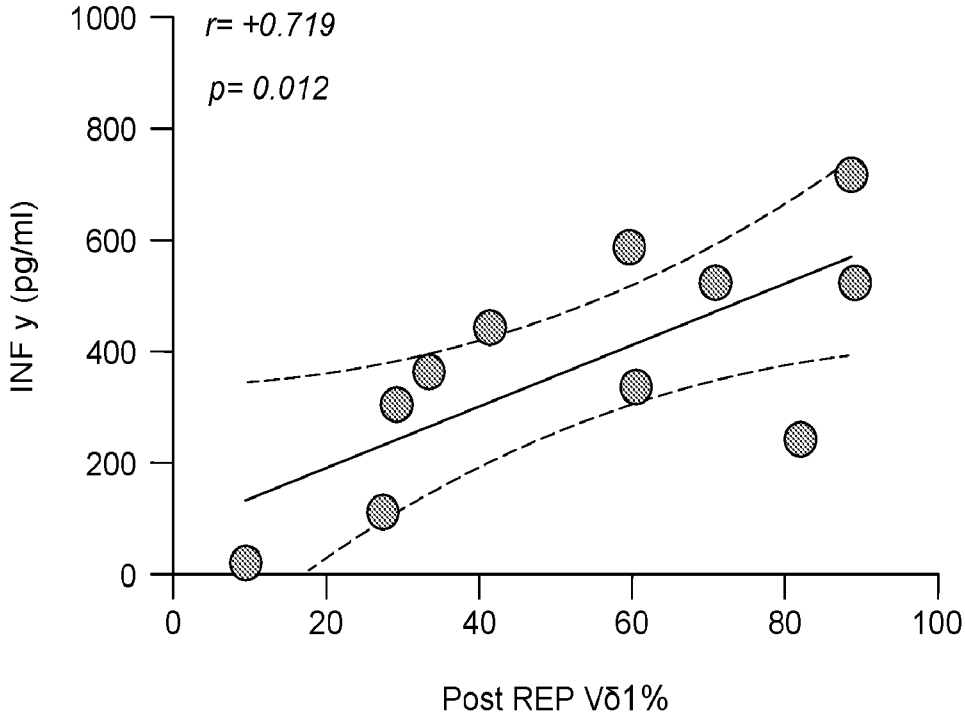

FIG. 12D

Cell Line	Natural Killer Receptor Ligand mRNA Z-Score				
	MICA	MICB	ULBP1	ULBP2	ULBP3
K562	-0.42	0.86	3.33	-0.32	-0.81
HCT116	0.13	1.08	0.12	-0.04	1.67
RKO	0.50	0.09	-0.56	0.27	1.48
SW480	0.01	0.25	-0.20	0.14	0.43
SW48	-0.35	-1.01	3.14	-0.43	0.52
Legend	-4	-2	0	2	4

FIG. 13

Melanoma Tumor Digest Cohort	N=15
Age at Surgery (years)	58.9± 15.8
Female Gender	4 (26.6%)
Tumor Location	Axillary Lymph Node (53.3%) Soft Tissue (20%) Adrenal (13.3%)) Other (13.3%)
Tumor Genetics	BRAF WT (30%) BRAF V600E (20%) Other (13.3%) NA (33.3%)
Stage	I (20%) II (20%) III (26.6%) IV (30%)
Prior Therapies	0 (20%) 1-2 (40%) > 2 (40%)
LDH (U/L)	229.7±73.4
NLR	4.04±2.7
PFS (months)	31.1±34.5
OS (months)	66.1±42.1

FIG. 14

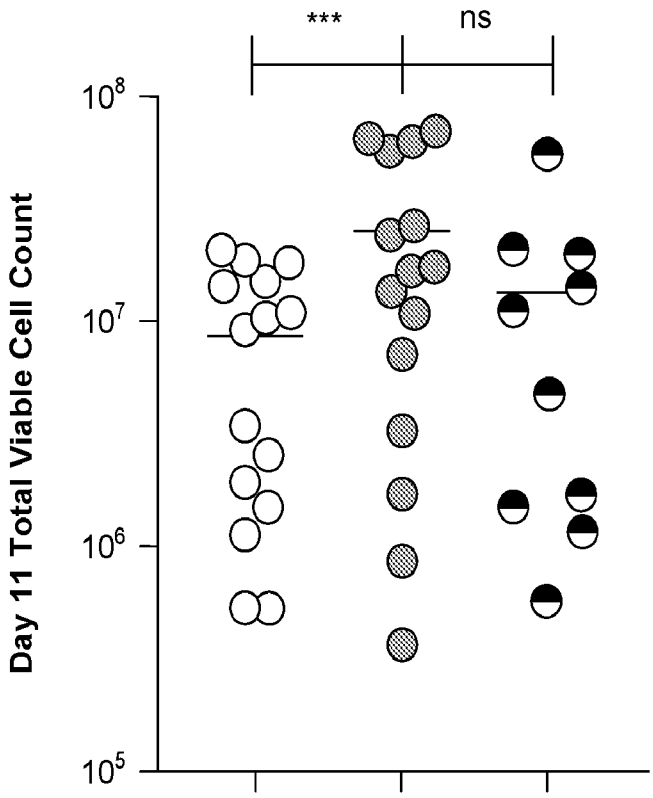

FIG. 15A

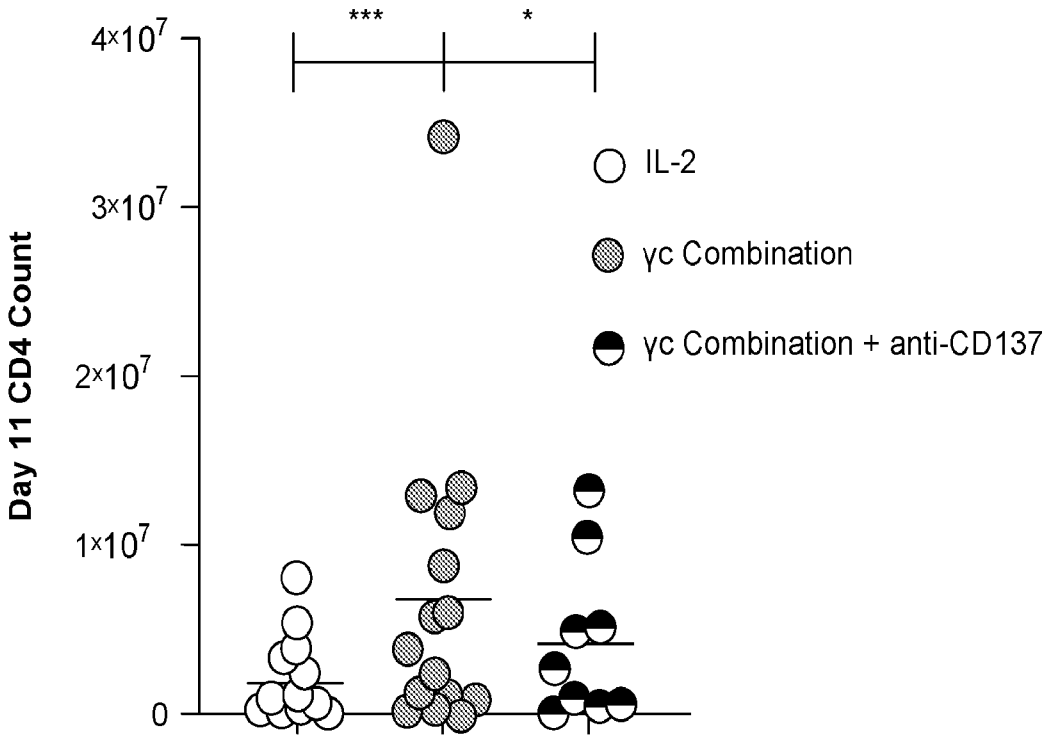

FIG. 15B

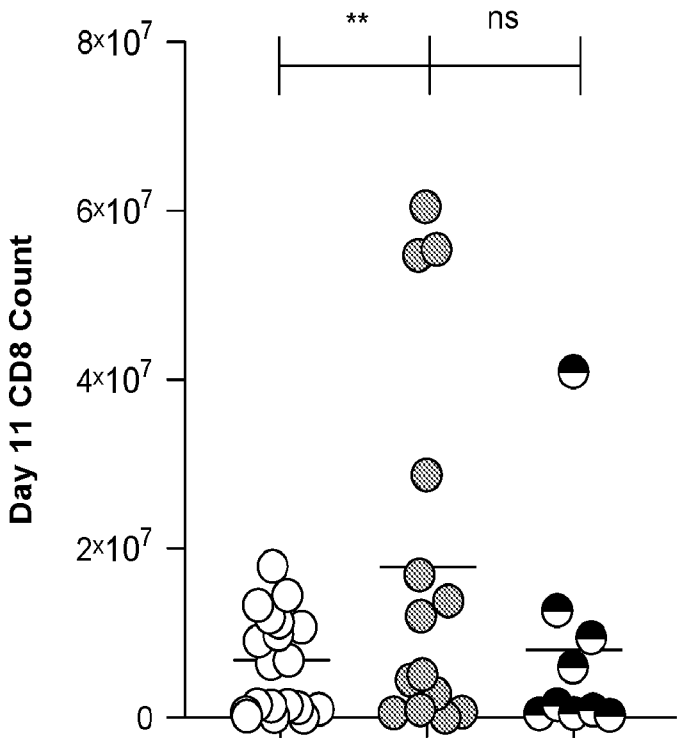

FIG. 15C

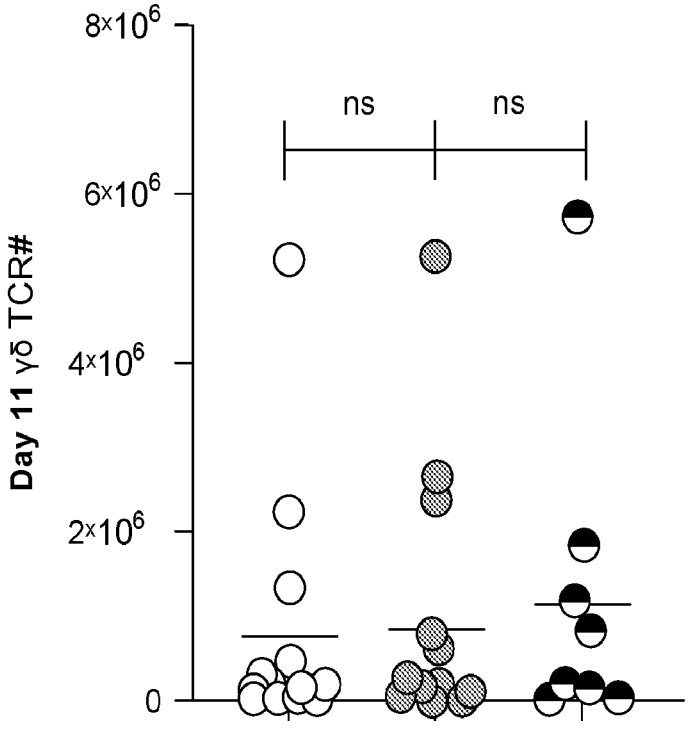

FIG. 15D

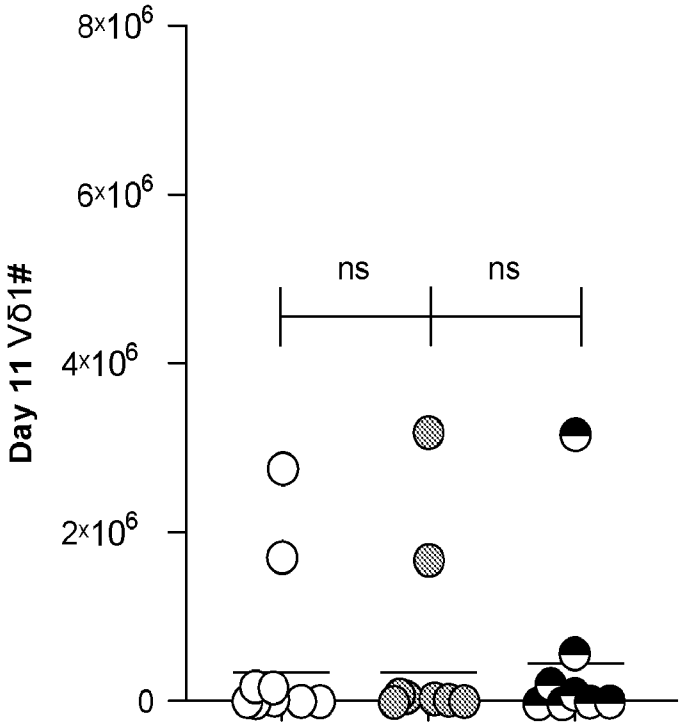

FIG. 15E

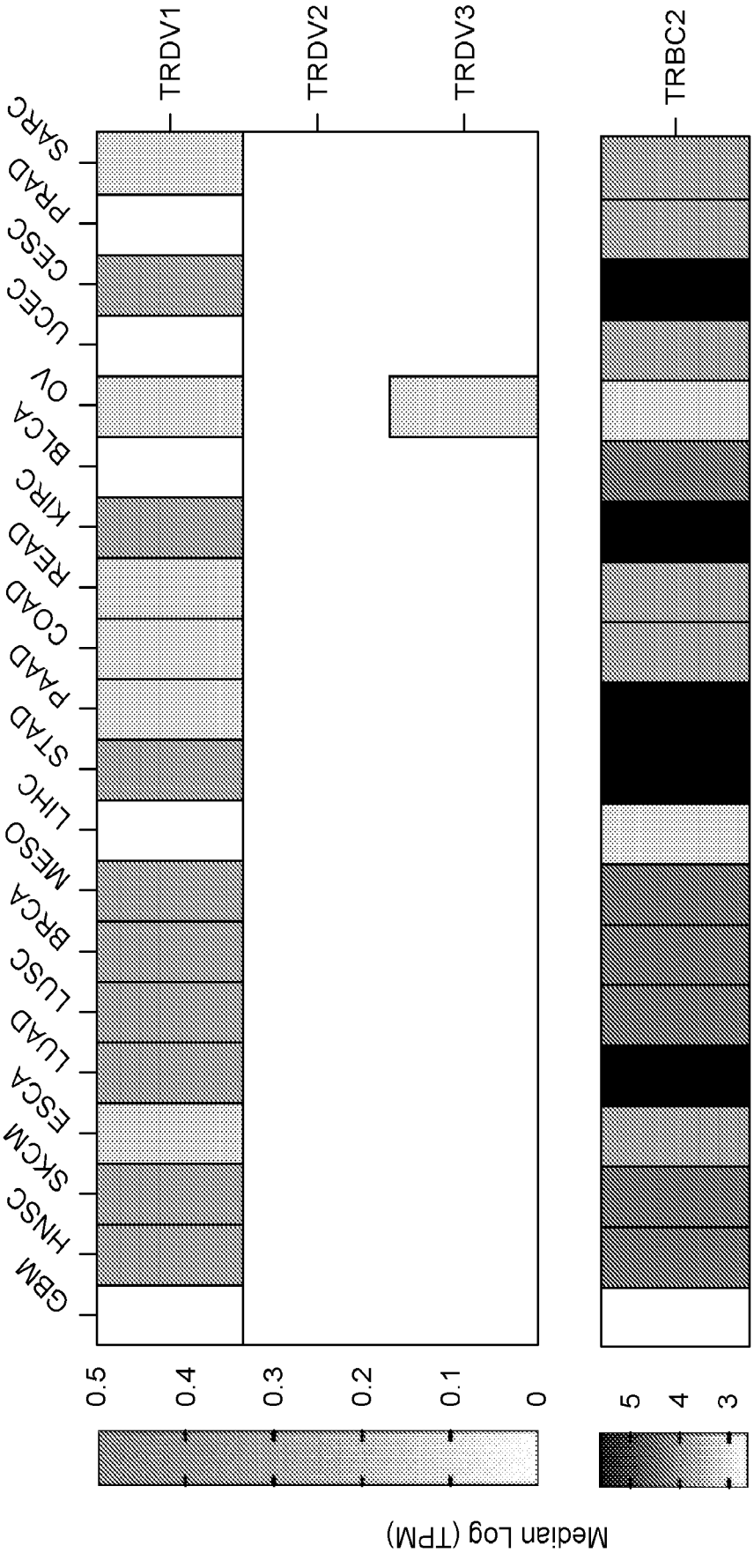
TRDV1
TRDV2
TRDV3
TRBC2
GBM
HNSC
SKCM
ESCA
LUAD
LUSC
BRCA
MESO
LIHC
STAD
PAAD
COAD
READ
KIRC
BLCA
OV
UCEC
CESC
PRAD
SARC
0.5
0.4
0.3
0.2
0.1
0
5
4
3
Median Log (TPM)

FIG. 16A

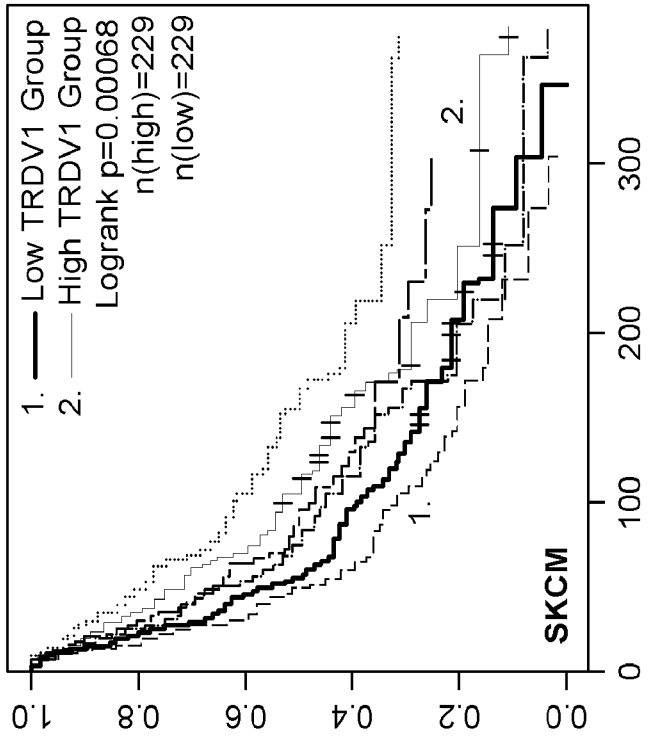

FIG. 16C

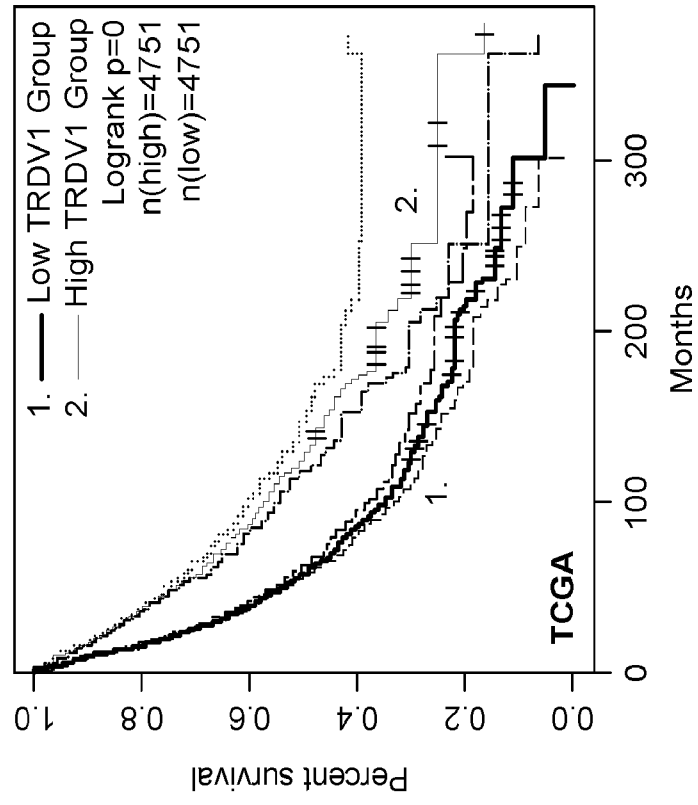

FIG. 16B

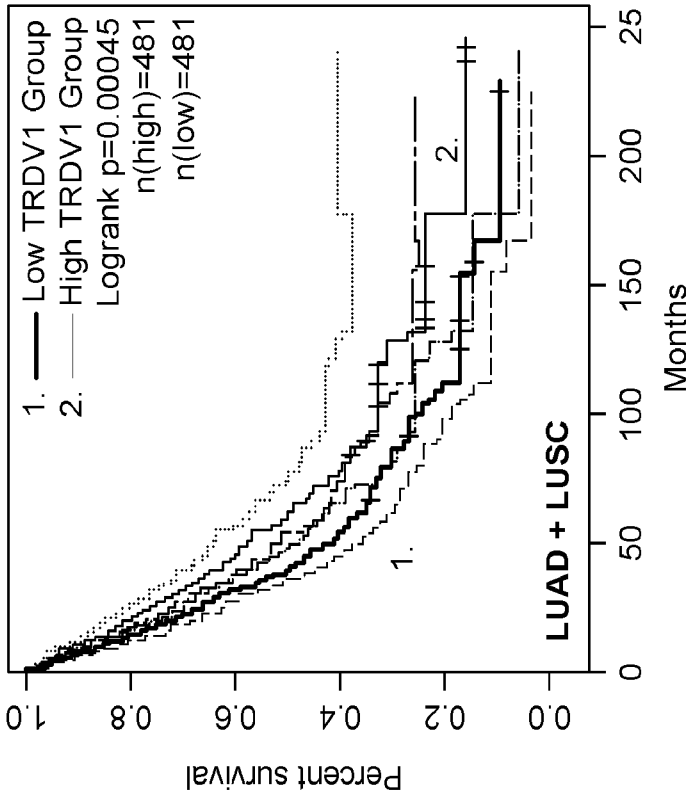

FIG. 16E

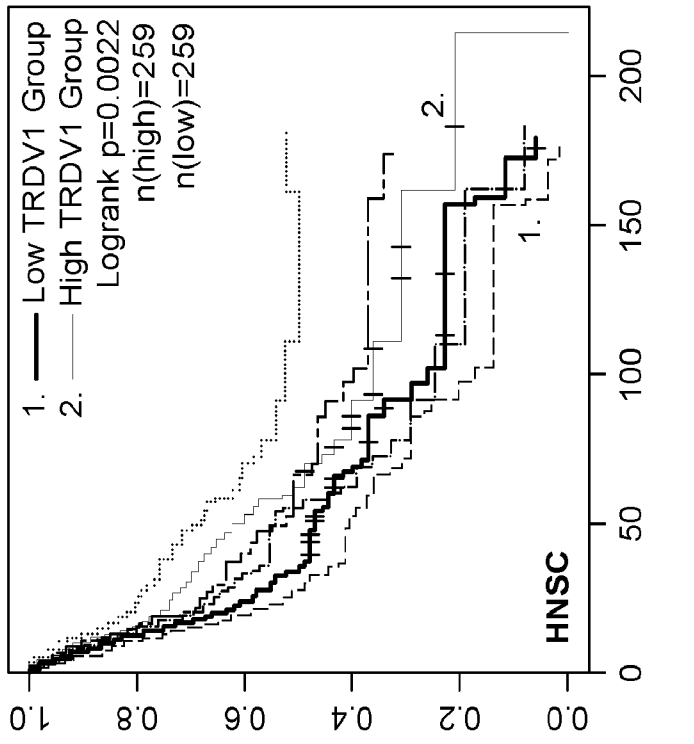

FIG. 16D

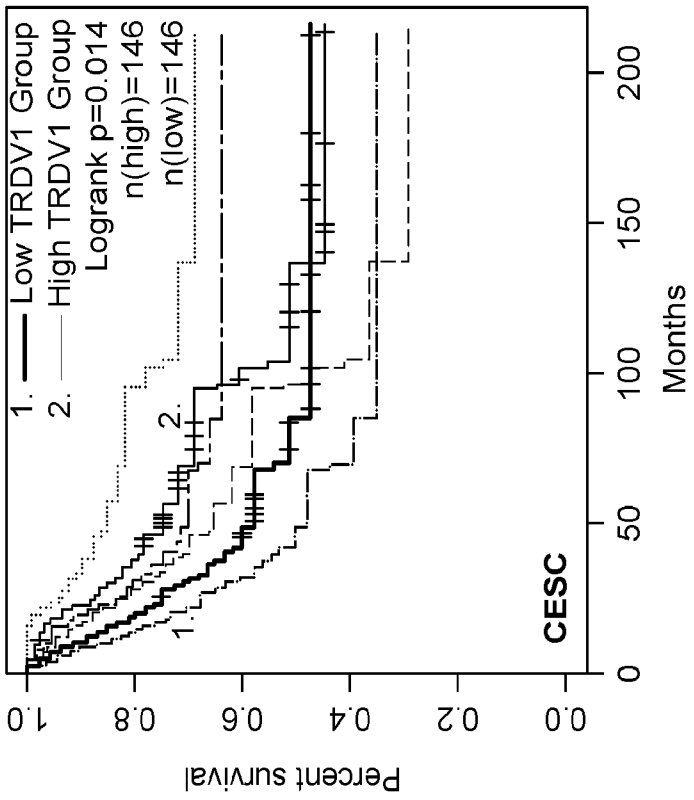

FIG. 16G

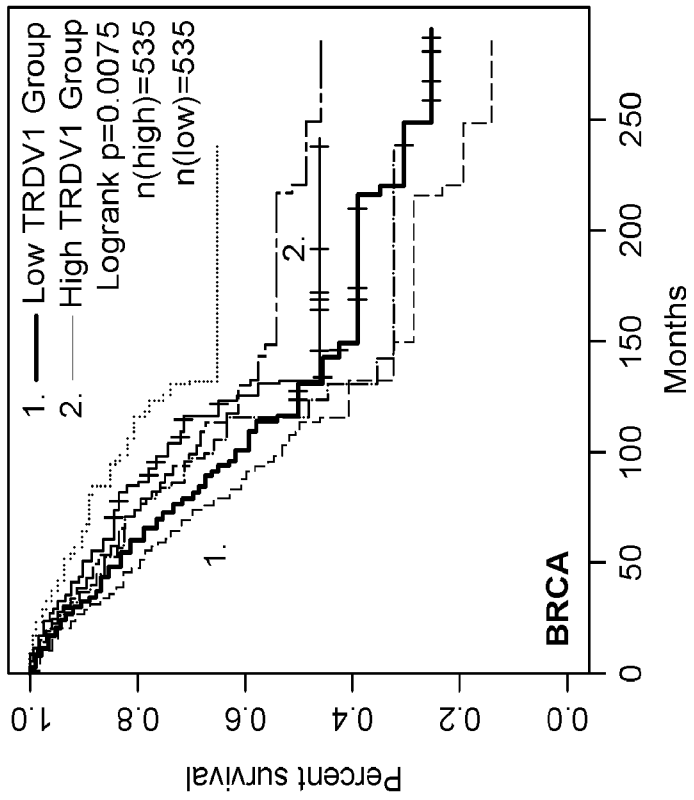

FIG. 16F

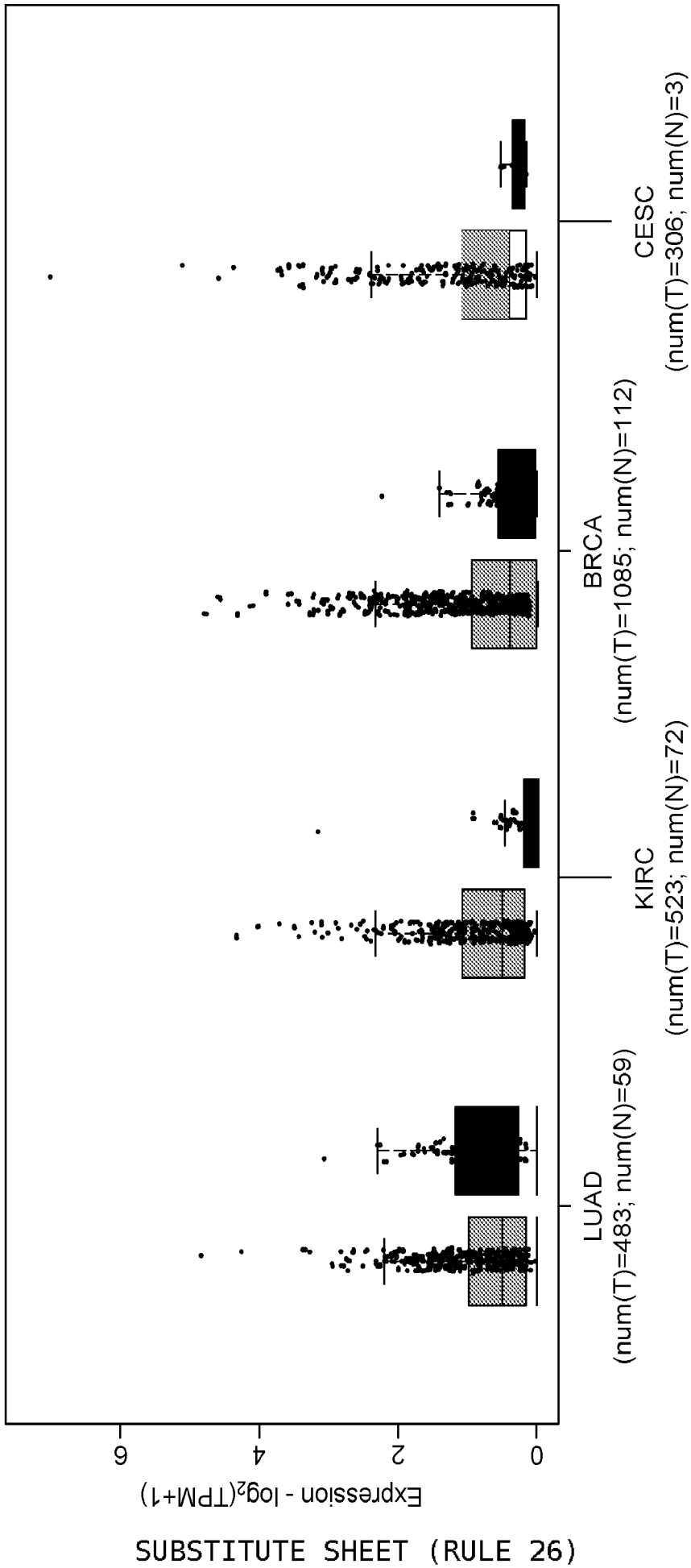
Expression - log2(TPM+1)
0
2
4
6
LUAD
(num(T)=483; num(N)=59)
KIRC
(num(T)=523; num(N)=72)
BRCA
(num(T)=1085; num(N)=112)
CESC
(num(T)=306; num(N)=3)

FIG. 17A

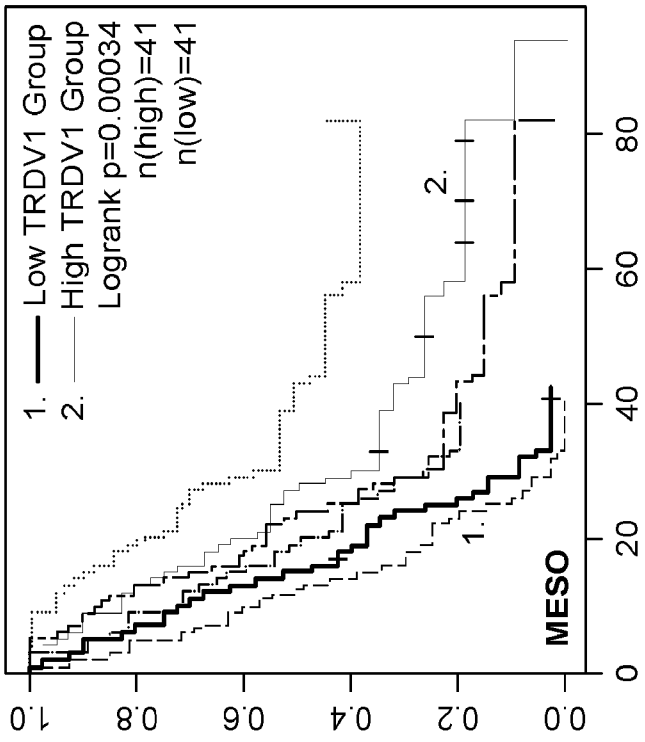

FIG. 17C

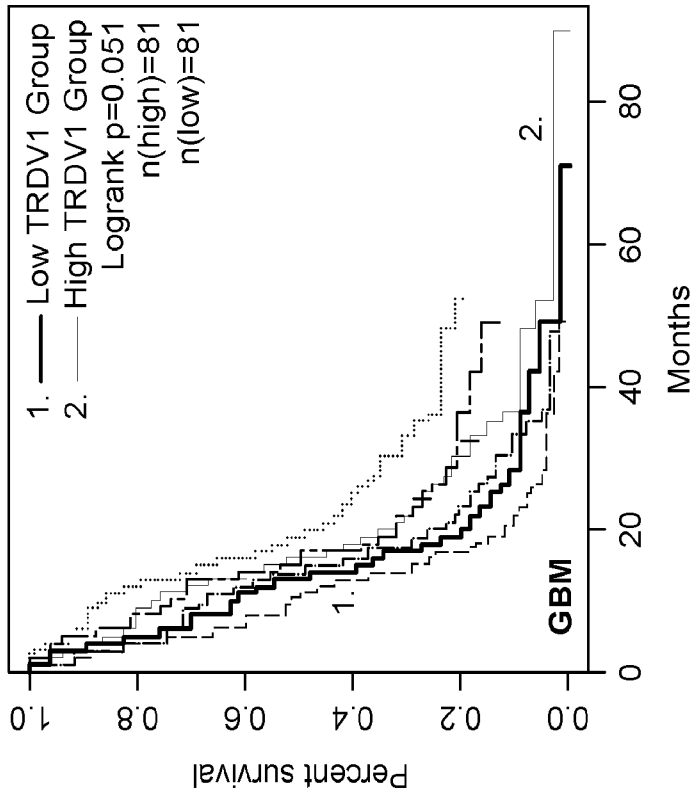

FIG. 17B

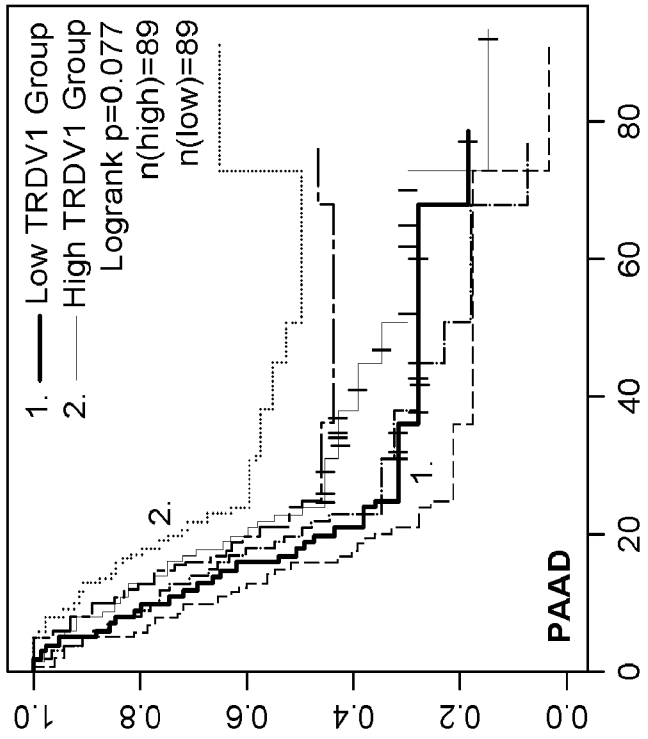

FIG. 17E

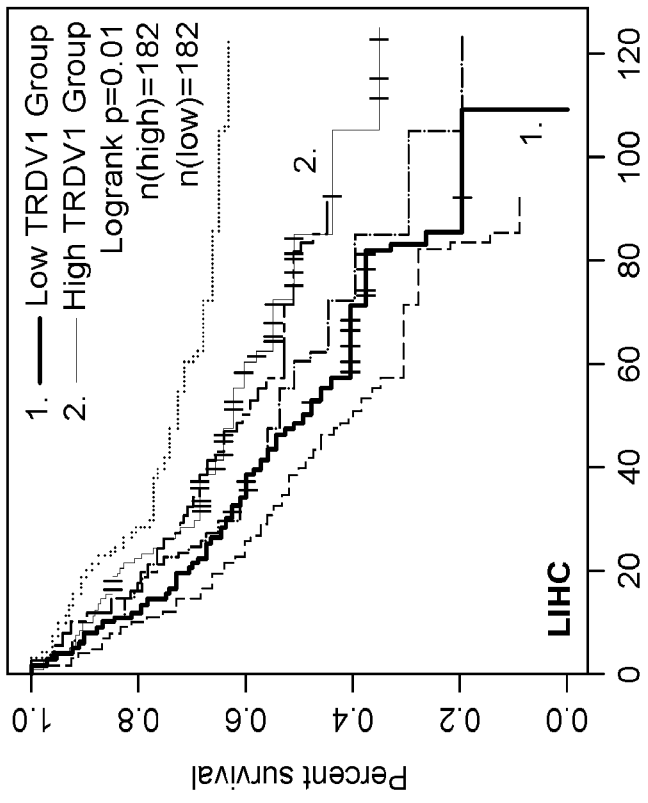

FIG. 17D

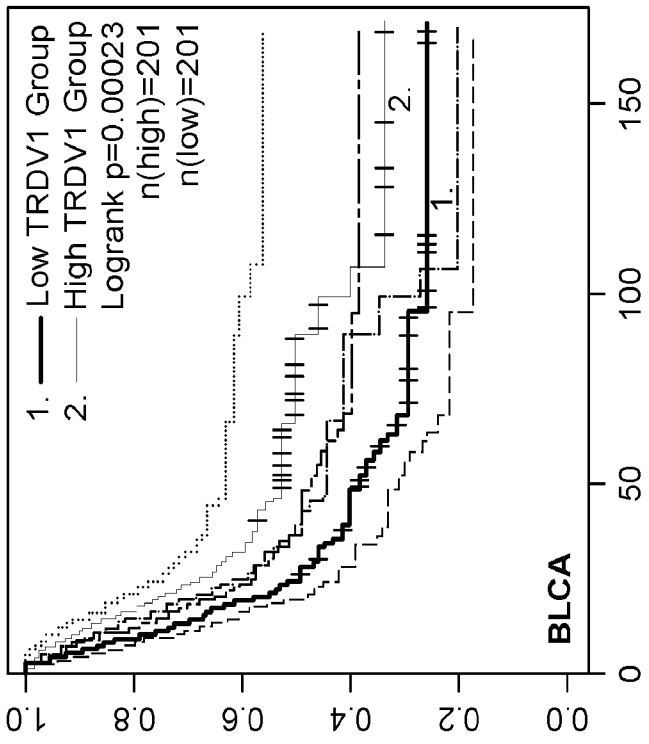

FIG. 17G

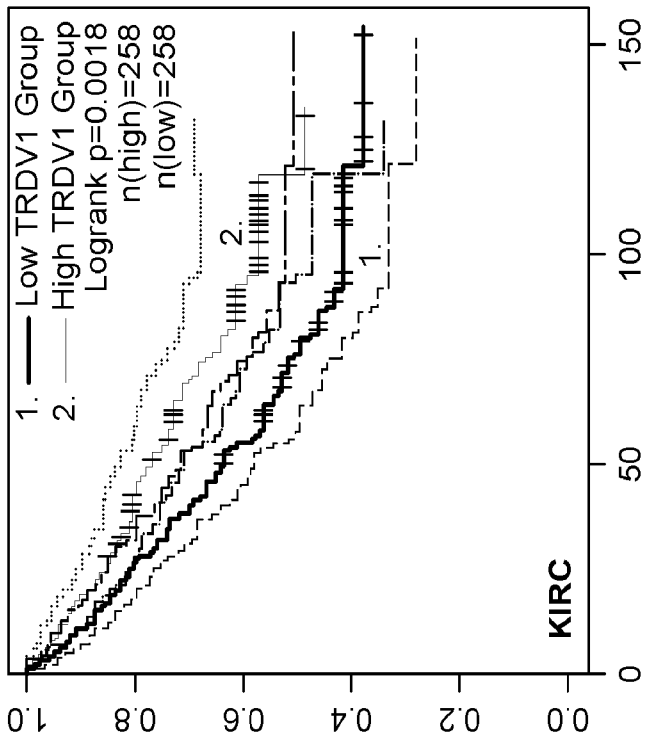

FIG. 17F

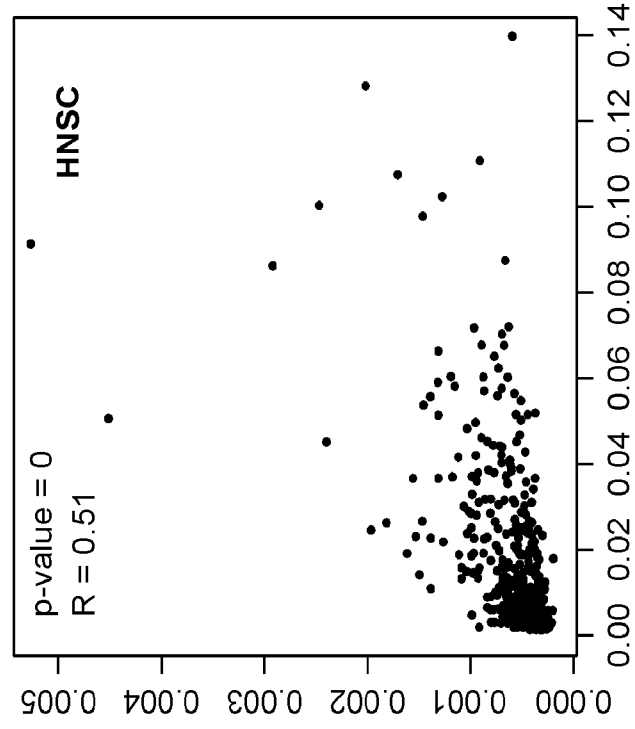

FIG. 18B

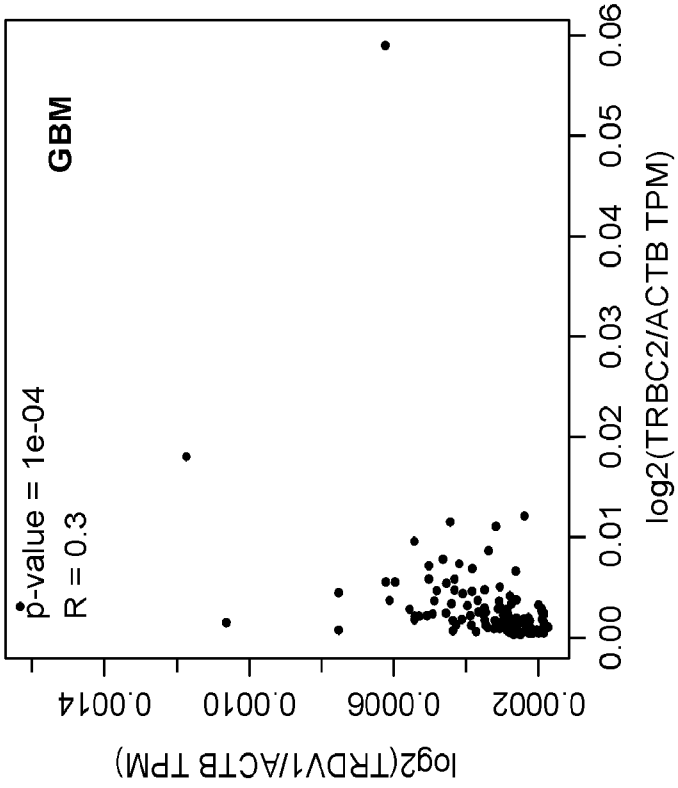

FIG. 18A

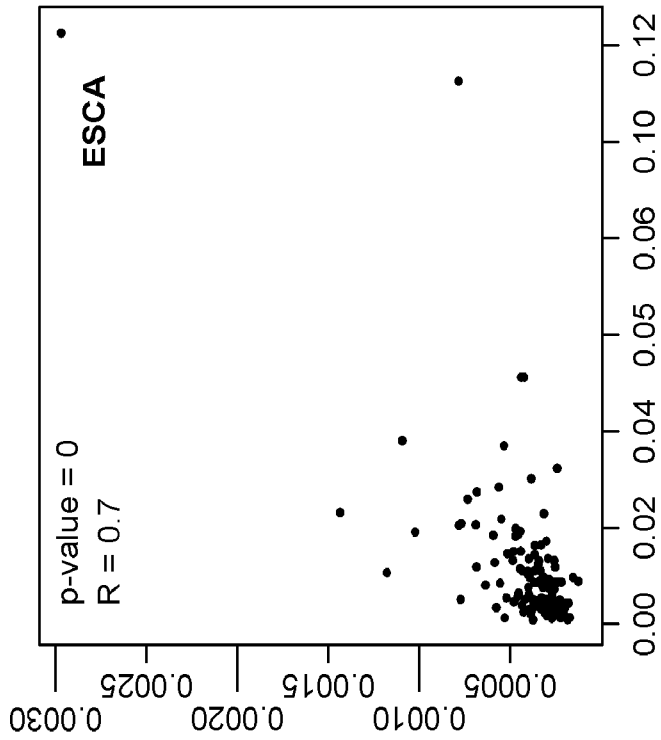

FIG. 18D

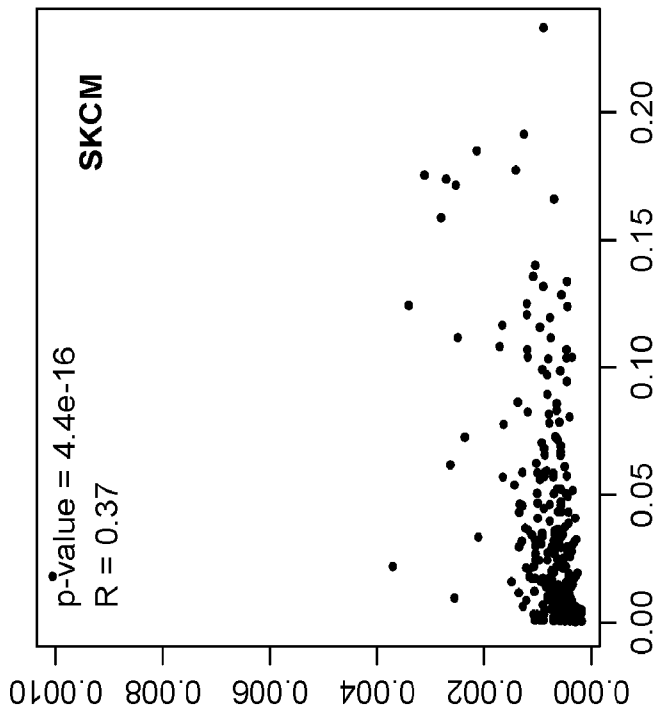

FIG. 18C

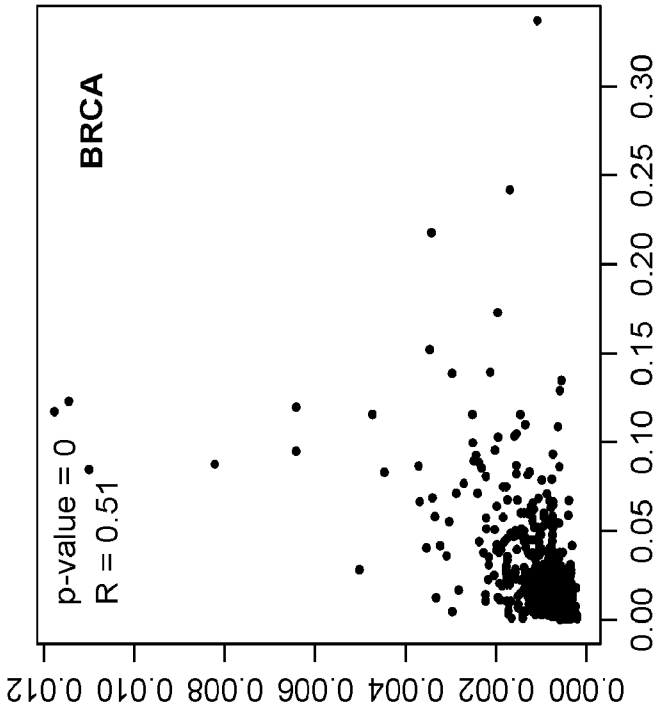

FIG. 18F

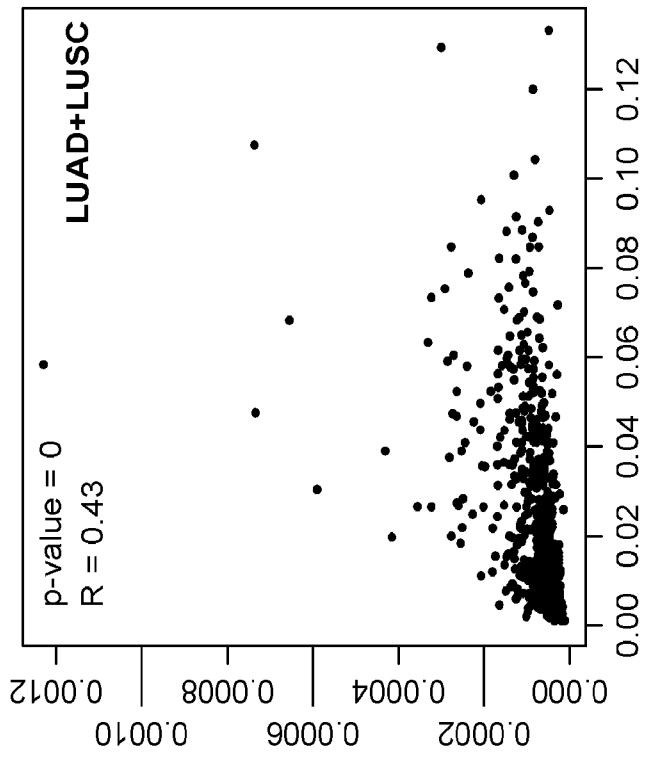

FIG. 18E

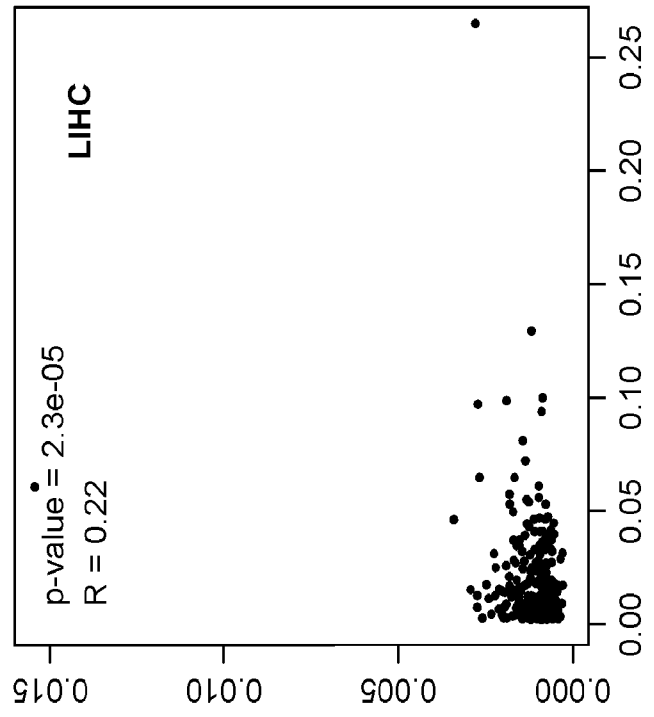

FIG. 18H

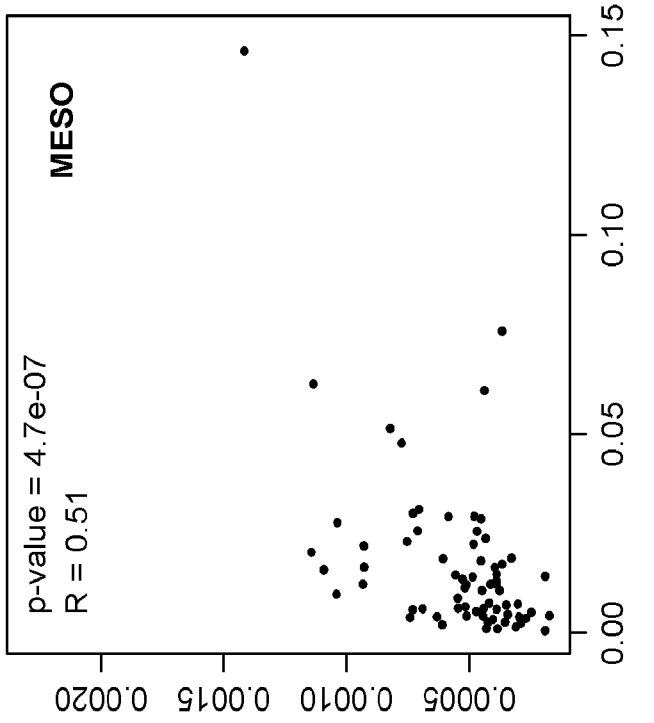

FIG. 18G

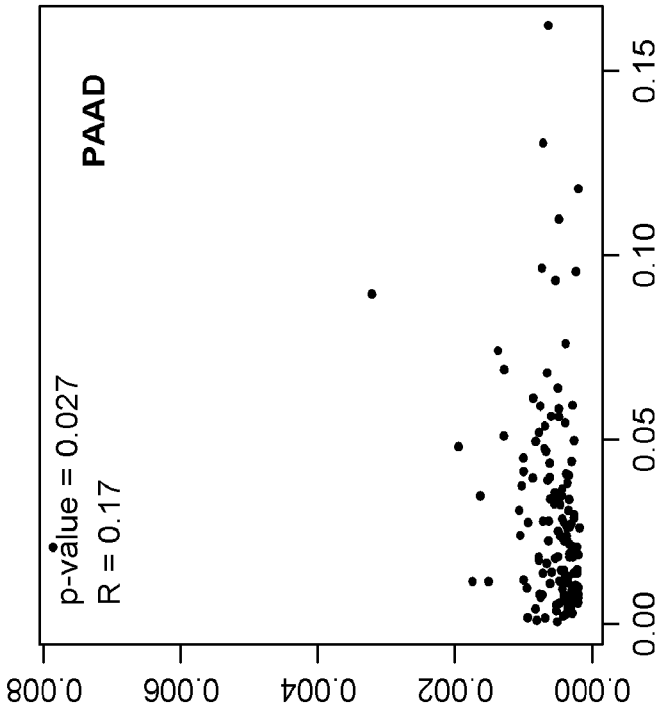

FIG. 18J

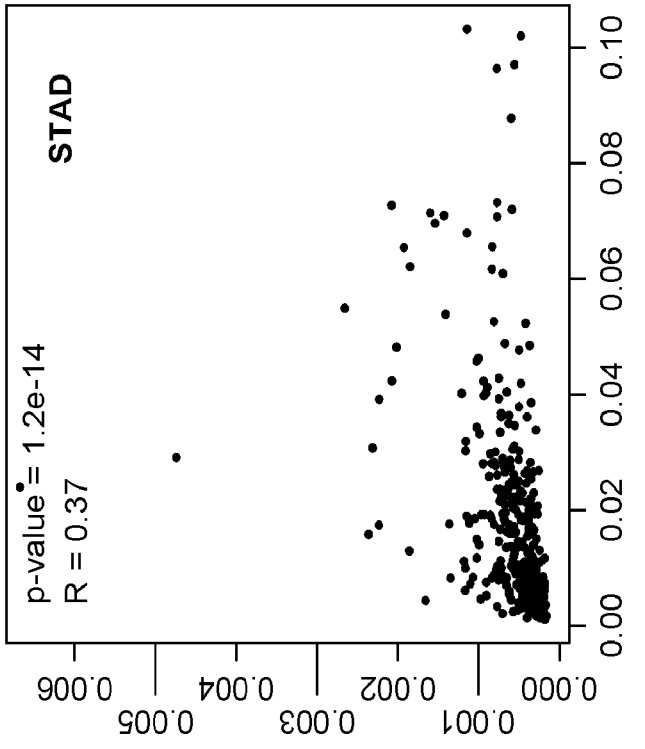

FIG. 18I

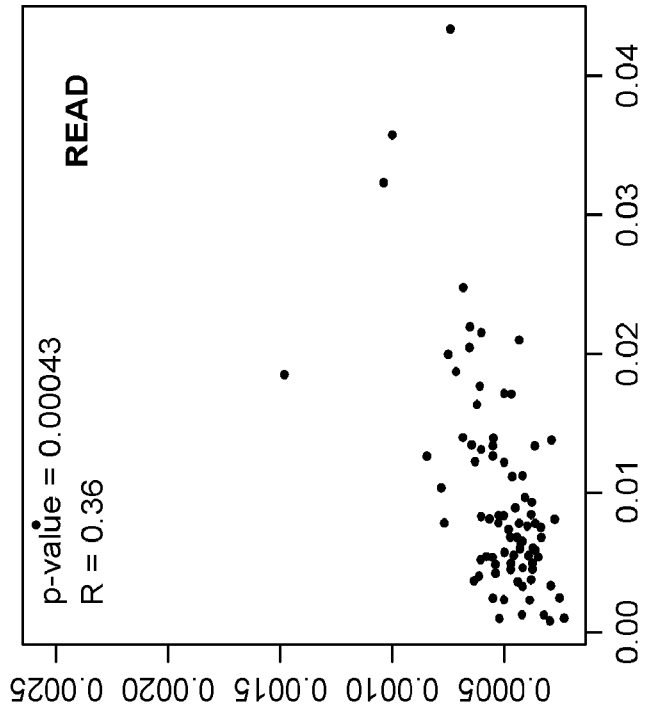

FIG. 18L

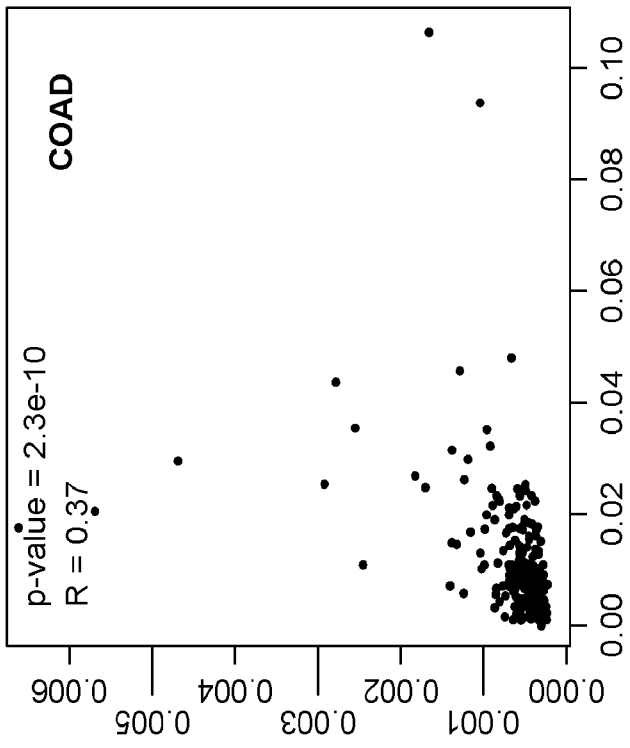

FIG. 18K

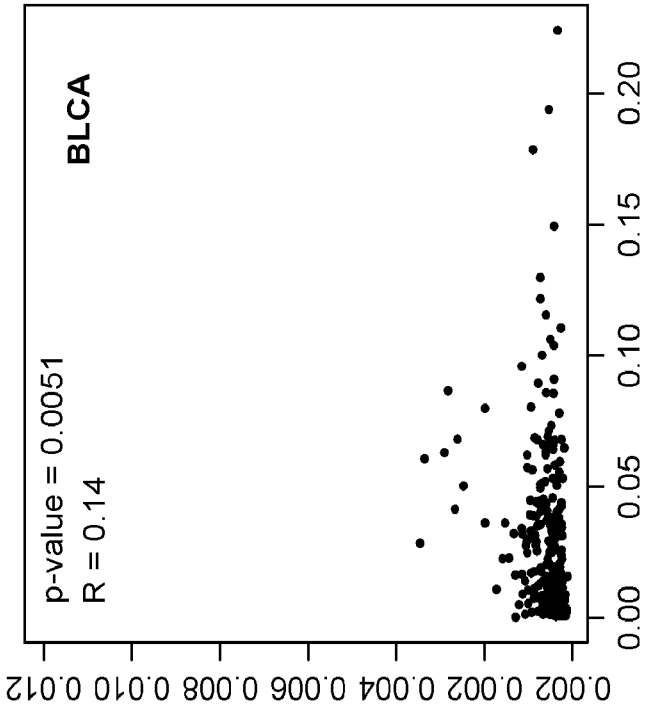

FIG. 18N

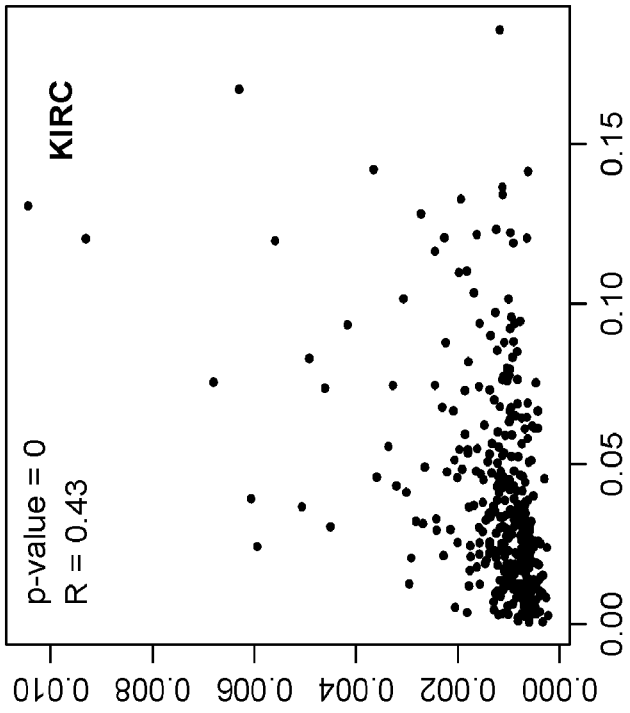

FIG. 18M

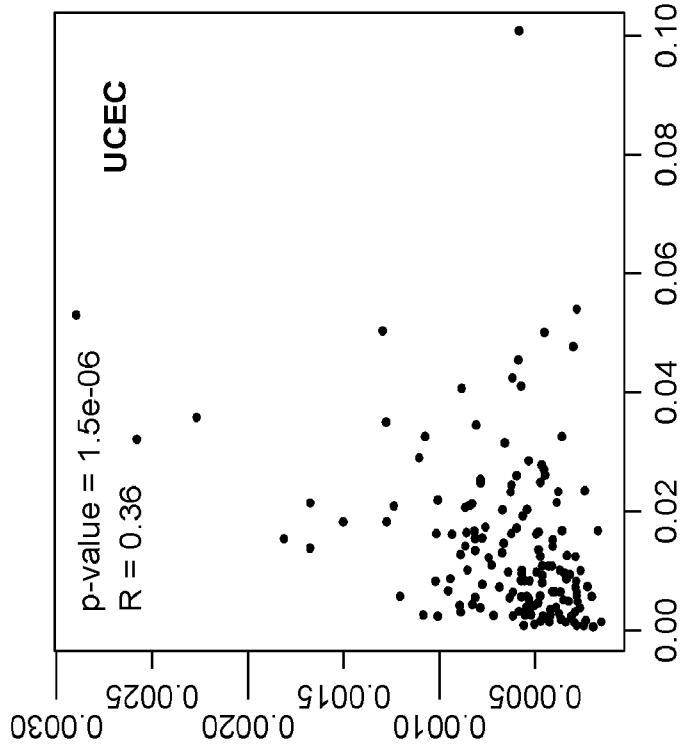

FIG. 18P

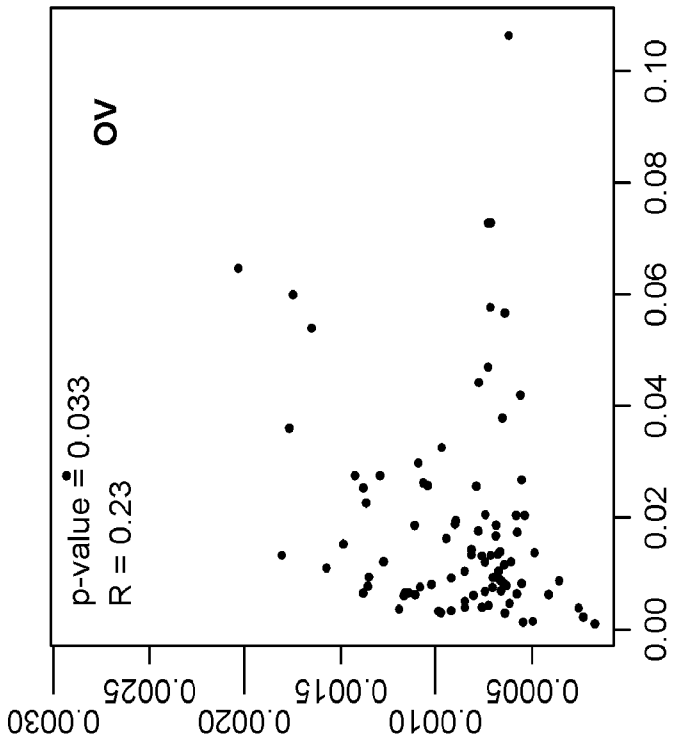

FIG. 18O

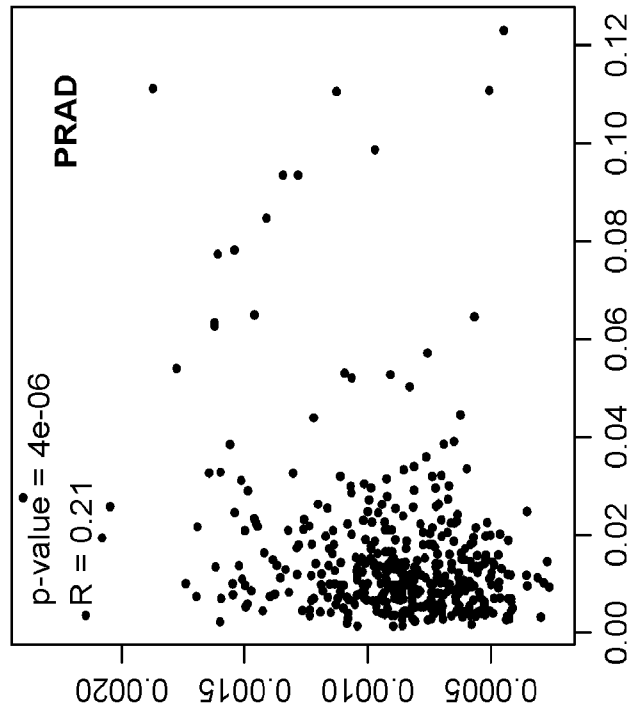

FIG. 18R

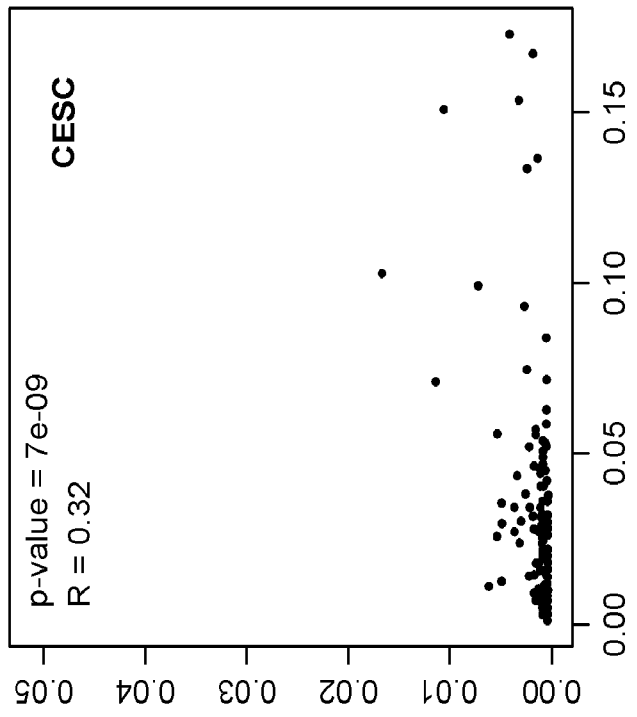

FIG. 18Q

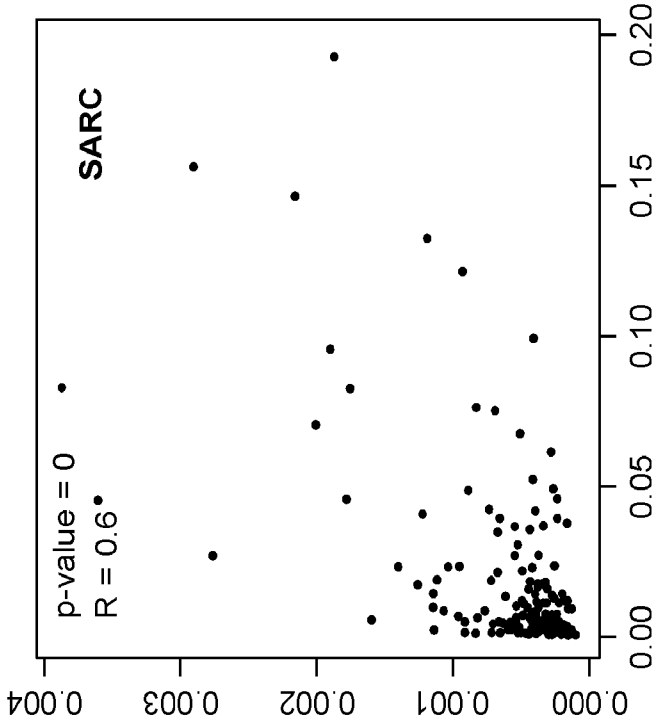

FIG. 18S

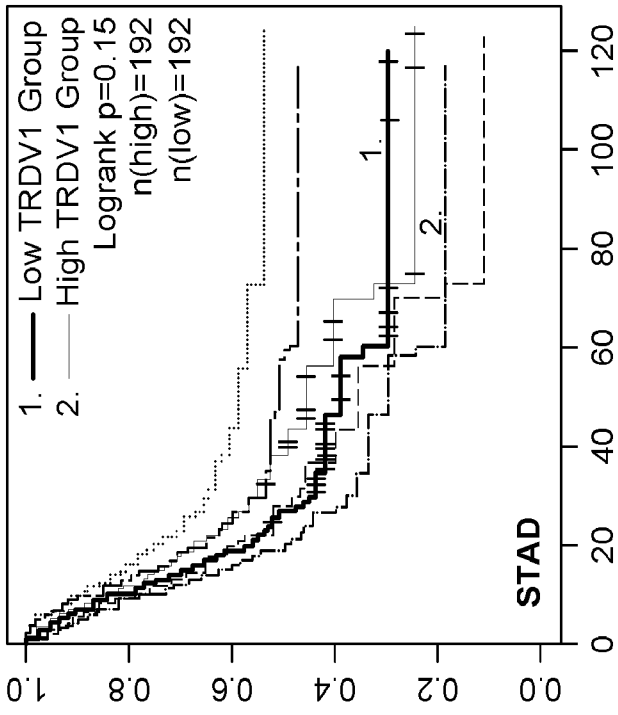

FIG. 19B

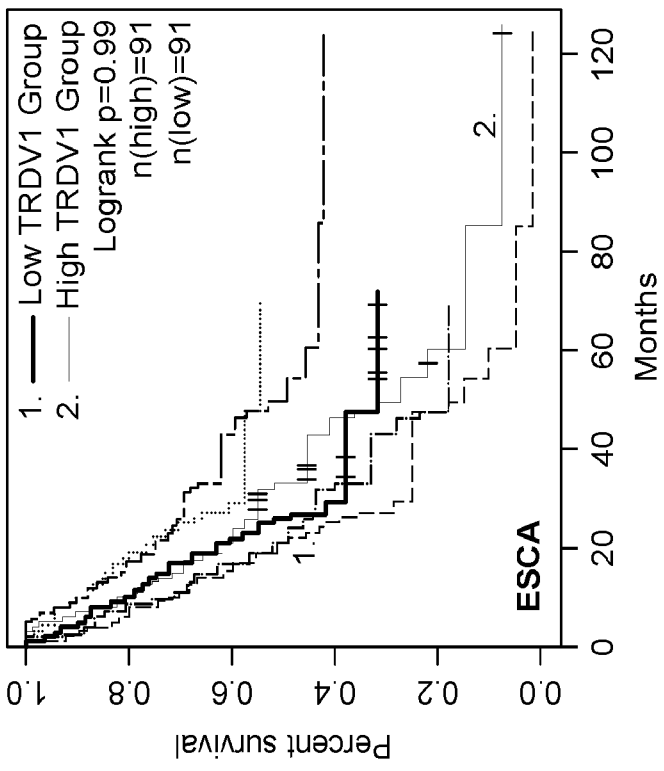

FIG. 19A

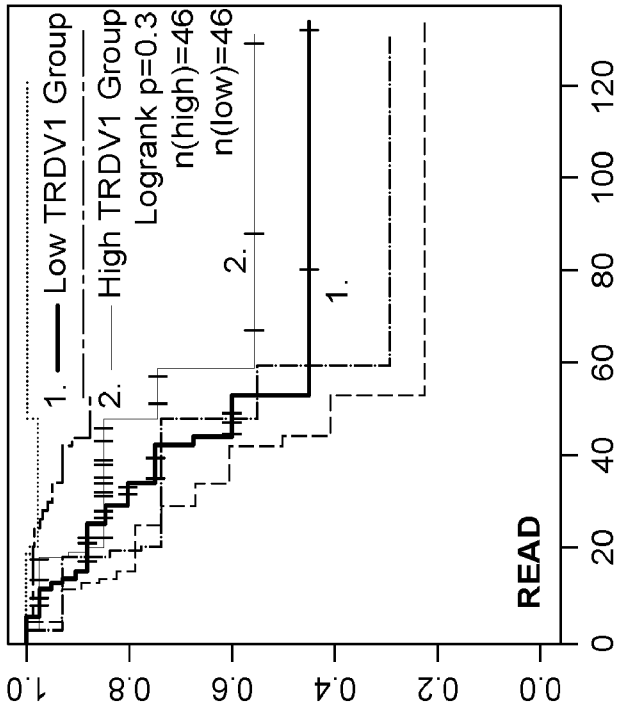

FIG. 19D

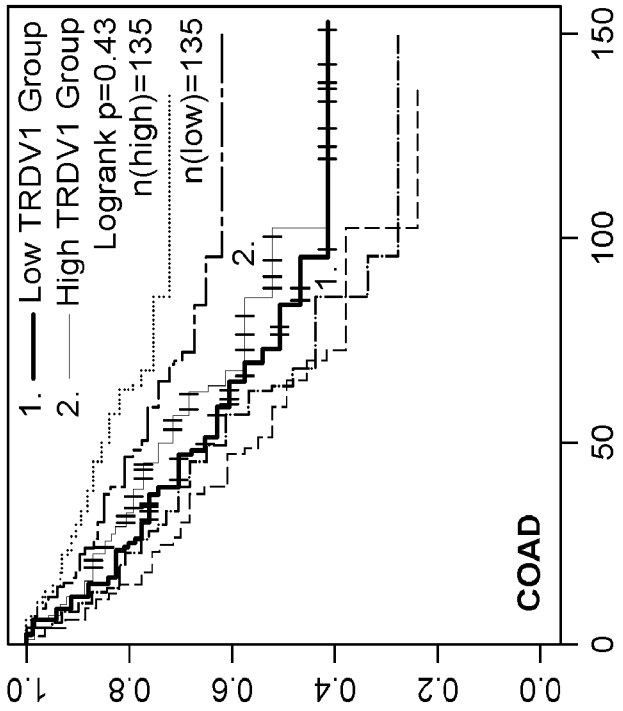

FIG. 19C

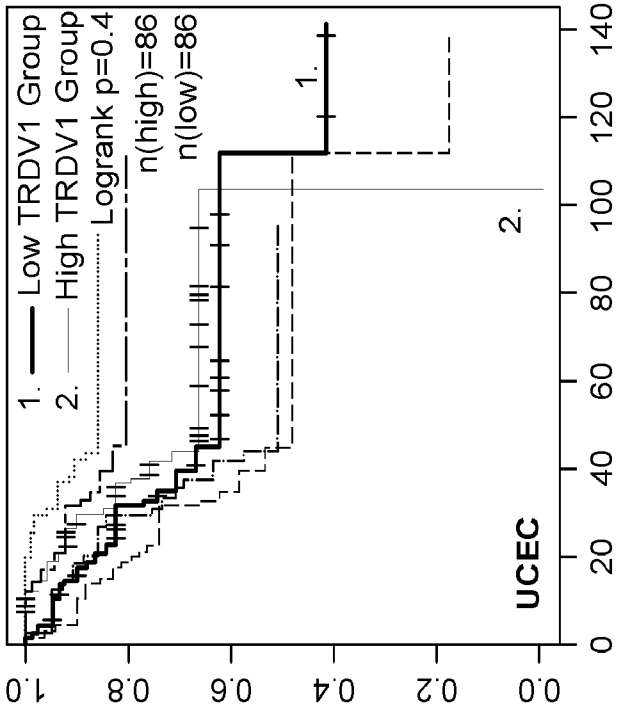

FIG. 19F

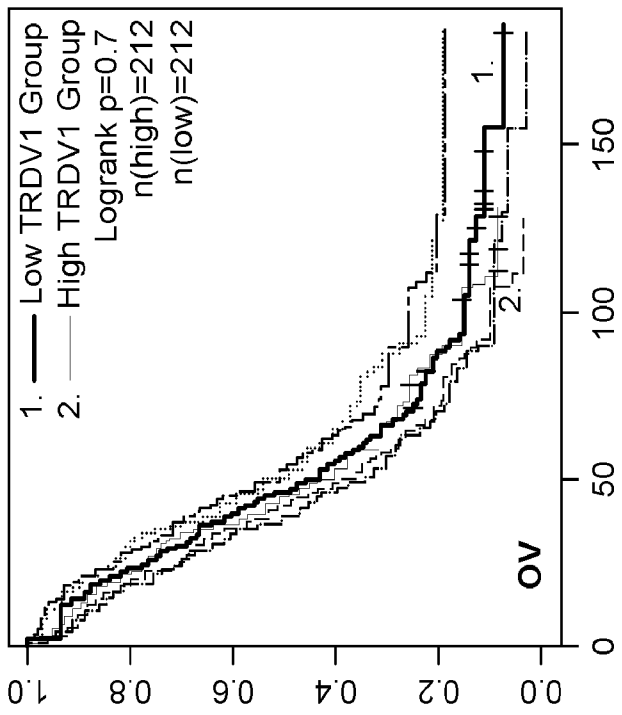

FIG. 19E

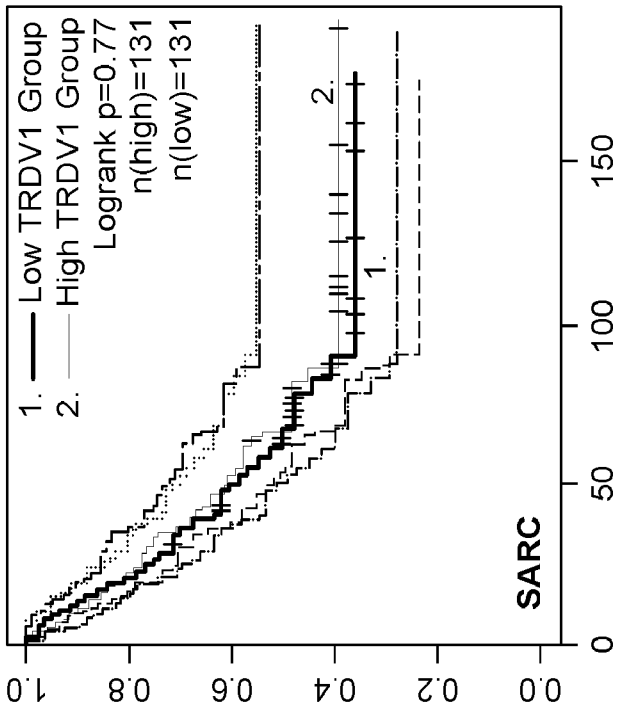

FIG. 19H

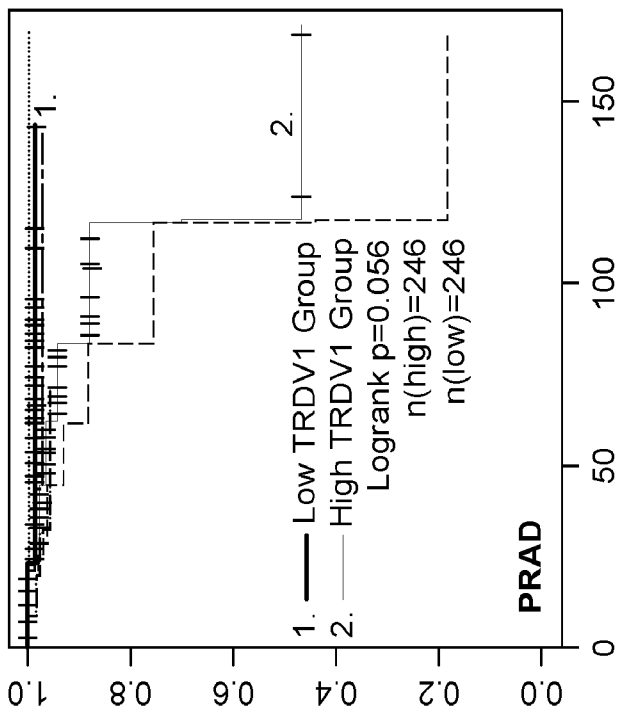

FIG. 19G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/046745

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - A61K 35/17; A61P 31/00; A61P 35/00; A61P 37/06; C12N 5/0783 (2022.01)
ADD.

CPC - INV. - A61K 35/17; C12N 5/0636; A61P 31/00; A61P 35/00; A61P 37/06 (2022.08)

ADD. - C12N 2501/515; C12N 2501/2302; C12N 2501/2304; C12N 2501/2315 (2022.08)

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)
See Search History document

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

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/170260 A1 (GAMIDA-CELL LTD.) 27 August 2020 (27.08.2020) entire document	1-3
X	WO 2015/061694 A2 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 30 April 2015 (30.04.2015) entire document	38-40
A	WO 2016/081518 A2 (ADICET BIO INC.) 26 May 2016 (26.05.2016) entire document	1-3, 38-40
A	WO 2016/166544 A1 (TC BIOPHARM LTD) 20 October 2016 (20.10.2016) entire document	1-3, 38-40
A	WO 2021/032961 A1 (GAMMADELTA THERAPEUTICS LIMITED) 25 February 2021 (25.02.2021) entire document	1-3, 38-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 2022	JAN 24 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Taina Matos Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/046745

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-37, 41-81
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.