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(54) Benævnelse: **ANTI-PVRIG-ANTISTOFFER OG ANVENDELSESFREMGANGSMÅDER**

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DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] Naive T cells must receive two independent signals from antigen-presenting cells (APC) in order to become productively activated. The first, Signal 1, is antigen-specific and occurs when T cell antigen receptors encounter the appropriate antigen-MHC complex on the APC. The fate of the immune response is determined by a second, antigen-independent signal (Signal 2) which is delivered through a T cell costimulatory molecule that engages its APC-expressed ligand. This second signal could be either stimulatory (positive costimulation) or inhibitory (negative costimulation or coinhibition). In the absence of a costimulatory signal, or in the presence of a coinhibitory signal, T-cell activation is impaired or aborted, which may lead to a state of antigen-specific unresponsiveness (known as T-cell anergy), or may result in T-cell apoptotic death.

[0002] Costimulatory molecule pairs usually consist of ligands expressed on APCs and their cognate receptors expressed on T cells. The prototype ligand/receptor pairs of costimulatory molecules are B7/CD28 and CD40/CD40L. The B7 family consists of structurally related, cell-surface protein ligands, which may provide stimulatory or inhibitory input to an immune response. Members of the B7 family are structurally related, with the extracellular domain containing at least one variable or constant immunoglobulin domain.

[0003] Both positive and negative costimulatory signals play critical roles in the regulation of cell-mediated immune responses, and molecules that mediate these signals have proven to be effective targets for immunomodulation. Based on this knowledge, several therapeutic approaches that involve targeting of costimulatory molecules have been developed, and were shown to be useful for prevention and treatment of cancer by turning on, or preventing the turning off, of immune responses in cancer patients and for prevention and treatment of autoimmune diseases and inflammatory diseases, as well as rejection of allogenic transplantation, each by turning off uncontrolled immune responses, or by induction of "off signal" by negative costimulation (or coinhibition) in subjects with these pathological conditions.

[0004] Manipulation of the signals delivered by B7 ligands has shown potential in the treatment of autoimmunity, inflammatory diseases, and transplant rejection. Therapeutic strategies include blocking of costimulation using monoclonal antibodies to the ligand or to the receptor of a costimulatory pair, or using soluble fusion proteins composed of the costimulatory receptor that may bind and block its appropriate ligand. Another approach is induction of co-inhibition using soluble fusion protein of an inhibitory ligand. These approaches rely, at least partially, on the eventual deletion of auto- or allo-reactive T cells (which are responsible for the pathogenic processes in autoimmune diseases or transplantation, respectively), presumably because in the absence of costimulation (which induces cell survival genes) T cells become highly susceptible to induction of apoptosis. Thus, novel agents that are capable of modulating costimulatory signals, without compromising the immune system's ability to defend against pathogens, are highly advantageous for treatment and prevention of such pathological conditions.

[0005] Costimulatory pathways play an important role in tumor development. Interestingly, tumors have been shown to evade immune destruction by impeding T cell activation through inhibition of co-stimulatory factors in the B7-CD28 and TNF families, as well as by attracting regulatory T cells, which inhibit anti-tumor T cell responses (see Wang (2006), "Immune Suppression by Tumor Specific CD4+ Regulatory T cells in Cancer", Semin. Cancer. Biol. 16:73-79; Greenwald, et al. (2005), "The B7 Family Revisited", Ann. Rev. Immunol. 23:515-48; Watts (2005), "TNF/TNFR Family Members in Co-stimulation of T Cell Responses", Ann. Rev. Immunol. 23:23-68; Sadum, et al., (2007) "Immune Signatures of Murine and Human Cancers Reveal Unique Mechanisms of Tumor Escape and New Targets for Cancer Immunotherapy", Clin. Canc. Res. 13(13): 4016-4025). Such tumor expressed co-stimulatory molecules have become attractive cancer biomarkers and may serve as tumor-associated antigens (TAAs). Furthermore, costimulatory pathways have been identified as immunologic checkpoints that attenuate T cell dependent immune responses, both at the level of initiation and effector function within tumor metastases. As engineered cancer vaccines continue to improve, it is becoming clear that such immunologic checkpoints are a major barrier to the vaccines' ability to induce therapeutic anti-tumor responses. In that regard, costimulatory molecules can serve as adjuvants for active (vaccination) and passive (antibody-mediated) cancer immunotherapy, providing strategies to thwart immune tolerance and stimulate the immune system.

[0006] Over the past decade, agonists and/or antagonists to various costimulatory proteins have been developed for treating autoimmune diseases, graft rejection, allergy and cancer. For example, CTLA4-Ig (Abatacept, Orencia®) is approved for treatment of RA, mutated CTLA4-Ig (Belatacept, Nulojix®) for prevention of acute kidney transplant rejection and by the anti-CTLA4 antibody (Ipilimumab, Yervoy®), recently approved for the treatment of melanoma. Other costimulation regulators have been approved, such as the anti-PD-1 antibodies of Merck (Keytruda®) and BMS (Opdivo®), have been approved for cancer treatments and are in testing for viral infections as well. WO 2012/178128 discloses the over-expression of the PVRIG transcripts in human cancer specimens.

[0007] Accordingly, it is an object of the invention to provide PVRIG immunomodulatory antibodies for use in the treatment of cancer.

BRIEF SUMMARY OF THE INVENTION

[0008] The invention is defined by the claims and any other aspects, configurations or embodiments set forth herein not falling within the scope of the claims are for information only.

[0009] The invention provides an anti-PVRIG antibody for use in the treatment of cancer, wherein the antibody activates T cells and/or NK cells, the antibody specifically binding to human PVRIG and competing with an antibody that comprises the vhCDR1 as set out in SEQ ID NO:885, vhCDR2 as set out in SEQ ID NO:886, vhCDR3 as set out in SEQ ID NO:887, vlCDRl as set out in SEQ ID NO:889, vlCDR2 as set out in SEQ ID NO:890 and vlCDR3 as set out in SEQ ID NO:891 to specifically bind to the PVRIG molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

Figure 1 Schematic presentation of the mechanisms of action of the invention.

Figure 2 presents mRNA Expression of PVRIG in various normal human tissues.

Figure 3 presents mRNA expression of PVRIG in various immune population derived from peripheral blood and bone marrow (based on GSE49910).

Figure 4 presents mRNA expression of PVRIG in various CD3+ lymphocyte population (based on GSE47855).

Figure 5 A, 5B and 5C presents mRNA expression of PVRIG in specific cell populations. Figure 5A resents mRNA expression of PVRIG in specific cell populations obtained by laser capture microscopy (based on GSE39397). Figure 5B presents mRNA expression of PVRIG in CD4 T-cells from normal and cancer patient as well as expression form CD4 T-cell expression from draining lymph nodes and TILs form breast cancer patients (based on GSE36765). Figure 5C presents mRNA expression of PVRIG from CD8 and CD4 T-cells derived from follicular lymphoma tumor and tonsil (based on GSE27928).

Figure 6 presents PVRIG expression in normal tissues based on GTEx. Expression levels are shown in log2(RPKM) values (fragments identified per million reads per kilobase). Values above 1 are considered high expression. Tissues are ranked from top to bottom by the median expression. Each dot on the plot represent a single sample.

Figure 7 presents PVRIG expression in cancerous tissues based on TCGA. Expression levels are shown in log2(RPKM) values (fragments identified per million reads per kilobase). Values above 1 are considered high expression. Tissues are ranked from top to bottom by the median expression. Each dot on the plot represent a single sample

Figure 8 shows a heatmap representation of the enrichment analysis results in three categories: protein interactions, pathways and disease associations. Results are ranked from top to bottom by average p-value per row. Only the top 10 results from each category are shown. Gray squares indicate p-values<0.05. Each column in the heatmap corresponds to a normal or cancer tissue from which a list of highly correlated genes was derived (r>0.55 using at least 50 samples). As shown in the heatmap, PVRIG correlates with a T cell gene expression signature which is strongly associated with the immune response and immune diseases.

Figure 9 presents PVRIG expression in normal skin vs. melanoma (GTEx and TCGA analysis). Such over-expression was observed in additional solid tumors and results from infiltrating lymphocytes and NK cells in the tumor microenvironment. In normal condictions, no infiltrating immune cells are present and therefore PVRIG expression levels are very low.

Figure 10 presents the correlations of PVRIG and PD1 in melanoma from TCGA samples, with several T cell makers in lung adenocarcinoma, colon adenocarcinoma and melanoma. The marker CD3 is a general markers for T cells and is also expressed on NKT cells. CD4 and CD8 markers are used to characterized subpopulation of T cells.

Figure 11 shows expression of PVRIG on human PBLs. Human PBLs derived from two donors were evaluated for PVRIG expression. Both donor 61 and donor 40 showed significant staining with anti-PVRIG specific Ab.

Figure 12 shows PVRIG-Ig exhibits strong binding to all four human melanoma cell lines MEL-23, Mel-624 and Mel-624.38 and mel-888 tested. Binding is not affected by co-culture with engineered melanoma specific T cells. Grey line corresponds to isotype control, solid black line corresponds to PVRIG-ECD-Ig.

Figure 13 Correlation of PVRIG with T cells and subpopulations of T cells. CD3G is component of the T cell receptor complex, CD4 is a maker for T helper cells and CD8A is component of CD8 protein used to identify cytotoxic T cells. PVRIG highly correlated with T cells in many types of tumors including lung adenocarcinoma, colon adenocarcinoma and melanoma which are shown here.

Figure 14 presents representative images from the Confirmation/Specificity screen. All hits from the Primary screen, and EGFR-expressing vector (negative control), were rearrayed/expressed in duplicate and probed with PVRIG at 20ug/ml. A specific hit with strong intensity is shown in green (PVRL2). Non-specific hits are shown in black. Another weak hit (MAG) was later shown to bind also other ligands, thus suggesting that it is not specific.

Figure 15A-15E presents effect of various PVRIG-ECD-Ig M:M proteins on mouse CD4 T cell activation. Plates were coated with anti-CD3 mAb (2µg/mL) in the presence of 10µg/ml PVRIG-ECD Ig (batch #198) or control mIgG2a as described in materials and methods. Wells were plated with 1×10^5 CD4+CD25- mouse T cells per well in the presence of 2ug/ml of soluble anti-CD28. (A) The expression of CD69 was analyzed by flow cytometry at 48h post-stimulation, representative histograms are shown. Each bar is the mean of duplicate cultures, the error bars indicating the standard deviation. (B-C) Culture supernatants were collected at 48 h post-stimulation and mouse IL-2 and IPNγ levels were analyzed by ELISA. Results are shown as Mean ± Standard errors of duplicate samples. (D) Dose response effect of immobilized PVRIG-ECD Ig (Figure 92BB on surface CD69 (D) and IPNγ secretion (E) is presented. Each bar is the mean of triplicate cultures, the error bars indicating the standard errors.

Figure 16 presents FACS analysis on PVRIG transduced PBLs using a specific antibody. The percent of cells staining positive (relative to empty vector transduced) for the protein is provided.

Figure 17 presents FACS analysis on PVRIG (either co-expressed with F4 TCR or in a bi-cystronic vector with F4 TCR and NGFR transduced PBLs using a specific antibody. The percent of cells staining positive (relative to empty vector transduced) for the protein is provided.

Figure 18A-18B presents FACS analysis performed on TCR transduced stimulated PBLs for experiment 1 (Figure 18A) and in experiment 2 (Figure 18B) using a specific monoclonal antibody that recognizes the extra-cellular domain of the beta-chain from the transduced specific TCR. The percentage of cells staining positive is provided.

Figure 19 shows expression of PVRIG on F4 expressing PBLs causes a reduction of IPNγ secretion upon co-culture with SK-MEL23, MEL-624 and MEL-624.38 in comparison to expression of an empty vector.

Figure 20A-20B shows expression of PVRIG and F4 in PBLs by co-transduction (Figure 20A) does not affect IPNγ secretion in co-culture with melanoma cell lines. Expression of PVRIG and F4 in PBLs using a bi-cystronic vector (Figure 20B) causes a reduction of IFNγ secretion upon co-culture with SK-MEL23, MEL-624 and MEL-624.38 in comparison to expression of an empty vector.

Figure 21 shows expression of PVRIG and F4 in PBLs using a bi-cystronic vector causes a reduction in T cell mediated cytotoxicity upon co-culture with melanoma cell lines.

Figure 22 shows PVRIG expression in 3 subgroups of low, no change and high levels of exhausted T cells. Exhausted T cells were selected based on high level expression of 4 markers: CD8A, PD-1, TIM-3 and TIGIT. Low expressing samples are not shown since none had any detectable levels of PVRIG.

Figure 23A-23B: Western blot analysis of ectopically expressed human PVRIG protein. Whole cell extracts of HEK293 cell pools, previously transfected with expression construct encoding human PVRIG-flag (lane 2) or with empty vector (lane 1) were analyzed by WB using an anti-flag antibody (23A) or anti-PVRIG antibodies (23B).

Figure 24: Cell surface expression of HEK293 cells ectopically expressed human PVRIG-flag protein by FACS analysis. Anti-PVRIG pAb (Abnova) was used to analyze HEK293 cells stably expressing the human PVRIG-flag protein. Cells expressing the empty vector were used as negative control. Detection was carried out by Goat Anti-mouse PE-conjugated secondary Ab and analyzed by FACS.

Figure 25 depicts the full length sequence of human PVRIG (showing two different methionine starting points) and the PVRIG Fc fusion protein used in the Examples. The signal peptide is underlined, the ECD is double underlined, and the Fc domain is the dotted underlining.

Figure 26 depicts the sequence of the human Poliovirus receptor-related 2 protein (PVLR2, also known as nectin-2, CD112 or herpesvirus entry mediator B, (HVEB)), the binding partner of PVRIG as shown in **Example 5.** PVLR2 is a human plasma membrane glycoprotein.

Figure 27 PVRIG antibody specificity towards HEK cells engineered to overexpress PVRIG. Data shows absolute geometric MFI (gMFI) measurements as a function of increasing antibody concentration. The broken black line with squares shows staining of HEK hPVRIG cells with a representative anti-human PVRIG antibody (CPA.7.021), and the solid black line with circles shows staining of HEK parental cells with the same antibody.

Figure 28 PVRIG RNA was assessed in various cancer cell lines by qPCR. Data shown is relative expression of PVRIG RNA in cell lines as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method.

Figure 29 PVRIG RNA was assessed in sorted PBMC subsets by qPCR. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in HEK GFP cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. D47-D49 denote three individual donors. CD4 denotes CD4 T cells, CD8 denotes CD8 T cells, CD14 denotes monocytes, and CD56 denotes NK cells.

Figure 30A-30B. Figure 30A: PVRIG RNA was assessed in sorted CD4 T cells (CD4) and NK cells (NK) under naive and activated conditions by qPCR. CD4 T cells were stimulated with human T cell stimulator dynabeads and 50U/ml IL-2 for 3 days. NK cells were stimulated in 50U/ml IL-2 for 3 days. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. Jurkat is included as a positive control. D47-D49 denote three individual donors. Figure 30B PVRIG RNA was assessed in sorted CD8 T cells under naive and activated conditions by qPCR. CD8 T cells were stimulated with human T cell stimulator dynabeads and 100U/ml IL-2 for 3 days. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. Jurkat is included as a positive control. D49, 70, and 71 indicate three individual donors.

Figure 31A-31B PVRIG binding characteristics to HEK hPVRIG engineered cell lines, HEK parental cells, CA46 cells, and Jurkat cells. HEK OE denotes HEK hPVRIG cells, HEK par denotes HEK parental cells. For Jurkat and CA46 data, gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. Not reliable fit indicates antibody binding characteristics do meet appropriate mathematical fitting requirements. Some antibodies were not tested in some conditions due to poor binding characteristics, specificity, or manufacturability.

Figure 32A-32B PVRIG binding characteristics to primary human PBMC, cyno transient over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. Some antibodies were not tested in some conditions due to poor binding characteristics, specificity, or manufacturability as in Figure 31. Additionally, select antibodies were triaged for screening on cyno PBMC subsets based on their ability to bind cPVRIG transient cells or functionality. Expression of PVRIG on CD4 T cells is similar to that described in the table for CD8 T cells.

Figure 33 PVRIG antibody specificity towards CA46 and Jurkat cells. Data shows absolute geometric MFI (gMFI) measurements by FACS as a function of increasing antibody concentration. The solid black line with triangles shows staining of CA46 cells with anti-human PVRIG antibody (CPA.7.021) and the solid black line with squares shows staining of Jurkat cells. OV-90 (broken line with upside down triangles) and

NCI-H4411 (broken line with diamonds) are shown as negative controls.

Figure 34A-34D PVRIG antibody cross-reactivity towards cPVRIG transient cells. Data shows an example of an antibody that is a negative binder (a-b, CPA.7.021) and a positive binder (c-d, CPA.7.024) on cPVRIG transient cells. Solid grey histograms indicate control antibody, open black histograms indicate the antibody of interest. Cells were stained with each antibody at a concentration of 5ug/ml.

Figure 35 cPVRIG RNA was assessed in sorted cyno PBMC subsets by qPCR. Data shown is the average Ct values from three cyno donors as detected by two primer sets directed at two distinct areas of the cPVRIG gene.

Figure 36A-36C cPVRIG protein was assessed on a) CD16+ lymphocytes (NK cells), b) CD14+ CD56+ myeloid cells (monocytes), and c) CD3+ lymphocytes (T cells) by FACS. Data is shown as absolute geometric MFI, with the solid black line indicating background fluorescence levels. Data is representative of a sample of our panel of anti-human PVRIG antibodies tested in three cyno donors.

Figure 37A-37B shows the CDR sequences for Fabs that were determined to successfully block interaction of the PVRIG with its counterpart PVRL2, as described in **Example 5.**

Figure 38A-38AA shows the amino acid sequences of the variable heavy and light domains, the full length heavy and light chains, and the variable heavy and variable light CDRs for the enumerated human CPA anti-PVRIG sequences that both bind PVRIG and block binding of PVRIG and PVLR2.

Figure 39A-39H depicts the amino acid sequences of the variable heavy and light domains, the full length heavy and light chains, and the variable heavy and variable light CDRs for eight human CPA anti-PVRIG sequences that bind PVRIG and but do not block binding of PVRIG and PVLR2.

Figure 40A-40D depicts the CDRs for all CPA anti-PVRIG antibody sequences that were generated that bind PVRIG, including those that do not block binding of PVRIG and PVLR2.

Figure 41A to 41DD depicts the variable heavy and light chains as well as the vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of each of the enumerated CHA antibodies of the invention, CHA.7.516, CHA.7.518, CHA.7.520.1, CHA.7.520.2, CHA.7.524, CHA.7.528, CHA.7.530, CHA.7.537, CHA.7.538.1, CHA.7.538.2, and CHA.7.548 (these include the variable heavy and light sequences from mouse sequences (from Hybridomas).

Figure 42 depicts the binning results from **Example 11.** Not binned: CPA.7.029 and CPA.7.026 (no binding to the antigen).

Figure 43 Binary matrix of pair-wise blocking ("0", red box) or sandwiching ("1", green box) of antigen for 35 anti-PVRIG mAbs. MAbs listed vertically on the left of the matrix are mAbs covalently immobilized to the ProteOn array. MAbs listed horizontally across the top of the matrix were analytes injected with pre-mixed antigen. Clone CPA.7.041 was studied only as an analyte. The black boxes outline four epitope bins according to the vertical blocking patterns of the mAbs.

Figure 44 Hierarchical clustering dendrogram of the vertical binding patterns of each mAb in the binary matrix in Figure 43. There are four bins of mAbs with identical epitope blocking patterns within each group. The only difference between bins 1 and 2 is mAbs in bin 1 block antigen binding to clone CPA.7.039 while mAbs in bin 2 can sandwich the antigen with CPA.7.039. Clone CPA.7.050 can sandwich the antigen with all other clones.

Figure 45A-45JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.036 with all other immobilized mAbs, which are representative data for Bin #1. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.036 in molar excess of antigen. Gray responses are mAb-only control injections. CPA.7.36 blocks antigen binding to all other mAbs except for CPA.7.050 (JJ).

Figure 46A-46JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.034 with all other immobilized mAbs, which are representative data for Bin #2. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.34 in molar excess of antigen. Gray responses are mAb-only control injections. CPA.7.34 blocks antigen binding to all other mAbs except for CPA.7.039 (DD) and CPA.7.050 (JJ).

Figure 47A-47JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.039 with all other immobilized mAbs. CPA.7.039 is the only mAb in Bin #3. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.039 in molar excess of antigen. Gray responses are mAb-only control injections. Panels C, F, H, J, L, N, R, S, Z, EE, GG, HH, II, and JJ show sandwiching of the antigen.

Figure 48A-48JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.050 with all other immobilized mAbs. CPA.7.050 is the only mAb in Bin #4. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.50 in molar excess of antigen. Gray responses are mAb-only control injections. Only panel JJ shows antigen blocking which is where CPA.7.050 was injected w/antigen over itself.

Figure 49 show the results of the SPR experiments of **Example 12.**

Figure 50A-50Q SPR sensorgram data of multiple concentrations of anti PVRIG fabs in supernatant injected over captured human PVRIG fusion protein (black lines). The red lines show the 1:1 global kinetic fit to multiple concentrations of the fabs to estimate the ka and k_d of the interactions. Letters indicate the clone listed in Table 1, which also lists the resulting rate constants and calculated K_D

Figure 51A-51C SPR sensorgrams for clones CPA.7.009 (A), CPA.7.003 (B), and CPA.7.014 (C) binding to captured human PVRIG fusion protein. These are examples where the sensorgrams showed complex, multi-phasic kinetics and therefore the rate constants could not be reliably estimated.

Figure 52A-52B shows the results of the blocking studies from "Additional Validation Study 4" in **Example 5.**

Figure 53 shows that following allo-activation, the expression of PVRIG was upregulated on CD4+ T cells as well as on CD8+ T cells and double negative gamma delta T cells. This upregulation was observed in PBMCs of one out of two donors tested.

Figure 54 shows the human cell lines tested in **Example 1**G.

Figure 55 shows the mouse cell lines tested in **Example 1**G.

Figure 56A-56C. Transcript expression of human PVRIG in various Human cancer cell lines. Verification of the human transcript in several cell lines was performed by qRT-PCR using TaqMan probe. Column diagram represents data observed using TaqMan probe Hs04189293_g1. Ct values are detailed in the table. Analysis indicating high transcript in Jurkat, HUT78 and HL60, and lower levels in THP1 and RPMI8226 cell lines.

Figure 57A-57B Transcript expression of mouse PVRIG in various mouse cell lines. Verification of the mouse transcript in several cell lines was performed by qRT-PCR using TaqMan probe. Column diagram represents data observed using TaqMan probe CC70L8H. Ct values are detailed in the table. Analysis indicating high transcript in NIH/3T3, Renca, Sal/N and J774A.1, and lower levels in CT26 and B104-1-1 cell lines.

Figure 58 Endogenous expression of PVRIG protein was analyzed by WB with the commercial anti-human PVRIG rabbit polyclonal antibody (Sigma, cat# HPA047497), using whole cell extracts of various cell lines. Extracts of HEK293 cells ectopically over-expressing human PVRIG (lane 2) or cells transfected with empty vector (lane 1), were used as positive and negative controls, respectively.

Figure 59 qRT-PCR analysis of human PVRIG transcript in Jurkat cell line transfected with PVRIG siRNA. Jurkat human cancer cell line, transfected with human PVRIG siRNA or with scrambled siRNA were analyzed by qRT-PCR using human PVRIG TaqMan probe # Hs04189293_g1, and was normalized with geo-mean of two housekeeping genes indicated in table above. Ct values are detailed in the table. Standard deviation of technical triplicates of the PCR reaction are indicated.

Figure 60 Membrane expression of human PVRIG protein in Jurkat human cell line transfected with human PVRIG siRNA. Jurkat cells transfected with Human PVRIG siRNA were stained with monoclonal anti-PVRIG Ab Inc, CPA.7.021 (left panel, green line) or with IgG2 isotype control antibody (left panel, blue line) and with Sigma Ab (right panel, red line) or with IgG (right panel, blue line). Cells transfected with Scrambled siRNA were stained with the same anti-PVRIG (orange) or isotype control (left panel red line for mAb staining; right panel green line for Sigma Ab). Following cell washing, PE-Goat anti-mouse secondary conjugated Ab was added to Sigma Ab only.

Figure 61 indicates the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down, HSKG- housekeeping gene, +- Positive, NT-Not Tested, X-negative, KD-knockdown.

Figure 62 indicates the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down. HSKG- housekeeping gene, +- Positive, NT-Not Tested, X-negative, KD-knockdown.

Figure 63A-63D depicts the vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of each of the enumerated CPA antibodies CPA.7.001 to CPA.7.050 are human sequences (from **P**hage display).

Figure 64A-64B shows the results of the screening in Example 1B.

Figure 65 Antibodies specifics and staining concentration used in **Example 1I.**

Figure 66A-66C depicts the sequences of human IgG1, IgG2, IgG3 and IgG4.

Figure 67 depicts a number of human PVRIG ECD fragments.

Figure 68 depicts the binding curve for CPA.7.021 as shown in **EXAMPLE 13**.

Figure 69A-69C **Detection of CD137 and PD-1 surface expression**. CD8+ T cells, CD4+ T cells and TILs were activated and monitored over time at 4 time-points as described in M&M. Resting or activated cells were first gated for lymphocytes (FSC-A vs. SSC-A), followed by live cells gate, further gated for singlets (FSC-H vs. FSC-A), CD4/CD8 positive cells and further gated for CD137 and PD1. Surface expression of PD-1 (left) and CD137 (right) on (**A**) CD8+ T cells (**B**) CD4+ T cells and (**C**) TILs at different time-points normalized to isotype control over the time course of activation.

Figure 70A-70C PVRIG expression on resting and activated CD4+ T and CD8+ T cells. CD4+ and CD8+ T cells were activated and monitored over time at 4 time-points as described in M&M. Cells were stained with viability dye, then incubated with anti-PVRIG and isotype control (7.5 □g/ml), and evaluated by flow cytometry. (A) Expression on CD4+ T cells. Expression of PVRIG on live resting (time 0) and activated CD4+ cells following singlet gating for 24, 48, 72h and 144h compared to isotype control. (B) Expression on CD8+ T cells. Expression of PVRIG on live resting (time 0) and activated CD8+ cells following singlet gating for 24, 48, 72h and 144h compared to isotype control. Shown are the Geometric Mean of the fluorescent intensity values obtained. (C) Normalization of fold induction staining with anti-PVRIG-CPA.7.021 ab compared to human IgG2 isotype over the time course of activation.

Figure 71A-71C PVRIG expression on resting and activated TILs. TILs Mart1 and 209 were activated and monitored over time at 4 time-points as described in M&M. Cells were stained with viability dye, then incubated with anti-PVRIG and isotype control (7.5 □g/ml), and evaluated by flow cytometry. (A) Expression on TIL Mart1. Expression of PVRIG on live resting (time 0) and activated TIL following singlet gating for 24, 48, 72h and 144h compared to isotype control. (B) Expression on TIL 209. Expression of PVRIG on live resting (time 0) and activated TIL following singlet gating for 24, 48, 72h and 144h compared to isotype control. Shown are the Geometric Mean of the fluorescent intensity values obtained. (C) Normalization of fold induction staining with anti PVRIG-CPA.7.021 ab compared with human IgG2 isotype control over the time course of activation.

Figure 72 Expression of PVRL2 on monocyte-derived DC. PVRL2 expression (triangles with broken line) as a function of time (days) relative to isotype control (circles with solid line) is shown. Day after differentiation indicates time after addition of GM-CSF and IL-4 to monocytes.

Figure 73A-73B Expression of PVRIG on CD4 and CD8 T cells in the MLR. The expression of PVRIG on proliferating (CFSE low) and non-proliferating T cells (CFSE high) is shown. Data is derived from three individual CD3 T cell donors and from a range of PVRIG antibodies. CFSE is measured on the X axis and PVRIG expression is measured on the Y axis. The top 3 series of scatter plots indicates PVRIG expression on CD4 T cells, and the bottom 3 series indicates expression on CD8 T cells.

Figure 74A-74B Normalised expression of PVRIG on CD4 and CD8 T cells in the MLR. The expression of PVRIG relative to mIgG1 isotype control is shown from three individual CD3 T cell donors across all antibodies analysed.

Figure 75A-75B PVRIG antibodies increase T cell proliferation in the MLR. The percentages of CFSE low cells are shown from MLR assays treated with the indicated PVRIG antibodies. Each graph represents one individual CD3 T cell donor.

Figure 76 FACS-based epitope analysis of PVRIG antibodies on T cells. The level of binding of conjugated CPA.7.021 (derived from phage campaign) is indicated after preincubation of T cells with unconjugated PVRIG antibodies derived from our hybridoma campaign, as well as relevant controls. Analysis was performed on CFSE low T cells derived from the MLR.

Figure 77 PVRIG antibody specificity towards HEK cells engineered to overexpress PVRIG. Data shows absolute geometric MFI (gMFI) measurements as a function of increasing antibody concentration. The broken black line with squares shows staining of HEK hPVRIG cells with a representative anti-human PVRIG antibody (CHA.7.518), and the solid black line with circles shows staining of HEK parental cells with the same antibody.

Figure 78 PVRIG antibodies show specificity towards Jurkat cells. Data shows absolute geometric MFI (gMFI) measurements by FACS as a function of increasing antibody concentration. The broken black line with squares shows staining of Jurkat cells with anti-human PVRIG antibody (CHA.7.518) and the solid black line with circles shows staining with an mIgG1 control antibody.

Figure 79A-79B PVRIG hybridoma antibody binding characteristics to HEK hPVRIG engineered cell lines, HEK parental cells, and Jurkat cells. HEK OE denotes HEK hPVRIG cells, HEK par denotes HEK parental cells. For Jurkat data, gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. No binding indicates antibody does not bind to the tested cell line. Highlighted antibodies are the 'top four' antibodies of interest.

Figure 80A-80B PVRIG hybridoma antibody binding characteristics to primary human PBMC, cyno over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentrations indicate that at which the gMFIr was calculated. Not tested indicates antibodies that were not tested due to an absence of binding to human HEK hPVRIG, expi cPVRIG cells, or not meeting binding requirements to PBMC subsets. Highlighted antibodies are the 'top four' antibodies of interest.

Figure 81A-81B Summary of blocking capacity of PVRIG antibodies in the FACS-based competition assay. The IC_{50} of inhibition is indicated. No IC_{50} indicates that these antibodies are non-blockers. Highlighted antibodies are the 'top four' antibodies of interest.

Figure 82 KD validation performed in TILs 24hr post-electroporation with siRNA. TILs were stained with anti PVRIG or anti PD-1 analyzed by FACS. Percentage of the KD population is calculated relative to SCR stained with the relevant Ab.

Figure 83A-83C KD TILs (MART-1 specific) were co-cultured with melanoma cells 624 in 1:1 E:T for 18hr and stained with anti CD8a antibody as well as anti CD137 antibody and analyzed by FACS. Geometric mean fluorescence intensity are plotted (A). Co-culture supernatant was collected as well and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IPNγ and TNF levels were detected (B,C). The percentage effect of a treatment is calculated by comparing each treatment to SCR control. The figure shows representative data of 2 independent experiments. Treatments were compared by Student's t-test (*$P \leq 0.05$, **$P \leq 0.01$) of triplicate samples.

Figure 84A-84B KD TILs (F4 gp100 specific) were co-cultured with melanoma cells 624 in 1:3 E:T for 18hr and stained with anti CD8a antibody as well as anti CD137 antibody and analyzed by FACS. Geometric mean fluorescence intensity are plotted (**A**). Co-culture supernatant was collected as well and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IPNγ levels were detected (**B**). Percentage of the effect a treatment has is calculated by comparing each treatment to SCR control. Figure shows representative data of 2 independent experiments. Treatments were compared by Student's t-test (*$P \leq 0.05$, **$P \leq 0.01$) of triplicate samples.

Figure 85A-85B TILs from were co-cultured with melanoma cells 624 at 1:1 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-TIGIT (10A7 clone; 10ug/ml) or in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IPNγ (**A**) and TNF (**B**) levels were detected. Treatments were compared by Student's t-test (*$P \leq 0.05$, **$P \leq 0.01$) of triplicate samples.

Figure 86A-86F MART-1 or 209 TILs were co-cultured with melanoma cells 624 at 1:1 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-DNAM1 (DX11 clone; 10ug/ml) or in in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IPNγ (**A,D**) and TNF (**B,E**) levels were detected. TILs were stained for surface expression of CD137 (**C,F**).

Figure 87A-87B TILs (F4) were co-cultured with melanoma cells 624 at 1:3 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-TIGIT (10A7 clone; 10ug/ml), anti-PD1 (mAb 1B8, Merck;

10ug/ml) or in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IPNγ (**A**) and TNF (**B**) levels were detected.

Figures 88A-88I I depict four humanized sequences for each of CHA.7.518, CHA.7.524, CHA.7.530, CHA.7.538_1 and CHA.7.538_2. Note that the light chain for CHA.7.538_2 is the same as for CHA.7.538_1. The "H1" of each is a "CDR swap" with no changes to the human framework. Subsequent sequences alter framework changes shown in larger bold font. CDR sequences are noted in bold. CDR definitions are AbM from website www.bioinf.org.uk/abs/. Human germline and joining sequences from IMGT® the international ImMunoGeneTics® information system www.imgt.org (founder and director: Marie-Paule Lefranc, Montpellier, France). Residue numbering shown as sequential (seq) or according to Chothia from website www.bioinf.org.uk/abs/ (AbM). "b" notes buried sidechain; "p" notes partially buried; "i" notes sidechain at interface between VH and VL domains. Sequence differences between human and murine germlines noted by asterisk (*). Potential additional mutations in frameworks are noted below sequence. Potential changes in CDR sequences noted below each CDR sequence as noted on the figure (# deamidation substitutions: Q/S/A; these may prevent asparagine (N) deamidation. @ tryptophan oxidation substitutions: Y/F/H; these may prevent tryptophan oxidation; @ methionine oxidation substitutions: L/F/A).

Figures 89A-E depicts a collation of the humanized sequences of five CHA antibodies.

Figure 90 depicts schemes for combining the humanized VH and VL CHA antibodies of Figures 88 and Figures 89. The "chimVH" and "chimVL" are the mouse variable heavy and light sequences attached to a human IgG constant domain.

Figure 91 PVRIG hybridoma antibody binding characteristics to primary human PBMC, cyno over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentrations indicate that at which the gMFIr was calculated. Not tested indicates antibodies that were not tested due to an absence of binding to human HEK hPVRIG, expi cPVRIG cells, or not meeting binding requirements to PBMC subsets. Highlighted antibodies are four antibodies for which humanization was done (See Figure 90).

Figure 92 Summary of blocking capacity of PVRIG antibodies in the FACS-based competition assay. The IC50 of inhibition is indicated. No IC50 indicates that these antibodies are non-blockers. Highlighted antibodies are four antibodies for which humanization was done (See Figure 90).

Figure 93A-93C Effect of PVRIG antibodies in blocking the interaction between PVRIG and PVRL2. (a-b) Data shows changes in absolute gMFI representing changes in binding of soluble PVRIG to HEK cells when four PVRIG antibodies are added to disrupt the interaction. Also indicated are the IC_{50} values of each antibody in each assay. A) Data shows disruption of soluble PVRIG with HEK cells when the antibodies are pre-incubated with antigen. B) Data shows disruption of soluble PVRIG with HEK cells when the antibodies are added concomitantly with antigen. C) Data shows changes in absolute gMFI representing changes in binding of soluble PVRL2 Fc to HEK hPVRIG cells when four PVRIG antibodies are added to disrupt the interaction. IC_{50} values of each antibody are indicated. ND denotes not determined.

Figure 94A-94H NK cell receptor and ligand expression on Reh cells. Expression of NK cell receptors such as a) PVRIG, b) DNAM-1, c) TIGIT are shown. Expression of NK receptor ligands such as d) PVR, e) PVRL2, f) ULBP2/5/6, g) ULBP3, and h) MICA/B are shown. Solid grey histograms represent isotype controls and open black histograms represent the antibody of interest.

Figure 95 Effect of PVRIG antibodies on enhancing NK cell-mediated cytotoxicity against Reh cells. The effect of 5ug/ml CPA.7.002 (a), CPA.7.005 (b), CPA.7.021 (a-c), and CPA.7.050 (c) was examined in NK cell cytotoxicity assays against Reh cells where the number of NK cells was titrated against a constant number of Reh cells. d) The effect of varying the concentration of CPA.7.002 and CPA.7.021 on NK cell-mediated cytotoxicity with a constant number of NK to Reh cells (5:1) was examined. DNAM-1 (e) and TIGIT (f) were examined in assays with conditions as outlined in panels a-c.

Figure 96A-96H NK cell receptor and ligand expression on MOLM-13 cells. Expression of NK cell receptors such as a) PVRIG, b) DNAM-1, c) TIGIT are shown. Expression of NK receptor ligands such as d) PVR, e) PVRL2, f) ULBP2/5/6, g) ULBP3, and h) MICA/B are shown. Solid grey histograms represent isotype controls and open black histograms represent the antibody of interest.

Figure 97A-97B Effect of PVRIG antibodies on enhancing NK cell-mediated cytotoxicity against MOLM-13 cells. a) The effect of 5ug/ml CPA.7.002, CPA.7.005, and CPA.7.021 was examined in NK cell cytotoxicity assays against MOLM-13 cells where the number of NK cells was titrated against a constant number of MOLM-13 cells. b) TIGIT was examined similar to panel a.

Figure 98 Summary of blocking capacity of PVRIG antibodies in the cellular biochemical assay. Assay permutation and orientation, and the IC_{50} of inhibition are indicated. (P) indicates the assay permutation where PVRIG antibodies are pre-incubated with PVRIG antigen prior to addition to HEK cells. (NP) indicates the concomitant addition of PVRIG antibodies and PVRIG antigen to HEK cells. Increased binding indicates that PVRL2 Fc binding to HEK hPVRIG cells was enhanced, rather than inhibited.

Figure 99: Summary of the activity of select PVRIG antibodies in NK cell cytotoxicity assays against Reh and MOLM-13 cells. Fold change in cytotoxicity relative to control was calculated by dividing the absolute level of killing (%) in the condition with PVRIG antibody, by the absolute level of killing (%) with control antibody. Fold change is calculated from the 5:1 effector to target ratio.

Figure 100 **Sequence alignment of PVRIG orthologs.** Aligned sequences of the human, cynomolgus, marmoset, and rhesus PVRIG extra-cellular domain. The differences between human and cynomolgus are highlighted in yellow.

Figure 101 Binding of anti human PVRIG antibodies to cyno, human, cyno/human hybrid PVRIG variants. Binding of antibodies to wild type cyno PVRIG (•), H61R cyno PVRIG (■), P67S cyno PVRIG (▲), L95R/T97I cyno PVRIG (▼), and wild type human PVRIG (◆) are shown. The ELISA signals are plotted as a function of antibody concentration.

Figure 102 Correlation of epitope group and cyno cross-reactivity of anti-human PVRIG antibodies.

Figure 103A-103BX shows a number of sequences of use.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0011] Cancer can be considered as an inability of the patient to recognize and eliminate cancerous cells. In many instances, these transformed (e.g. cancerous) cells counteract immunosurveillance. There are natural control mechanisms that limit T-cell activation in the body to prevent unrestrained T-cell activity, which can be exploited by cancerous cells to evade or suppress the immune response. Restoring the capacity of immune effector cells-especially T cells-to recognize and eliminate cancer is the goal of immunotherapy. The field of immuno-oncology, sometimes referred to as "immunotherapy" is rapidly evolving, with several recent approvals of T cell checkpoint inhibitory antibodies such as Yervoy, Keytruda and Opdivo. These antibodies are generally referred to as "checkpoint inhibitors" because they block normally negative regulators of T cell immunity. It is generally understood that a variety of immunomodulatory signals, both costimulatory and coinhibitory, can be used to orchestrate an optimal antigen-specific immune response. Generally, these antibodies bind to checkpoint inhibitor proteins such as CTLA-4 and PD-1, which under normal circumstances prevent or suppress activation of cytotoxic T cells (CTLs). By inhibiting the checkpoint protein, for example through the use of antibodies that bind these proteins, an increased T cell response against tumors can be achieved. That is, these cancer checkpoint proteins suppress the immune response; when the proteins are blocked, for example using antibodies to the checkpoint protein, the immune system is activated, leading to immune stimulation, resulting in treatment of conditions such as cancer and infectious disease.

[0012] The present disclosure is directed to the use of antibodies to human Poliovirus Receptor Related Immunoglobulin Domain Containing Protein, or "PVRIG", sometimes also referred to herein as "PV protein". PVRIG is expressed on the cell surface of NK and T-cells and shares several similarities to other known immune checkpoints.

[0013] Computational algorithms were used to analyze the human genome in order to identify novel immune checkpoints. Genes were identified that are predicted to be cell surface proteins, have an Ig domain and are expressed on immune cells within the tumor microenvironment, specifically on tumor infiltrating lymphocytes (TILs), which are presumed to be receptors. Proteins that have a single IgV domain and have an intracellular ITIM-like motif were identified, which suggests that they are acting as immune checkpoint and have an inhibitory effect on T cells and/or NK cells. Once identified computationally, various validation experiments were done, including: expression studies demonstrating that PVRIG is expressed on lymphocytes and on lymphocytes within the tumor microenvironment and has an inhibitory effect on NK and T cells (demonstrated both with knockdown experiments and with antibodies directed at PVRIG). PVRL2 was identified/confirmed to be the counterpart of PVRIG. Antibodies that bind to PVRIG were generated, and then a subset of those were identified that both bind to PVRIG and block the interaction of PVRIG and PVLR2.

[0014] Accordingly, when PVRIG is bound by its ligand (PVRL2), an inhibitory signal is elicited which acts to attenuate the immune response of NK and T-cells against a target cell (i.e. analogous to PD-1/PDL1). Blocking the binding of PVRL2 to PVRIG shuts-off this inhibitory signal of PVRIG and as a result modulates the immune response of NK and T-cells. Utilizing an antibody against PVRIG that blocks binding to PVRL2 is a therapeutic approach that could enhance the killing of cancer cells by NK and T-cells. Blocking antibodies have been generated which bind PVRIG and block the binding of its ligand, PVRL2.

[0015] As shown in the Example section, the expression of PVRIG has been positively correlated to expression of PD-1, a known immune checkpoint protein. Additionally, introduction of PVRIG (as a extracellular domain (ECD) fusion protein) was shown to inhibit the activation of T cells, and thus the use of anti-PVRIG antibodies leads to T cell activation. Accordingly, anti-PVRIG antibodies can be used to treat conditions for which T cell or NK cell activation is desired such as cancer.

[0016] Functional effects of PVRIG blocking antibodies on NK and T-cells can be assessed in vitro (and in some cases in vivo, as described more fully below) by measuring changes in the following parameters: proliferation, cytokine release and cell-surface makers. For NK cells, increases in cell proliferation, cytotoxicity (ability to kill target cells as measured by increases in CD107a, granzyme, and perforin expression, or by directly measuring target cells killing), cytokine production (e.g. IFN-y and TNF), and cell surface receptor expression (e.g. CD25) is indicative of immune modulation, e.g. enhanced killing of cancer cells. For T-cells, increases in proliferation, increases in expression of cell surface markers of activation (e.g. CD25, CD69, CD137, and PD1), cytotoxicity (ability to kill target cells), and cytokine production (e.g. IL-2, IL-4, IL-6, IFNγ, TNF-a, IL-10, IL-17A) are indicative of immune modulation, e.g. enhanced killing of cancer cells.

[0017] Accordingly, the present disclosure provides antibodies, including antigen binding domains, that bind to human PVRIG pps and methods of activating T cells and/or NK cells to treat diseases such as cancer.

II. PVRIG Proteins

[0018] The present disclosure provides antibodies that specifically bind to PVRIG proteins. "Protein" in this context is used interchangeably with "polypeptide", and includes peptides as well. PVRIG is a transmembrane domain protein of 326 amino acids in length, with a signal peptide (spanning from amino acid 1 to 40), an extracellular domain (spanning from amino acid 41 to 171), a transmembrane domain (spanning from amino acid 172 to 190) and a cytoplasmic domain (spanning from amino acid 191 to 326). The full length human PVRIG protein is shown in Figure 25. There are two methionines that can be start codons, but the mature proteins are identical.

[0019] Accordingly, as used herein, the term "PVRIG" or "PVRIG protein" or "PVRIG polypeptide" may optionally include any such protein, or variants, conjugates, or fragments thereof, including but not limited to known or wild type PVRIG, as described herein, as well as any naturally occurring splice variants, amino acid variants or isoforms, and in particular the ECD fragment of PVRIG. The term "soluble" form of PVRIG is also used interchangeably with the terms "soluble ectodomain (ECD)" or "ectodomain" or "extracellular domain (ECD) as well as "fragments of PVRIG polypeptides", which may refer broadly to one or more of the following optional polypeptides:

[0020] The PVRIG proteins contain an immunoglobulin (Ig) domain within the extracellular domain, which is a PVR-like Ig fold domain. The PVR-like Ig fold domain may be responsible for functional counterpart binding, by analogy to the other B7 family members. The PVR-like Ig fold domain of the extracellular domain includes one disulfide bond formed between intra domain cysteine residues, as is typical for this fold and may be important for structure-function. These cysteines are located at residues 22 and 93 (or 94). In one embodiment, there is provided a soluble fragment of PVRIG that can be used in testing of PVRIG antibodies.

[0021] Included within the definition of PVRIG proteins are PVRIG ECD fragments. Optionally, the PVRIG ECD fragments refer also to any one of the polypeptide sequences listed in Figure 67, which are reasonably expected to comprise functional regions of the PVRIG protein. This expectation is based on a systematic analysis of a set of protein complexes with solved 3D structures, which contained complexes of Ig proteins (for example PDB ID 1i85 which describe the complex of CTLA4 AND CD86). The intermolecular contact residues from each "co-structure" from each PDB were collected and projected on the sequence of PVRIG. Several regions with clusters of interacting residues supported by several contact maps were identified and synthesized as a series of peptides and are reasonably expected to mimic the structure of the intact full length protein and thereby modulate one or more of the effects of PVRIG on immunity and on specific immune cell types. According to at least some embodiments of the invention, the PVRIG ECD fragments represented by polypeptide sequences listed in Figure 67, are located as follows (as compared to human PVRIG ECD of Figure 25, counting from the first amino acid of the ECD): PVRIG Fragment A is located at positions 46 to 66; PVRIG Fragment B is located at positions 46 to 79; PVRIG

Fragment C is located at positions 63 to 79; PVRIG Fragment D is located at positions 91 to 106; PVRIG Fragment E is located at positions 91 to 114; PVRIG Fragment F is located at positions 11 to 25; PVRIG Fragment G is located at positions 3 to 24; PVRIG Fragment H is located at positions 18 to 36; PVRIG Fragment I is located at positions 29 to 52; PVRIG Fragment J is located at positions 73-98.

[0022] As noted herein and more fully described below, anti-PVRIG antibodies (including antigen-binding fragments) that both bind to PVRIG and prevent activation by PVRL2 (e.g. most commonly by blocking the interaction of PVRIG and PVLR2), are used to enhance T cell and/or NK cell activation and be used in treating diseases such as cancer and pathogen infection.

III. Antibodies

[0023] Accordingly, the disclosure provides anti-PVRIG antibodies. PVRIG, also called Poliovirus Receptor Related Immunoglobulin Domain Containing Protein, Q6DKI7 or C7orf15, relates to amino acid and nucleic acid sequences shown in RefSeq accession identifier NP_076975, shown in Figure 25. The antibodies are specific for the PVRIG extracellular domain as more fully outlined herein.

[0024] As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. In general, the term "antibody" includes any polypeptide that includes at least one antigen binding domain, as more fully described below. Antibodies may be polyclonal, monoclonal, xenogeneic, allogeneic, syngeneic, or modified forms thereof, as described herein, with monoclonal antibodies finding particular use in many embodiments. In some embodiments, antibodies of the invention bind specifically to PVRIG molecules. The terms "monoclonal antibodies" and "monoclonal antibody composition", as used herein, refer to a population of antibody molecules that contain only one species of an antigen-binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody molecules that contain multiple species of antigen-binding sites capable of interacting with a particular antigen. A monoclonal antibody composition, typically displays a single binding affinity for a particular antigen with which it immunoreacts.

[0025] Traditional full length antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. While the exemplary antibodies herein designated "CPA" are based on IgG1 heavy constant regions, as shown in Figure 38, the anti-PVRIG antibodies of the invention include those using IgG2, IgG3 and IgG4 sequences, or combinations thereof. For example, as is known in the art, different IgG isotypes have different effector functions which may or may not be desirable. Accordingly, the CPA antibodies of the invention can also swap out the IgG1 constant domains for IgG2, IgG3 or IgG4 constant domains (depicted in Figure 66), with IgG2 and IgG4 finding particular use in a number of situations, for example for ease of manufacture or when reduced effector function is desired, the latter being desired in some situations.

[0026] For the enumerated antibodies of the CHA designation, these are murine antibodies generated in hybridomas (the "H" designation), and thus in general they are humanized as is known in the art, generally in the framework regions (F1 to F4 for each of the heavy and light variable regions), and then grafted onto human IgG1, IgG2, IgG3 or IgG4 constant heavy and light domains (depicted in Figure 66), again with IgG4 finding particular use, as is more fully described below.

[0027] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions".

[0028] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[0029] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region, although sometimes the numbering is shifted slightly as will be appreciated by those in the art; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5 th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below and shown in Figure 40.

[0030] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5 th edition, NIH publication, No. 91-3242, E. A. Kabat et al., entirely incorporated by reference).

[0031] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[0032] Accordingly, the disclosure provides variable heavy domains, variable light domains, heavy constant domains, light constant domains and Fc domains to be used as outlined herein. By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the Vκ or Vλ, and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively. Accordingly, the variable heavy domain comprises vhFR1-vhCDR1-vhFR2-vhCDR2-vhFR3-vhCDR3-vhFR4, and the variable light domain comprises vlFR1-vlCDR1-

vlFR2-vlCDR2-vlFR3-vlCDR3-vlFR4. By "heavy constant region" herein is meant the CH1-hinge-CH2-CH3 portion of an antibody. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0033] Thus, "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index.

[0034] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody. As will be appreciated by those in the art, these generally are made up of two chains.

[0035] Throughout the present specification, either the IMTG numbering system or the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat et al., supra (1991)). EU numbering as in Kabat is generally used for constant domains and/or the Fc domains.

[0036] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[0037] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[0038] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0039] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning". Specific bins are described below.

[0040] Included within the definition of "antibody" is an "antigen-binding portion" of an antibody (also used interchangeably with "antigen-binding fragment", "antibody fragment" and "antibody derivative"). That is, for the purposes of the invention, an antibody of the invention has a minimum functional requirement that it bind to a PVRIG antigen. As will be appreciated by those in the art, there are a large number of antigen fragments and derivatives that retain the ability to bind an antigen and yet have alternative structures, including, but not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., 1988, Science 242:423-426, Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883, entirely incorporated by reference), (iv) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson et. al., 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448, all entirely incorporated by reference), (v) "domain antibodies" or "dAb" (sometimes referred to as an "immunoglobulin single variable domain", including single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and Camelid V-HH dAbs, (vi) SMIPs (small molecule immunopharmaceuticals), camelbodies, nanobodies and IgNAR.

[0041] Still further, an antibody or antigen-binding portion thereof (antigen-binding fragment, antibody fragment, antibody portion) may be part of a larger immunoadhesion molecules (sometimes also referred to as "fusion proteins"), formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules. Antibody portions, such as Fab and F(ab')2 fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

[0042] In general, the anti-PVRIG antibodies of the present disclosure are recombinant. "Recombinant" as used herein, refers broadly with reference to a product, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0043] The term "recombinant antibody", as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo.*

A. Optional Antibody Engineering

[0044] The antibodies can be modified, or engineered, to alter the amino acid sequences by amino acid substitutions.

[0045] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0046] As discussed herein, amino acid substitutions can be made to alter the affinity of the CDRs for the PVRIG protein (including both increasing and decreasing binding, as is more fully outlined below), as well as to alter additional functional properties of the antibodies. For example, the antibodies may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody according to at least some embodiments of the invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Such embodiments are described further below. The numbering of residues in the Fc region is that of the EU index of Kabat.

[0047] In one embodiment, the hinge region of C_{H1} is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Pat. No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[0048] In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired *Staphylococcyl* protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Pat. No. 6,165,745 by Ward et al.

[0049] In some embodiments, amino acid substitutions can be made in the Fc region, in general for altering binding to FcγR receptors. By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII-1 (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[0050] There are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in U.S. Ser. Nos. 11/124,620 (particularly FIG. 41) and U.S. Patent No. 6,737,056, both of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 299T and 297N.

[0051] In addition, the antibodies are modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Pat. No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the C_{H1} or C_L region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Pat. Nos. 5,869,046 and 6,121,022 by Presta et al. Additional mutations to increase serum half life are disclosed in U.S. Patent Nos. 8,883,973, 6,737,056 and 7,371,826, and include 428L, 434A, 434S, and 428L/434S.

[0052] In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos. 5,624,821 and 5,648,260, both by Winter et al.

[0053] In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Pat. Nos. 6,194,551 by Idusogie et al.

[0054] In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in

PCT Publication WO 94/29351 by Bodmer et al.

[0055] In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R. L. et al. (2001) J. Biol. Chem. 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 are shown to improve binding to FcγRIII. Additionally, the following combination mutants are shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A. Furthermore, mutations such as M252Y/S254T/T256E or M428L/N434S improve binding to FcRn and increase antibody circulation half-life (see Chan CA and Carter PJ (2010) Nature Rev Immunol 10:301-316).

[0056] In still another embodiment, the antibody can be modified to abrogate *in vivo* Fab arm exchange. Specifically, this process involves the exchange of IgG4 half-molecules (one heavy chain plus one light chain) between other IgG4 antibodies that effectively results in bispecific antibodies which are functionally monovalent. Mutations to the hinge region and constant domains of the heavy chain can abrogate this exchange (see Aalberse, RC, Schuurman J., 2002, Immunology 105:9-19).

[0057] In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen or reduce effector function such as ADCC. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence, for example N297. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site.

[0058] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies according to at least some embodiments of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (α (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 FUT8 cell lines are created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane et al. and Yamane-Ohnuki et al. (2004) Biotechnol Bioeng 87:614-22). As another example, EP 1,176,195 by Hanai et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the α 1,6 bond-related enzyme. Hanai et al. also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R. L. et al. (2002) J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., β(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al. (1999) Nat. Biotech. 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase α-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A. L. et al. (1975) Biochem. 14:5516-23).

[0059] Another modification of the antibodies herein that is is pegylation or the addition of other water soluble moieties, typically polymers, e.g., in order to enhance half-life. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies according to at least some embodiments of the invention. See for example, EP 0 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

[0060] In addition to substitutions made to alter binding affinity to FcγRs and/or FcRn and/or increase in vivo serum half life, additional antibody modifications can be made, as described in further detail below.

[0061] In some cases, affinity maturation is done. Amino acid modifications in the CDRs are sometimes referred to as "affinity maturation". An "affinity matured" antibody is one having one or more alteration(s) in one or more CDRs which results in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some cases, although rare, it may be desirable to decrease the affinity of an antibody to its antigen, but this is generally not preferred.

[0062] In some embodiments, one or more amino acid modifications are made in one or more of the CDRs of the VISG1 antibodies In general, only 1 or 2 or 3-amino acids are substituted in any single CDR, and generally no more than from 1, 2, 3. 4, 5, 6, 7, 8 9 or 10 changes are made within a set of CDRs. However, it should be appreciated that any combination of no substitutions, 1, 2 or 3 substitutions in any CDR can be independently and optionally combined with any other substitution.

[0063] Affinity maturation can be done to increase the binding affinity of the antibody for the PVRIG antigen by at least about 10% to 50-100-150% or more, or from 1 to 5 fold as compared to the "parent" antibody. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the PVRIG antigen. Affinity matured antibodies are produced by known procedures. See, for example, Marks et al., 1992, Biotechnology 10:779-783 that describes affinity maturation by variable heavy chain (VH) and variable light chain (VL) domain shuffling. Random mutagenesis of CDR and/or framework residues is described in: Barbas, et al. 1994, Proc. Nat. Acad. Sci, USA 91:3809-3813; Shier et al., 1995, Gene 169:147-155; Yelton et al., 1995, J. Immunol. 155:1994-2004; Jackson et al., 1995, J. Immunol. 154(7):3310-9; and Hawkins et al, 1992, J. Mol. Biol. 226:889-896, for example.

[0064] Alternatively, amino acid modifications can be made in one or more of the CDRs of the antibodies of the invention that are "silent", e.g. that do not significantly alter the affinity of the antibody for the antigen. These can be made for a number of reasons, including optimizing expression (as can be done for the nucleic acids encoding the antibodies of the invention).

[0065] Thus, included within the definition of the CDRs and are variant CDRs and antibodies; that is, the antibodies of the invention can include amino acid modifications in one or more of the CDRs of the enumerated antibodies of the invention. In addition, as outlined below, amino acid modifications can also independently and optionally be made in any region outside the CDRs, including framework and constant regions.

IV. PVRIG Antibodies

[0066] Provided are anti-PVRIG antibodies. (For convenience, "anti-PVRIG antibodies" and "PVRIG antibodies" are used interchangeably). The anti-PVRIG antibodies specifically bind to human PVRIG, and preferably the ECD of human VISG1, as depicted in Figure 25.

[0067] Specific binding for PVRIG or a PVRIG epitope can be exhibited, for example, by an antibody having a KD of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the PVRIG antigen or epitope.

[0068] However, as shown in the Examples, for optimal binding to PVRIG expressed on the surface of NK and T-cells, the antibodies preferably have a KD less 50 nM and most preferably less than 1 nM, with less than 0.1 nM and less than 1 pM and 0.1 pM finding use in the methods of the invention.

[0069] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for a PVRIG antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.

[0070] In some embodiments, the anti-PVRIG antibodies bind to human PVRIG with a K_D of 100 nM or less, 50 nM or less, 10 nM or less, or 1 nM or less (that is, higher binding affinity), or 1pM or less, wherein K_D is determined by known methods, e.g. surface plasmon resonance (SPR, e.g. Biacore assays), ELISA, KINEXA, and most typically SPR at 25° or 37° C.

A. Specific anti-PVRIG antibodies

[0071] Also provided are antigen binding domains, including full length antibodies, which contain a number of specific, enumerated sets of 6 CDRs.

[0072] The antibodies described herein as labeled as follows. The antibodies have reference numbers, for example "CPA.7.013". This represents the combination of the variable heavy and variable light chains, as depicted in Figure 38 and Figure 39 for example. "CPA.7.013.VH" refers to the variable heavy portion of

CPA.7.013, while "CPA.7.013.VL" is the variable light chain. "CPA.7.013.vhCDR1", "CPA.7.013.vhCDR2", "CPA.7.013.vhCDR3", "CPA.7.013.v1CDR1", "CPA.7.013.v1CDR2", and "CPA.7.013.vlCDR3", refers to the CDRs are indicated. "CPA.7.013.HC" refers to the entire heavy chain (e.g. variable and constant domain) of this molecule, and "CPA.7.013.LC" refers to the entire light light chain (e.g. variable and constant domain) of the same molecule. "CPA.7.013.H1" refers to a full length antibody comprising the variable heavy and light domains, including the constant domain of **H**uman IgG**1** (hence, the H1; IgG1, IgG2, IgG3 and IgG4 sequences are shown in Figure 66). Accordingly, "CPA.7.013.H2" would be the CPA.7.013 variable domains linked to a **H**uman IgG**2**. "CPA.7.013.H3" would be the CPA.7.013 variable domains linked to a **H**uman IgG**3**, and "CPA.7.013.H4" would be the CPA.7.013 variable domains linked to a **H**uman IgG**4**.

[0073] Further provided are variable heavy and light domains as well as full length heavy and light chains.

[0074] In many embodiments, the antibodies are human (derived from phage) and block binding of PVRIG and PVLR2. As shown in Figure 52, the CPA antibodies that both bind and block the receptor-ligand interaction are as below, with their components outlined as well:

CPA.7.001, CPA.7.001.VH, CPA.7.001.VL, CPA.7.001.HC, CPA.7.001.LC and CPA.7.001.H1, CPA.7.001.H2, CPA.7.001.H3, CPA.7.001.H4; CPA.7.001.vhCDR1, CPA.7.001.vhCDR2, CPA.7.001.vhCDR3, CPA.7.001.vlCDR1, CPA.7.001.vlCDR2, and CPA.7.001.vlCDR3;

CPA.7.003, CPA.7.003.VH, CPA.7.003.VL, CPA.7.003.HC, CPA.7.003.LC, CPA.7.003.H1, CPA.7.003.H2, CPA.7.003.H3, CPA.7.003.H4; CPA.7.003.vhCDR1, CPA.7.003.vhCDR2, CPA.7.003.vhCDR3, CPA.7.003.vlCDR1, CPA.7.003.vlCDR2, and CPA.7.003.vlCDR3;

CPA.7.004, CPA.7.004.VH, CPA.7.004.VL, CPA.7.004.HC, CPA.7.004.LC, CPA.7.004.H1, CPA.7.004.H2, CPA.7.004.H3 CPA.7.004.H4; CPA.7.004.vhCDR1, CPA.7.004.vhCDR2, CPA.7.004.vhCDR3, CPA.7.004.vlCDR1, CPA.7.004.vlCDR2, and CPA.7.004.vlCDR3;

CPA.7.006, CPA.7.006.VH, CPA.7.006.VL, CPA.7.006.HC, CPA.7.006.LC, CPA.7.006.H1, CPA.7.006.H2, CPA.7.006.H3 CPA.7.006.H4; CPA.7.006.vhCDR1, CPA.7.006.vhCDR2, CPA.7.006.vhCDR3, CPA.7.006.vlCDR1, CPA.7.006.vlCDR2, and CPA.7.006.vlCDR3;

CPA.7.008, CPA.7.008.VH, CPA.7.008.VL, CPA.7.008.HC, CPA.7.008.LC, CPA.7.008.H1, CPA.7.008.H2, CPA.7.008.H3 CPA.7.008.H4; CPA.7.008.vhCDR1, CPA.7.008.vhCDR2, CPA.7.008.vhCDR3, CPA.7.008.vlCDR1, CPA.7.008.vlCDR2, and CPA.7.008.vlCDR3;

CPA.7.009, CPA.7.009.VH, CPA.7.009.VL, CPA.7.009.HC, CPA.7.009.LC, CPA.7.009.H1, CPA.7.009.H2, CPA.7.009.H3 CPA.7.009.H4; CPA.7.009.vhCDR1, CPA.7.009.vhCDR2, CPA.7.009.vhCDR3, CPA.7.009.vlCDR1, CPA.7.009.vlCDR2, and CPA.7.009.vlCDR3;

CPA.7.010, CPA.7.010.VH, CPA.7.010.VL, CPA.7.010.HC, CPA.7.010.LC, CPA.7.010.H1, CPA.7.010.H2, CPA.7.010.H3 CPA.7.010.H4; CPA.7.010.vhCDR1, CPA.7.010.vhCDR2, CPA.7.010.vhCDR3, CPA.7.010.vlCDR1, CPA.7.010.vlCDR2, and CPA.7.010.vlCDR3;

CPA.7.011, CPA.7.011.VH, CPA.7.011.VL, CPA.7.011.HC, CPA.7.011.LC, CPA.7.011.H1, CPA.7.011.H2, CPA.7.011.H3 CPA.7.011.H4; CPA.7.011.vhCDR1, CPA.7.011.vhCDR2, CPA.7.011.vhCDR3, CPA.7.011.vlCDR1, CPA.7.011.vlCDR2, and CPA.7.011.vlCDR3;

CPA.7.012, CPA.7.012.VH, CPA.7.012.VL, CPA.7.012.HC, CPA.7.012.LC, CPA.7.012.H1, CPA.7.012.H2, CPA.7.012.H3 CPA.7.012.H4; CPA.7.012.vhCDR1, CPA.7.012.vhCDR2, CPA.7.012.vhCDR3, CPA.7.012.vlCDR1, CPA.7.012.vlCDR2, and CPA.7.012.vlCDR3;

CPA.7.013, CPA.7.013.VH, CPA.7.013.VL, CPA.7.013.HC, CPA.7.013.LC, CPA.7.013.H1, CPA.7.013.H2, CPA.7.013.H3 CPA.7.013.H4; CPA.7.013.vhCDR1, CPA.7.013.vhCDR2, CPA.7.013.vhCDR3, CPA.7.013.vlCDR1, CPA.7.013.vlCDR2, and CPA.7.013.vlCDR3;

CPA.7.014, CPA.7.014.VH, CPA.7.014.VL, CPA.7.014.HC, CPA.7.014.LC, CPA.7.014.H1, CPA.7.014.H2, CPA.7.014.H3 CPA.7.014.H4; CPA.7.014.vhCDR1, CPA.7.014.vhCDR2, CPA.7.014.vhCDR3, CPA.7.014.vlCDR1, CPA.7.014.vlCDR2, and CPA.7.014.vlCDR3;

CPA.7.015, CPA.7.015.VH, CPA.7.015.VL, CPA.7.015.HC, CPA.7.015.LC, CPA.7.015.H1, CPA.7.015.H2, CPA.7.015.H3 CPA.7.015.H4; CPA.7.015.vhCDR1, CPA.7.015.vhCDR2, CPA.7.015.vhCDR3, CPA.7.015.vlCDR1, CPA.7.015.vlCDR2, and CPA.7.015.vlCDR3;

CPA.7.017, CPA.7.017.VH, CPA.7.017.VL, CPA.7.017.HC, CPA.7.017.LC, CPA.7.017H1, CPA.7.017.H2, CPA.7.017.H3 CPA.7.017.H4; CPA.7.017.vhCDR1, CPA.7.000171.vhCDR2, CPA.7.017.vhCDR3, CPA.7.017.vlCDR1, CPA.7.017.vlCDR2, and CPA.7.017.vlCDR3;

CPA.7.018, CPA.7.018.VH, CPA.7.018.VL, CPA.7.018.HC, CPA.7.018.LC, CPA.7.018.H1, CPA.7.018.H2, CPA.7.018.H3 CPA.7.018.H4; CPA.7.017.vhCDR1, CPA.7.017.vhCDR2, CPA.7.017.vhCDR3, CPA.7.017.vlCDR1, CPA.7.017.vlCDR2, and CPA.7.017.vlCDR3;

CPA.7.019, CPA.7.019.VH, CPA.7.019.VL, CPA.7.019.HC, CPA.7.019.LC, CPA.7.019.H1, CPA.7.019.H2, CPA.7.019.H3 CPA.7.019.H4; CPA.7.019.vhCDR1, CPA.7.019.vhCDR2, CPA.7.019.vhCDR3, CPA.7.019.vlCDR1, CPA.7.019.vlCDR2, and CPA.7.019.vlCDR3;

CPA.7.021, CPA.7.021.VH, CPA.7.021.VL, CPA.7.021.HC, CPA.7.021.LC, CPA.7.021.H1, CPA.7.021.H2, CPA.7.021.H3 CPA.7.021.H4; CPA.7.021.vhCDR1, CPA.7.021.vhCDR2, CPA.7.021.vhCDR3, CPA.7.021.vlCDR1, CPA.7.021.vlCDR2, and CPA.7.021.vlCDR3;

CPA.7.022, CPA.7.022.VH, CPA.7.022.VL, CPA.7.022.HC, CPA.7.022.LC, CPA.7.022.H1, CPA.7.022.H2, CPA.7.022.H3 CPA.7.022.H4; CPA.7.022.vhCDR1, CPA.7.022.vhCDR2, CPA.7.002201.vhCDR3, CPA.7.022.vlCDR1, CPA.7.022.vlCDR2, and CPA.7.022.vlCDR3;

CPA.7.023, CPA.7.023.VH, CPA.7.023.VL, CPA.7.023.HC, CPA.7.023.LC, CPA.7.023.H1, CPA.7.023.H2, CPA.7.023.H3 CPA.7.023.H4; CPA.7.023.vhCDR1, CPA.7.023.vhCDR2, CPA.7.023.vhCDR3, CPA.7.023.vlCDR1, CPA.7.023.vlCDR2, and CPA.7.023.vlCDR3;

CPA.7.024, CPA.7.024.VH, CPA.7.024.VL, CPA.7.024.HC, CPA.7.024.LC, CPA.7.024.H1, CPA.7.024.H2, CPA.7.024.H3 CPA.7.024.H4; CPA.7.024.vhCDR1, CPA.7.024.vhCDR2, CPA.7.024.vhCDR3, CPA.7.024.vlCDR1, CPA.7.024.vlCDR2, and CPA.7.024.vlCDR3;

CPA.7.033, CPA.7.033.VH, CPA.7.033.VL, CPA.7.033.HC, CPA.7.033.LC, CPA.7.033.H1, CPA.7.033.H2, CPA.7.033.H3 CPA.7.033.H4; CPA.7.033.vhCDR1, CPA.7.033.vhCDR2, CPA.7.033.vhCDR3, CPA.7.033.vlCDR1, CPA.7.033.vlCDR2, and CPA.7.033.vlCDR3;

CPA.7.034, CPA.7.034.VH, CPA.7.034.VL, CPA.7.034.HC, CPA.7.034.LC, CPA.7.034.H1, CPA.7.034.H2, CPA.7.034.H3 CPA.7.034.H4; CPA.7.034.vhCDR1, CPA.7.034.vhCDR2, CPA.7.034.vhCDR3, CPA.7.034.vlCDR1, CPA.7.034.vlCDR2, and CPA.7.034.vlCDR3;

CPA.7.036, CPA.7.036.VH, CPA.7.036.VL, CPA.7.036.HC, CPA.7.036.LC, CPA.7.036.H1, CPA.7.036.H2, CPA.7.036.H3 CPA.7.036.H4; CPA.7.036.vhCDRI, CPA.7.036.vhCDR2, CPA.7.036.vhCDR3, CPA.7.036.vlCDR1, CPA.7.036.vlCDR2, and CPA.7.036.vlCDR3;

CPA.7.040, CPA.7.040.VH, CPA.7.040.VL, CPA.7.040.HC, CPA.7.040.LC, CPA.7.040.H1, CPA.7.040.H2, CPA.7.040.H3 and CPA.7.040.H4; CPA.7.040.vhCDR1, CPA.7.040.vhCDR2, CPA.7.040.vhCDR3, CPA.7.040.vlCDR1, CPA.7.040.vlCDR2, and CPA.7.040.vlCDR3;

CPA.7.046, CPA.7.046.VH, CPA.7.046.VL, CPA.7.046.HC, CPA.7.046.LC, CPA.7.046.H1, CPA.7.046.H2, CPA.7.046.H3 CPA.7.046.H4; CPA.7.046.vhCDR1, CPA.7.046.vhCDR2, CPA.7.046.vhCDR3, CPA.7.046.vlCDR1, CPA.7.046.vlCDR2, and CPA.7.046.vlCDR3;

CPA.7.047, CPA.7.047.VH, CPA.7.047.VL, CPA.7.047.HC, CPA.7.047.LC, CPA.7.047.H1, CPA.7.047.H2, CPA.7.047.H3 CPA.7.047.H4; CPA.7.047.vhCDR1, CPA.7.047.vhCDR2, CPA.7.047.vhCDR3, CPA.7.047.vlCDR1, CPA.7.004701.vlCDR2, and CPA.7.047.vlCDR3;

CPA.7.049, CPA.7.049.VH, CPA.7.049.VL, CPA.7.049.HC, CPA.7.049.LC, CPA.7.049.H1, CPA.7.049.H2, CPA.7.049.H3 CPA.7.049.H4; CPA.7.049.vhCDR1, CPA.7.049.vhCDR2, CPA.7.049.vhCDR3, CPA.7.049.vlCDR1, CPA.7.049.vlCDR2, and CPA.7.049.vlCDR3; and

CPA.7.050, CPA.7.050.VH, CPA.7.050.VL, CPA.7.050.HC, CPA.7.050.LC, CPA.7.050.H1, CPA.7.050.H2, CPA.7.050.H3 CPA.7.050.H4, CPA.7.050.vhCDR1, CPA.7.050.vhCDR2, CPA.7.050.vhCDR3, CPA.7.050.vlCDR1, CPA.7.050.vlCDR2, and CPA.7.050.vlCDR3.

In addition, there are a number of CPA antibodies generated herein that bound to PVRIG but did not block the interaction of PVRIG and PVLR2 as shown in Figure 52, only eight of which sequences are included herein in Figure 40, the components of which are

CPA.7.028, CPA.7.028.VH, CPA.7.028.VL, CPA.7.028.HC, CPA.7.028.LC, CPA.7.028.H1, CPA.7.028.H2, CPA.7.028.H3 and CPA.7.028.H4; CPA.7.028.vhCDR1, CPA.7.028.vhCDR2, CPA.7.028.vhCDR3, CPA.7.028.vlCDR1, CPA.7.028.vlCDR2, and CPA.7.028.vlCDR3.

CPA.7.030, CPA.7.030.VH, CPA.7.030.VL, CPA.7.030.HC, CPA.7.030.LC, CPA.7.030.H1, CPA.7.030.H2, CPA.7.030.H3 and CPA.7.030.H4; CPA.7.030.vhCDR1, CPA.7.030.vhCDR2, CPA.7.030.vhCDR3, CPA.7.030.vlCDR1, CPA.7.030.vlCDR2, and CPA.7.030.vlCDR3.

CPA.7.041, CPA.7.041.VH, CPA.7.041.VL, CPA.7.041.HC, CPA.7.041.LC, CPA.7.041.H1, CPA.7.041.H2, CPA.7.041.H3 and CPA.7.041.H4; CPA.7.041.vhCDR1, CPA.7.041.vhCDR2, CPA.7.041.vhCDR3, CPA.7.041.vlCDR1, CPA.7.041.vlCDR2, and CPA.7.041.vlCDR3.

CPA.7.016, CPA.7.016.VH, CPA.7.016.VL, CPA.7.016.HC, CPA.7.016.LC, CPA.7.016.H1, CPA.7.016.H2, CPA.7.016.H3 and CPA.7.016.H4; CPA.7.016.vhCDR1, CPA.7.016.vhCDR2, CPA.7.016.vhCDR3, CPA.7.016.vlCDR1, CPA.7.016.vlCDR2, and CPA.7.016.vlCDR3.

CPA.7.020, CPA.7.020.VH, CPA.7.020.VL, CPA.7.020.HC, CPA.7.020.LC, CPA.7.020.H1, CPA.7.020.H2, CPA.7.020.H3 and CPA.7.020.H4; CPA.7.020.vhCDR1, CPA.7.020.vhCDR2, CPA.7.020.vhCDR3, CPA.7.020.vlCDR1, CPA.7.020.vlCDR2, and CPA.7.020.vlCDR3.

CPA.7.038, CPA.7.038.VH, CPA.7.038.VL, CPA.7.038.HC, CPA.7.038.LC, CPA.7.038.H1, CPA.7.038.H2, CPA.7.038.H3 and CPA.7.038.H4; CPA.7.038.vhCDR1, CPA.7.038.vhCDR2, CPA.7.038.vhCDR3, CPA.7.038.vlCDR1, CPA.7.038.vlCDR2, and CPA.7.038.vlCDR3.

CPA.7.044, CPA.7.044.VH, CPA.7.044.VL, CPA.7.044.HC, CPA.7.044.LC, CPA.7.044.H1, CPA.7.044.H2, CPA.7.044.H3 and CPA.7.044.H4; CPA.7.044.vhCDR1, CPA.7.044.vhCDR2, CPA.7.044.vhCDR3, CPA.7.044.vlCDR1, CPA.7.044.vlCDR2, and CPA.7.044.vlCDR3.

CPA.7.045, CPA.7.045.VH, CPA.7.045.VL, CPA.7.045.HC, CPA.7.045.LC, CPA.7.045.H1, CPA.7.045.H2, CPA.7.045.H3 and CPA.7.045.H4; CPA.7.045.vhCDR1, CPA.7.045.vhCDR2, CPA.7.045.vhCDR3, CPA.7.045.vlCDR1, CPA.7.045.vlCDR2, and CPA.7.045.vlCDR3.

[0075] As discussed herein, the invention further provides variants of the above components, including variants in the CDRs, as outlined above. In addition, variable heavy chains can be 80%, 90%, 95%, 98% or 99% identical to the "VH" sequences herein, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid changes, or more, when Fc variants are used. Variable light chains are provided that can be 80%, 90%, 95%, 98% or 99% identical to the "VL" sequences herein, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid changes, or more, when Fc variants are used. Similarly, heavy and light chains are provided that are 80%, 90%, 95%, 98% or 99% identical to the "HC" and "LC" sequences herein, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid changes, or more, when Fc variants are used.

[0076] Furthermore, the present disclosure provides a number of CHA antibodies, which are murine antibodies generated from hybridomas. As is well known the art, the six CDRs are useful when put into either human framework variable heavy and variable light regions or when the variable heavy and light domains are humanized.

[0077] Accordingly, the present disclosure provides antibodies, usually full length or scFv domains, that comprise the following CHA sets of CDRs, the sequences of which are shown in Figure 41:

CHA.7.516.vhCDR1, CHA.7.516.vhCDR2, CHA.7.516.vhCDR3, CHA.7.516.vlCDR1, CHA.7.516.vlCDR2, and CHA.7.516.vlCDR3.

CHA.7.518.vhCDR1, CHA.7.518.vhCDR2, CHA.7.518.vhCDR3, CHA.7.518.vlCDR1, CHA.7.518.vlCDR2, and CHA.7.518.vlCDR3.

CHA.7.524.vhCDR1, CHA.7.524.vhCDR2, CHA.7.524.vhCDR3, CHA.7.524.vlCDR1, CHA.7.524.vlCDR2, and CHA.7.524.vlCDR3.

CHA.7.528.vhCDR1, CHA.7.528.vhCDR2, CHA.7.528.vhCDR3, CHA.7.528.vlCDR1, CHA.7.528.vlCDR2, and CHA.7.528.vlCDR3.

CHA.7.530.vhCDR1, CHA.7.530.vhCDR2, CHA.7.530.vhCDR3, CHA.7.530.vlCDR1, CHA.7.530.vlCDR2, and CHA.7.530.vlCDR3.

CHA.7.537.vhCDR1, CHA.7.537.vhCDR2, CHA.7.537.vhCDR3, CHA.7.537.vlCDR1, CHA.7.537.vlCDR2, and CHA.7.537.vlCDR3.

CHA.7.538_1.vhCDR1, CHA.7.538_1.vhCDR2, CHA.7.538_1.vhCDR3, CHA.7.538_1.vlCDR1, CHA.7.538_1.vlCDR2, and CHA.7.538_1.vlCDR3.

CHA.7.538_2.vhCDR1, CHA.7.538_2.vhCDR2, CHA.7.538_2.vhCDR3, CHA.7.538 2.vlCDR1, CHA.7.538_2.vlCDR2, and CHA.7.538_2.vlCDR3.

CHA.7.548.vhCDR1, CHA.7.548.vhCDR2, CHA.7.548.vhCDR3, CHA.7.548.v1CDR1, CHA.7.548.v1CDR2, and CHA.7.548.vlCDR3.

[0078] As above, these sets of CDRs may also be amino acid variants as described above.

[0079] In addition, the framework regions of the variable heavy and variable light chains can be humanized as is known in the art (with occasional variants generated in the CDRs as needed), and thus humanized variants of the VH and VL chains of Figure 41 can be generated. Furthermore, the humanized variable heavy and light domains can then be fused with human constant regions, such as the constant regions from IgG1, IgG2, IgG3 and IgG4.

[0080] In particular, as is known in the art, murine VH and VL chains can be humanized as is known in the art, for example, using the IgBLAST program of the NCBI website, as outlined in Ye et al. Nucleic Acids Res. 41:W34-W40 (2013), herein incorporated by reference in its entirety for the humanization methods. IgBLAST takes a murine VH and/or VL sequence and compares it to a library of known human germline sequences. As shown herein, for the humanized sequences generated herein, the databases used were IMGT human VH genes (F+ORF, 273 germline sequences) and IMGT human VL kappa genes (F+ORF, 74 germline sequences). An exemplary five CHA sequences were chosen: CHA.7.518, CHA.7.530, CHA.7.538_1, CHA.7.538_2 and CHA.7.524 (see Figure 41 for the VH and VL sequences). For this embodiment of the humanization, human germline IGHV1-46(allele1) was chosen for all 5 as the acceptor sequence and the human heavy chain IGHJ4(allele1) joining region (J gene). For three of four (CHA.7.518, CHA.7.530, CHA.7.538_1 and CHA.7.538_2), human germline IGKV1-39(allele 1) was chosen as the acceptor sequence and human light chain IGKJ2(allele1) (J gene) was chosen. The J gene was chosen from human joining region sequences compiled at IMGT® the international ImMunoGeneTics information system as www.imgt.org. CDRs were defined according to the AbM definition (see www.bioinfo.org.uk/abs/). Figures 88 depicts humanized sequences as well as some potential changes to optimize binding to PVRIG.

[0081] Specific humanized antibodies of CHA antibodies include those shown in Figures 88, Figures 89 and Figure 90. As will be appreciated by those in the art, each humanized variable heavy (**H**umanized **H**eavy; HH) and variable light (**H**umanized **L**ight, HL) sequence can be combined with the constant regions of human IgG1, IgG2, IgG3 and IgG4. That is, CHA.7.518.HH1 is the first humanized variable heavy chain, and CHA.7.518.HH1.**1** is the full length heavy chain, comprising the "HH1" humanized sequence with a IgG**1** constant region (CHA.7.518.HH1.2 is CHA.7.518.HH1 with IgG2, etc,).

[0082] In some embodiments, the anti-PVRIG antibodies include anti-PVRIG antibodies wherein the V_H and V_L sequences of different anti-PVRIG antibodies can be "mixed and matched" to create other anti-PVRIG antibodies. PVRIG binding of such "mixed and matched" antibodies can be tested using the binding assays described above. e.g., ELISAs). In some embodiments, when V_H and V_L chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, in some embodiments, a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence. For example, the V_H and V_L sequences of homologous antibodies are particularly amenable for mixing and matching.

[0083] Accordingly, the antibodies comprise CDR amino acid sequences selected from the group consisting of (a) sequences as listed herein; (b) sequences that differ from those CDR amino acid sequences specified in (a) by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions; (c) amino acid sequences having 90% or greater, 95% or greater, 98% or greater, or 99% or greater sequence identity to the sequences specified in (a) or (b); (d) a polypeptide having an amino acid sequence encoded by a polynucleotide having a nucleic acid sequence encoding the amino acids as listed herein.

[0084] Additionally included in the definition of PVRIG antibodies are antibodies that share identity to the PVRIG antibodies enumerated herein. That is, in certain embodiments, an anti-PVRIG antibody according to the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to isolated anti-PVRIG amino acid sequences of preferred anti-PVRIG immune molecules, respectively, wherein the antibodies retain the desired functional properties of the parent anti-PVRIG antibodies. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology=# of identical positions/total # of positions X 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[0085] The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available commercially), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0086] Additionally or alternatively, the protein sequences can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the antibody molecules according to at least some embodiments of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0087] In general, the percentage identity for comparison between PVRIG antibodies is at least 75%, at least 80%, at least 90%, with at least about 95, 96, 97, 98 or 99% percent identity being preferred. The percentage identity may be along the whole amino acid sequence, for example the entire heavy or light chain or along a portion of the chains. For example, included within the definition of the anti-PVRIG antibodies of the invention are those that share identity along the entire variable region (for example, where the identity is 95 or 98% identical along the variable regions), or along the entire constant region, or along just the Fc domain.

[0088] In addition, also included are sequences that may have the identical CDRs but changes in the variable domain (or entire heavy or light chain). For example, PVRIG antibodies include those with CDRs identical to those shown in Figure 63 but whose identity along the variable region can be lower, for example 95 or 98% percent identical.

B. PVRIG Antibodies that Compete for binding with Enumerated Antibodies

[0089] The present disclosure provides not only the enumerated antibodies but additional antibodies that compete with the enumerated antibodies (the CPA and CHA numbers enumerated herein that specifically bind to PVRIG) to specifically bind to the PVRIG molecule. As is shown in **Example 11,** the PVRIG antibodies of the invention "bin" into different epitope bins. There are four separate bins outlined herein; 1) the epitope bin into which CPA.7.002, CPA.7.003, CPA.7.005, CPA.7.007, CPA.7.010, CPA.7.012, CPA.7.015, CPA.7.016, CPA.7.017, CPA.7.019, CPA.7.020, CPA.7.021, CPA.7.024, CPA.7.028, CPA.7.032, CPA.7.033, CPA.7.036, CPA.7.037, CPA.7.038, CPA.7.043, CPA.7.046 and CPA.7.041 all fall into; 2) the epitope bin into which CPA.7.004, CPA.7.009, CPA.7.011, CPA.7.014, CPA.7.018, CPA.7.022, CPA.7.023, CPA.7.034, CPA.7.040, CPA.7.045 and CPA.7.047 all fall; 3) CPA.7.039, which defines the distinction between bin 1 and bin 2, in that bin 1 blocks CPA.7.039 binding and bin 2 sandwiches the ligand with CPA.7.039, and bin 4) with CPA.7.050.

[0090] Thus, the disclosure provides anti-PVRIG antibodies that compete for binding with antibodies that are in bin 1.

[0091] Additional antibodies that compete with the enumerated antibodies are generated, as is known in the art and generally outlined below. Competitive binding studies can be done as is known in the art, generally using SPR/Biacore® binding assays, as well as ELISA and cell-based assays.

C. Generation of Additional Antibodies

[0092] Additional antibodies to human PVRIG can be done as is well known in the art, using well known methods such as those outlined in the examples. Thus, additional anti-PVRIG antibodies can be generated by traditional methods such as immunizing mice (sometimes using DNA immunization, for example, such as is used by Aldevron), followed by screening against human PVRIG protein and hybridoma generation, with antibody purification and recovery.

V. Formulations of Anti-PVRIG Antibodies

[0093] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl orbenzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN.TM., PLURONICS.TM. or polyethylene glycol (PEG).

[0094] The pharmaceutical composition that comprises the antibodies may be in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for in vivo administration are preferrably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

[0095] Administration of the pharmaceutical composition comprising antibodies of the present disclosure preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to subcutaneously and intravenously. Subcutaneous administration may be preferable in some circumstances because the patient may self-administer the pharmaceutical composition. Many protein therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and polysorbate (see WO 04091658). Fc polypeptides may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility.

[0096] As is known in the art, protein therapeutics are often delivered by IV infusion or bolus. The antibodies may also be delivered using such methods. For example, administration may venious be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

[0097] In addition, any of a number of delivery systems are known in the art and may be used to administer the Fc variants Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (eg. PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate, ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the LUPRON DEPOT.RTM., and poly-D-(-)-3-hydroxyburyric acid. The antibodies disclosed herein may also be formulated as immunoliposomes. A liposome is a small vesicle comprising various types of lipids, phospholipids and/or surfactant that is useful for delivery of a therapeutic agent to a mammal. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., 1985, Proc Natl Acad Sci USA, 82:3688; Hwang et al., 1980, Proc Natl Acad Sci USA, 77:4030; U.S. Pat. No. 4,485,045; U.S. Pat. No. 4,544,545; and PCT WO 97/38731. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. A chemotherapeutic agent or other therapeutically active agent is optionally contained within the liposome (Gabizon et al., 1989, J National Cancer Inst 81:1484).

[0098] The antibodies may also be entrapped in microcapsules prepared by methods including but not limited to coacervation techniques, interfacial polymerization (for example using hydroxymethylcellulose or gelatin-microcapsules, or poly-(methylmethacylate) microcapsules), colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), and macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymer, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT.RTM. (which are injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, and ProLease.RTM. (commercially available from Alkermes), which is a microsphere-based delivery system composed of the desired bioactive molecule incorporated into a matrix of poly-DL-lactide-co-glycolide (PLG).

[0099] The dosing amounts and frequencies of administration are, in a preferred embodiment, selected to be therapeutically or prophylactically effective. As is known in the art, adjustments for protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[0100] The concentration of the antibody in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the Fc variant is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the Fc variant of the present invention may be administered. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.0001 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10 mg/kg being preferred.

VI. Methods of Using Anti-PVRIG Antibodies

[0101] Once made, the anti-PVRIG antibodies find use in a number of different applications.

A. Therapeutic Uses

[0102] The anti-PVRIG antibodies find use in treating patients, such as human subjects, generally with a condition associated with PVRIG. The term "treatment" as used herein, refers to both therapeutic treatment and prophylactic or preventative measures, which relates to treatment of cancer. Those in need of treatment include those already with cancer as well as those in which the cancer is to be prevented. Hence, the mammal to be treated herein may have been diagnosed as having the cancer or may be predisposed or susceptible to the cancer. As used herein the term "treating" refers to preventing, delaying the onset of, curing, reversing, attenuating, alleviating, minimizing, suppressing, halting the deleterious effects or stabilizing of discernible symptoms of the above-described cancerous diseases, disorders or conditions. It also includes managing the cancer as described above. By "manage" it is meant reducing the severity of the disease, reducing the frequency of episodes of the disease, reducing the duration of such episodes, reducing the severity of such episodes, slowing/reducing cancer cell growth or proliferation, slowing progression of at least one symptom, amelioration of at least one measurable physical parameter and the like. For example, immunostimulatory anti-PVRIG immune molecules should promote T cell or NK or cytokine immunity against target cells, e.g., cancer, and thereby treat cancer by depleting the cells involved in the disease condition.

[0103] The PVRIG antibodies are provided in therapeutically effective dosages. A "therapeutically effective dosage" of an anti-PVRIG immune molecule according to at least some embodiments of the present invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, an increase in lifespan, disease remission, or a prevention or reduction of impairment or disability due to the disease affliction. For example, for the treatment of PVRIG positive tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject.

[0104] One of ordinary skill in the art would be able to determine a therapeutically effective amount based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

1. Cancer Treatment

[0105] Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy (or for diagnosis). The PVRIG antibodies find particular use in the treatment of cancer. In general, the antibodies are immunomodulatory, in that rather than directly attack cancerous cells, the anti-PVRIG antibodies stimulate the immune system, generally by inhibiting the action of PVRIG. Thus, unlike tumor-targeted therapies, which are aimed at inhibiting molecular pathways that are crucial for tumor growth and development, and/or depleting tumor cells, cancer immunotherapy is aimed to stimulate the patient's own immune system to eliminate cancer cells, providing long-lived tumor destruction. Various approaches can be used in cancer immunotherapy, among them are therapeutic cancer vaccines to induce tumor-specific T cell responses, and immunostimulatory antibodies (i.e. antagonists of inhibitory receptors = immune checkpoints) to remove immunosuppressive pathways.

[0106] Clinical responses with targeted therapy or conventional anti-cancer therapies tend to be transient as cancer cells develop resistance, and tumor recurrence takes place. However, the clinical use of cancer immunotherapy in the past few years has shown that this type of therapy can have durable clinical responses, showing dramatic impact on long term survival. However, although responses are long term, only a small number of patients respond (as opposed to conventional or targeted therapy, where a large number of patients respond, but responses are transient).

[0107] By the time a tumor is detected clinically, it has already evaded the immune-defense system by acquiring immunoresistant and immunosuppressive properties and creating an immunosuppressive tumor microenvironment through various mechanisms and a variety of immune cells.

[0108] Accordingly, the anti-PVRIG antibodies are useful in treating cancer. Due to the nature of an immuno-oncology mechanism of action, PVRIG does not necessarily need to be overexpressed on or correlated with a particular cancer type; that is, the goal is to have the anti-PVRIG antibodies de-suppress T cell and NK cell activation, such that the immune system will go after the cancers.

[0109] "Cancer," as used herein, refers broadly to any neoplastic disease (whether invasive or metastatic) characterized by abnormal and uncontrolled cell division causing malignant growth or tumor (e.g., unregulated cell growth.) The term "cancer" or "cancerous" as used herein should be understood to encompass any neoplastic disease (whether invasive, non-invasive or metastatic) which is characterized by abnormal and uncontrolled cell division causing malignant growth or tumor, non-limiting examples of which are described herein. This includes any physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer are exemplified in the working examples and also are described within the specification.

[0110] Non-limiting examples of cancer that can be treated using anti-PVRIG antibodies include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; multiple myeloma and post-transplant lymphoproliferative disorder (PTLD).

[0111] Other cancers amenable for treatment by the present invention include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include colorectal, bladder, ovarian, melanoma, squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of colorectal cancer, breast cancer, rectal cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. In an exemplary embodiment the cancer is an early or advanced (including metastatic) bladder, ovarian or melanoma. In another embodiment the cancer is colorectal cancer. The cancerous conditions amenable for treatment of the invention include cancers that express or do not express PVRIG and further include non-metastatic or non-invasive as well as invasive or metastatic cancers wherein PVRIG expression by immune, stromal or diseased cells suppress antitumor responses and anti-invasive immune responses. The method of the present invention is particularly suitable for the treatment of vascularized tumors.

[0112] As shown in the Examples, PVRIG is over expressed and/or correlates with tumor lymphocyte infiltration (as demonstrated by correlation to CD3, CD4, CD8 and PD-1 expression) in a number of different tumors of various origins, and thus is useful in treating any cancer, including but not limited to, prostate cancer, liver cancer (HCC), colorectal cancer, ovarian cancer, endometrial cancer, breast cancer, pancreatic cancer, stomach cancer, cervical cancer, head and neck cancer, thyroid cancer, testis cancer, urothelial cancer, lung cancer, melanoma, non melanoma skin cancer (squamous and basal cell carcinoma), glioma, renal cancer (RCC), lymphoma (non-Hodgkins' lymphoma (NHL) and Hodgkin's lymphoma (HD)), Acute myeloid leukemia (AML),), T cell Acute Lymphoblastic Leukemia (T-ALL), Diffuse Large B cell lymphoma, testicular germ cell tumors, mesothelioma and esophageal cancer

[0113] "Cancer therapy" herein refers to any method which prevents or treats cancer or ameliorates one or more of the symptoms of cancer. Typically such therapies will comprises administration of immunostimulatory anti-PVRIG antibodies (including antigen-binding fragments) either alone or in combination with chemotherapy or radiotherapy or other biologics and for enhancing the activity thereof, i.e., in individuals wherein expression of PVRIG suppresses antitumor responses and the efficacy of chemotherapy or radiotherapy or biologic efficacy.

2. Combination Therapies in Cancer

[0114] As is known in the art, combination therapies comprising a therapeutic antibody targeting an immunotherapy target and an additional therapeutic agent, specific for the disease condition, are showing great promise. For example, in the area of immunotherapy, there are a number of promising combination therapies using a chemotherapeutic agent (either a small molecule drug or an anti-tumor antibody) with immuno-oncology antibodies like anti-PD-1, and as such, the anti-PVRIG antibodies outlined herein can be substituted in the same way. Any chemotherapeutic agent exhibiting anticancer activity can be used according to the present invention; various non-limiting examples are described in the specification.

[0115] The underlying scientific rationale for the dramatic increased efficacy of combination therapy claims that immune checkpoint blockade as a monotherapy will induce tumor regressions only when there is pre-existing strong anti-tumor immune response to be 'unleashed' when the pathway is blocked. However, in most patients and tumor types the endogenous anti-tumor immune responses are weak, and thus the induction of anti-tumor immunity is required for the immune checkpoint blockade to be effective, as shown in the **Figure 1** According to at least some embodiments of the present invention, PVRIG-specific antibodies, antibody fragments, conjugates and compositions comprising same, are used for treatment of all types of cancer in cancer immunotherapy in combination therapy.

[0116] The terms "in combination with" and "co-administration" are not limited to the administration of said prophylactic or therapeutic agents at exactly the same time. Instead, it is meant that the anti-PVRIG antibody and the other agent or agents are administered in a sequence and within a time interval such that they may act together to provide a benefit that is increased versus treatment with only either anti-PVRIG antibody of the present invention or the other agent or agents. It is preferred that the anti-PVRIG antibody and the other agent or agents act additively, and especially preferred that they act synergistically. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The skilled medical practitioner can determine empirically, or by considering the pharmacokinetics and modes of action of the agents, the appropriate dose or doses of each therapeutic agent, as well as the appropriate timings and methods of administration.

[0117] Accordingly, the antibodies may be administered concomitantly with one or more other therapeutic regimens or agents. The additional therapeutic regimes or agents may be used to improve the efficacy or safety of the anti-PVRIG antibody. Also, the additional therapeutic regimes or agents may be used to treat the same disease or a comorbidity rather than to alter the action of the PVRIG antibody. For example, a PVRIG antibody may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and radiation therapy.

[0118] The PVRIG antibodies may be administered in combination with one or more other prophylactic or therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, agents that promote proliferation of hematological cells, angiogenesis inhibitors, protein tyrosine kinase (PTK) inhibitors, or other therapeutic agents.

[0119] According to at least some embodiments, the anti PVRIG immune molecules could be used in combination with any of the known in the art standard of care cancer treatment (as can be found, for example, in http://www.cancer.gov/cancertopics).

[0120] For example, the combination therapy can include an anti PVRIG antibody combined with at least one other therapeutic or immune modulatory agent, other compounds or immunotherapies, or immunostimulatory strategy as described herein. including, but not limited to, tumor vaccines, adoptive T cell therapy, Treg depletion, antibodies (e.g. bevacizumab, Erbitux), peptides, pepti-bodies, small molecules, chemotherapeutic agents such as cytotoxic and cytostatic agents (e.g. paclitaxel, cisplatin, vinorelbine, docetaxel, gemcitabine, temozolomide, irinotecan, 5FU, carboplatin), immunological modifiers such as interferons and interleukins, immunostimulatory antibodies, growth hormones or other cytokines, folic acid, vitamins, minerals, aromatase inhibitors, RNAi, Histone Deacetylase Inhibitors, proteasome inhibitors, doxorubicin (Adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/dose once every four weeks and Adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days.

[0121] According to at least some embodiments therapeutic agents that can be used in combination with anti-PVRIG antibodies are other potentiating agents that enhance anti-tumor responses, e.g. other anti-immune checkpoint antibodies or other potentiating agents that are primarily geared to increase endogenous anti-tumor responses, such as Radiotherapy, Cryotherapy, Conventional/classical chemotherapy potentiating anti-tumor immune responses, Targeted therapy potentiating anti-tumor immune responses, Anti-angiogenic therapy, Therapeutic agents targeting immunosuppressive cells such as Tregs and MDSCs, Immunostimulatory antibodies, Cytokine therapy, Therapeutic cancer vaccines, Adoptive cell transfer.

[0122] In some embodiments, anti-PVRIG antibodies are used in combination with Bisphosphonates, especially amino- bisphosphonates (ABP), which have shown to have anti-cancer activity. Some of the activities associated with ABPs are on human γδT cells that straddle the interface of innate and adaptive immunity and have potent anti-tumour activity.

[0123] Targeted therapies can also stimulate tumor-specific immune response by inducing the immunogenic death of tumor cells or by engaging immune effector mechanisms (Galluzzi et al, 2012, Nature Reviews - Drug discovery, Volume 11, pages 215-233).

[0124] According to at least some embodiments targeted therapies used as agents for combination with anti PVRIG immune molecules for treatment of cancer are as described herein.

[0125] In some embodiments, anti-PVRIG antibodies are used in combination with therapeutic agents targeting regulatory immunosuppressive cells such as regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs). A number of commonly used chemotherapeutics exert non-specific targeting of Tregs and reduce the number or the immunosuppressive capacity of Tregs or MDSCs (Facciabene A. et al 2012 Cancer Res; 72(9) 2162-71; Byrne WL. et al 2011, Cancer Res. 71:691520; Gabrilovich DI. and Nagaraj S, Nature Reviews 2009 Volume 9, pages 162-174). In this regard, metronomic therapy with some chemotherapy drugs results in immunostimulatory rather than immunosuppressive effects, via modulation of regulatory cells. Thus, according to at least some embodiments of the present invention, anti-PVRIG immune molecule for cancer immunotherapy is used in combination with drugs selected from but not limited to cyclophosphamide, gemcitabine, mitoxantrone, fludarabine, fludarabine, docetaxel, paclitaxel, thalidomide and thalidomide derivatives.

[0126] In some embodiments, anti-PVRIG antibodies are used in combination with novel Treg-specific targeting agents including: 1) depleting or killing antibodies that directly target Tregs through recognition of Treg cell surface receptors such as anti-CD25 mAbs daclizumab, basiliximab or 2) ligand-directed toxins such as denileukin diftitox (Ontak) - a fusion protein of human IL-2 and diphtheria toxin, or LMB-2 - a fusion between an scFv against CD25 and Pseudomonas exotoxin and 3) antibodies targeting Treg cell surface receptors such as CTLA4, PD-1, OX40 and GITR or 4) antibodies, small molecules or fusion proteins targeting other NK receptors such as previously identified.

[0127] In some embodiments, anti-PVRIG antibodies are used in combination with any of the options described below for disrupting Treg induction and/or function, including TLR (toll like receptors) agonists; agents that interfere with the adenosinergic pathway, such as ectonucleotidase inhibitors, or inhibitors of the A2A adenosine receptor; TGF-β inhibitors, such as fresolimumab, lerdelimumab, metelimumab, trabedersen, LY2157299, LY210976; blockade of Tregs recruitment to tumor tissues including chemokine receptor inhibitors, such as the CCR4/CCL2/CCL22 pathway.

[0128] In some embodiments, anti-PVRIG antibodies are used in combination with any of the options described below for inhibiting the immunosuppressive tumor microenvironment, including inhibitors of cytokines and enzymes which exert immunosuppressive activities, such as IDO (indoleamine-2,3-dioxygenase) inhibitors; inhibitors of anti-inflammatory cytokines which promote an immunosuppressive microenvironment, such as IL-10, IL-35, IL-4 and IL-13; Bevacizumab® which reduces Tregs and favors the differentiation of DCs.

[0129] In some embodiments, anti-PVRIG antibodies are used in combination with any of the options described below for targeting MDSCs (myeloid-derived suppressive cells), including promoting their differentiation into mature myeloid cells that do not have suppressive functions by Vitamin D3, or Vitamin A metabolites, such as retinoic acid, all-trans retinoic acid (ATRA); inhibition of MDSCs suppressive activity by COX2 inhibitors, phosphodiesterase 5 inhibitors like sildenafil, ROS inhibitors such as nitroaspirin.

[0130] In some embodiments, anti-PVRIG antibodies are used in combination with immunostimulatory antibodies or other agents which potentiate anti-tumor immune responses (Pardoll J Exp Med. 2012; 209(2): 201-209). Immunostimulatory antibodies promote anti-tumor immunity by directly modulating immune functions, i.e. blocking other inhibitory targets or enhancing immunostimulatory proteins. According to at least some embodiments of the present invention, anti--PVRIG immune molecules for cancer immunotherapy is used in combination with antagonistic antibodies targeting additional immune checkpoints including anti-CTLA4 mAbs, such as ipilimumab, tremelimumab; anti-PD-1 such as nivolumab

BMS-936558/ MDX-1106/ONO-4538, AMP224, CT-011, MK-3475, anti-PDL-1 antagonists such as BMS-936559/ MDX-1105, MEDI4736, RG-7446/MPDL3280A; Anti-LAG-3 such as IMP-321), anti-TIM-3, anti-BTLA, anti-B7-H4, anti-B7-H3, Anti-VISTA; Agonistic antibodies targeting immunostimulatory proteins, including anti-CD40 mAbs such as CP-870,893, lucatumumab, dacetuzumab; anti-CD137 mAbs such as BMS-663513 urelumab, PF-05082566; anti-OX40 mAbs, such as anti-OX40; anti-GITR mAbs such as TRX518; anti-CD27 mAbs, such as CDX-1127; and anti-ICOS mAbs.

[0131] In some embodiments, anti-PVRIG antibodies are used in combination with cytokines. A number of cytokines are in preclinical or clinical development as agents potentiating anti-tumor immune responses for cancer immunotherapy, including among others: IL-2, IL-7, IL-12, IL-15, IL-17, IL-18 and IL-21, IL-23, IL-27, GM-CSF, IFNα (interferon α), IFNβ, and IPNγ. However, therapeutic efficacy is often hampered by severe side effects and poor pharmacokinetic properties. Thus, in addition to systemic administration of cytokines, a variety of strategies can be employed for the delivery of therapeutic cytokines and their localization to the tumor site, in order to improve their pharmacokinetics, as well as their efficacy and/or toxicity, including antibody- cytokine fusion molecules (immunocytokines), chemical conjugation to polyethylene glycol (PEGylation), transgenic expression of cytokines in autologous whole tumor cells, incorporation of cytokine genes into DNA vaccines, recombinant viral vectors to deliver cytokine genes, etc. In the case of immunocytokines, fusion of cytokines to tumor-specific antibodies or antibody fragments allows for targeted delivery and therefore improved efficacy and pharmacokinetics, and reduced side effects.

[0132] In some embodiments, anti-PVRIG antibodies are used in combination with cancer vaccines. Therapeutic cancer vaccines allow for improved priming of T cells and improved antigen presentation, and can be used as therapeutic agents for potentiating anti-tumor immune responses (Mellman I. et al., 2011, Nature, 480:22-29; Schlom J, 2012, J Natl Cancer Inst;104:599-613).

[0133] Several types of therapeutic cancer vaccines are in preclinical and clinical development. These include for example:

1. 1) Whole tumor cell vaccines, in which cancer cells removed during surgery are treated to enhance their immunogenicity, and injected into the patient to induce immune responses against antigens in the tumor cells. The tumor cell vaccine can be autologous, i.e. a patient's own tumor, or allogeneic which typically contain two or three established and characterized human tumor cell lines of a given tumor type, such as the GVAX vaccine platforms.
2. 2) Tumor antigen vaccines, in which a tumor antigen (or a combination of a few tumor antigens), usually proteins or peptides, are administered to boost the immune system (possibly with an adjuvant and/or with immune modulators or attractants of dendritic cells such as GM-CSF). The tumor antigens may be specific for a certain type of cancer, but they are not made for a specific patient.
3. 3) Vector-based tumor antigen vaccines and DNA vaccines can be used as a way to provide a steady supply of antigens to stimulate an anti-tumor immune response. Vectors encoding for tumor antigens are injected into the patient (possibly with proinflammatory or other attractants such as GM-CSF), taken up by cells *in vivo* to make the specific antigens, which would then provoke the desired immune response. Vectors may be used to deliver more than one tumor antigen at a time, to increase the immune response. In addition, recombinant virus, bacteria or yeast vectors should trigger their own immune responses, which may also enhance the overall immune response.
4. 4) Oncolytic virus vaccines, such as OncoVex/T-VEC, which involves the intratumoral injection of replication-conditional herpes simplex virus which preferentially infects cancer cells. The virus, which is also engineered to express GM-CSF, is able to replicate inside a cancer cell causing its lysis, releasing new viruses and an array of tumor antigens, and secreting GM-CSF in the process. Thus,

such oncolytic virus vaccines enhance DCs function in the tumor microenvironment to stimulate anti-tumor immune responses.

5. 5) Dendritic cell vaccines (Palucka and Banchereau, 2102, Nat. Rev. Cancer, 12(4):265-277): Dendritic cells (DCs) phagocytose tumor cells and present tumor antigens to tumor specific T cells. In this approach, DCs are isolated from the cancer patient and primed for presenting tumor-specific T cells. To this end several methods can be used: DCs are loaded with tumor cells or lysates; DCs are loaded with fusion proteins or peptides of tumor antigens; coupling of tumor antigens to DC-targeting mAbs. The DCs are treated in the presence of a stimulating factor (such as GM-CSF), activated and matured *ex vivo,* and then re-infused back into the patient in order provoke an immune response to the cancer cells. Dendritic cells can also be primed *in vivo* by injection of patients with irradiated whole tumor cells engineered to secrete stimulating cytokines (such as GM-CSF). Similar approaches can be carried out with monocytes. Sipuleucel-T (Provenge), a therapeutic cancer vaccine which has been approved for treatment of advanced prostate cancer, is an example of a dendritic cell vaccine.

[0134] In some embodiments, anti-PVRIG antibodies are used in combination with adoptive T cell therapy or adoptive cell transfer (ACT), which involves the *ex vivo* identification and expansion of autologous naturally occurring tumor specific T cells, which are then adoptively transferred back into the cancer patient (Restifo et al, 2013, Cancer Immunol. Immunother.62(4):727-36 (2013) Epub Dec 4 2012). Cells that are infused back into a patient after *ex vivo* expansion can traffic to the tumor and mediate its destruction. Prior to this adoptive transfer, hosts can be immunodepleted by irradiation and/or chemotherapy. The combination of lymphodepletion, adoptive cell transfer, and a T cell growth factor (such as IL-2), can lead to prolonged tumor eradication in tumor patients. A more novel approach involves the *ex vivo* genetic modification of normal peripheral blood T cells to confer specificity for tumor-associated antigens. For example, clones of TCRs of T cells with particularly good anti-tumor responses can be inserted into viral expression vectors and used to infect autologous T cells from the patient to be treated. Another option is the use of chimeric antigen receptors (CARs) which are essentially a chimeric immunoglobulin-TCR molecule, also known as a T-body. CARs have antibody-like specificities and recognize MHC-nonrestricted structures on the surface of target cells (the extracellular target-binding module), grafted onto the TCR intracellular domains capable of activating T cells (Restifo et al Cancer Immunol. Immunother.62(4):727-36 (2013) Epub Dec 4 2012; and Shi et al, Nature 493:111-115 2013.

[0135] The PVRIG antibodies and the one or more other therapeutic agents can be administered in either order or simultaneously. The composition can be linked to the agent (as an immunocomplex) or can be administered separately from the agent. In the latter case (separate administration), the composition can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, e.g., an anti-cancer therapy, e.g., radiation.

[0136] Co-administration of the humanized anti-PVRIG immune molecules, according to at least some embodiments of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody. In other embodiments, the subject can be additionally treated with an agent that modulates, e.g., enhances or inhibits, the expression or activity of Fcγ or Fcγ receptors by, for example, treating the subject with a cytokine.

[0137] Target-specific effector cells, e.g., effector cells linked to compositions (e.g., human antibodies, multispecific and bispecific molecules) according to at least some embodiments of the present invention can also be used as therapeutic agents. Effector cells for targeting can be human leukocytes such as macrophages, neutrophils or monocytes. Other cells include eosinophils, natural killer cells and other IgG- or IgA-receptor bearing cells. If desired, effector cells can be obtained from the subject to be treated. The target-specific effector cells can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^{-8} to 10^{-9} but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell, e.g., a tumor cell expressing PVRIG proteins, and to effect cell killing e.g., by, e.g., phagocytosis. Routes of administration can also vary.

[0138] Therapy with target-specific effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy using the compositions (e.g., human antibodies, multispecific and bispecific molecules) according to at least some embodiments of the present invention and/or effector cells armed with these compositions can be used in conjunction with chemotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-PVRIG immune molecules linked to anti-Fc-y RI or anti-CD3 may be used in conjunction with IgG- or IgA-receptor specific binding agents.

[0139] Bispecific and multispecific molecules according to at least some embodiments of the present invention can also be used to modulate FcyR or FcyR levels on effector cells, such as by capping and elimination of receptors on the cell surface. Mixtures of anti-Fc receptors can also be used for this purpose.

[0140] The therapeutic compositions (e.g., human antibodies, alternative scaffolds multispecific and bispecific molecules and immunoconjugates) according to at least some embodiments of the present invention which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement, can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent according to at least some embodiments of the present invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with a binding agent according to at least some embodiments of the present invention can be improved by binding of complement proteins. In another embodiment target cells coated with the compositions (e.g., human antibodies, multispecific and bispecific molecules) according to at least some embodiments of the present invention can also be lysed by complement. In yet another embodiment, the compositions according to at least some embodiments of the present invention do not activate complement.

[0141] The therapeutic compositions (e.g., human antibodies, alternative scaffolds multispecific and bispecific molecules and immunoconjugates) according to at least some embodiments of the present invention can also be administered together with complement. Thus, according to at least some embodiments of the present invention there are compositions, comprising human antibodies, multispecific or bispecific molecules and serum or complement. These compositions are advantageous in that the complement is located in close proximity to the human antibodies, multispecific or bispecific molecules. Alternatively, the human antibodies, multispecific or bispecific molecules according to at least some embodiments of the present invention and the complement or serum can be administered separately.

[0142] The anti-PVRIG immune molecules, according to at least some embodiments of the present invention, can be used as neutralizing antibodies. A neutralizing antibody (Nabs), is an antibody that is capable of binding and neutralizing or inhibiting a specific antigen thereby inhibiting its biological effect.. NAbs will partially or completely abrogate the biological action of an agent by either blocking an important surface molecule needed for its activity or by interfering with the binding of the agent to its receptor on a target cell.

[0143] According to an additional aspect of the present invention the therapeutic agents can be used to prevent pathologic inhibition of T cell activity, such as that directed against cancer cells.

[0144] Thus, according to an additional aspect of the present invention there is provided a method of treating cancer as recited herein, and/or for promoting immune stimulation by administering to a subject in need thereof an effective amount of any one of the therapeutic agents and/or a pharmaceutical composition comprising any of the therapeutic agents and further comprising a pharmaceutically acceptable diluent or carrier.

[0145] According to at least some embodiments, immune cells, preferably T cells, can be contacted *in vivo* or *ex vivo* with the therapeutic agents to modulate immune responses. The T cells contacted with the therapeutic agents can be any cell which expresses the T cell receptor, including α/β and γ/δ T cell receptors. T-cells include all cells which express CD3, including T-cell subsets which also express CD4 and CDS. T-cells include both naive and memory cells and effector cells such as CD8+ cytotoxic T lymphocytes (CTL). T-cells also include cells such as Th1, Tc1, Th2, Tc2, Th3, Th9, Th17, Th22, Treg, follicular helper cells (T_{FH}) and Tr1 cells. T-cells also include NKT-cells iNKT, α/β NKT and γ/δ NKT cells, and similar unique classes of the T-cell lineage.

[0146] PVRIG blocking antibodies can also be used in combination with bispecific antibodies that target Fcα or Fcγ receptor-expressing effectors cells to tumor cells (see, e.g., U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti-tumor antigen (e.g., Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the use of PVRIG blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

[0147] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-β (Kehrl, J. et al. (1986) J. Exp. Med. 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) Immunology Today 13: 198-200), and Fas ligand (Hahne, M. et al. (1996) Science 274: 1363-1365). Antibodies to each of these entities may be used in combination with anti-PVRIG to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

[0148] Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-PVRIG. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. et al. (1998) Nature 393: 474-478) and can be used in conjunction with PVRIG antibodies (Ito, N. et al. (2000) Immunobiology 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules such as OX-40 (Weinberg, A. et al. (2000) Immunol 164: 2160-2169), 4-1BB (Melero, I. et al. (1997) Nature Medicine 3: 682-685 (1997), and ICOS (Hutloff, A. et al. (1999) Nature 397: 262-266) as well as antibodies which block the activity of negative costimulatory molecules such as CTLA-4 (e.g., U.S. Pat. No. 5,811,097, implimumab) or BTLA (Watanabe, N. et al. (2003) Nat Immunol 4:670-9), B7-H4 (Sica, G L et al. (2003) Immunity 18:849-61) PD-1 (may also provide for increased levels of T cell activation.
Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses.PVRIG blockade can be used to increase the effectiveness of the donor

engrafted tumor specific T cells.

[0149] There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) Science 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PVRIG immune molecules may be expected to increase the frequency and activity of the adoptively transferred T cells.

[0150] Optionally, antibodies to PVRIG can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al (2004) J. Immunol. 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of MUC1 for treatment of colon cancer, peptides of MUC-1/CEA/TRICOM for the treatment of ovary cancer, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

[0151] In humans, some tumors have been shown to be immunogenic such as RCC. It is anticipated that by raising the threshold of T cell activation by PVRIG blockade, we may expect to activate tumor responses in the host.

[0152] PVRIG blockade is likely to be most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita, V. et al. (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Editi*on*). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al. (1993) Proc. Natl. Acad. Sci U.S.A. 90: 3539-43).

[0153] The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so-called tumor specific antigens (Rosenberg, S A (1999) Immunity 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. PVRIG blockade may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self-antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N et al. (1994) Science 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (i.e. bcr-ab1 in the Philadelphia chromosome), or idiotype from B cell tumors.

[0154] Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PVRIG blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P (1995) Science 269:1585-1588; Tamura, Y. et al. (1997) Science 278:117-120).

[0155] Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. et al. (1998) Nature Medicine 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. et al. (2000) Nature Medicine 6:332-336). As a method of vaccination, DC immunization may be effectively combined with PVRIG blockade to activate more potent anti-tumor responses.

[0156] Use of the therapeutic agents according to at least some embodiments of the invention as adjuvant for cancer vaccination:

[0157] Immunization against tumor-associated antigens (TAAs) is a promising approach for cancer therapy and prevention, but it faces several challenges and limitations, such as tolerance mechanisms associated with self-antigens expressed by the tumor cells. Costimulatory molecules such as B7.1 (CD80) and B7.2 (CD86) have improved the efficacy of gene-based and cell-based vaccines in animal models and are under investigation as adjuvant in clinical trials. This adjuvant activity can be achieved either by enhancing the costimulatory signal or by blocking inhibitory signal that is transmitted by negative costimulators expressed by tumor cells (Neighbors et al., 2008 J Immunother.;31(7):644-55).

[0158] According to at least some embodiments any one of polyclonal or monoclonal antibody and/or antigen-binding fragments and/or conjugates containing same, and/or alternative scaffolds, specific to any one of PVRIG proteins, can be used as adjuvant for cancer vaccination. According to at least some embodiments, the invention provides methods for improving immunization against TAAs, comprising administering to a patient an effective amount of any one of polyclonal or monoclonal antibody and/or antigen-binding fragments and/or conjugates containing same, and/or alternative scaffolds, specific to any one of PVRIG proteins.

[0159] Also provided is the use of PVRIG antibodies to perform one or more of the following in a subject in need thereof: (a) upregulating pro-inflammatory cytokines; (b) increasing T-cell proliferation and/or expansion; (c) increasing interferon-γ or TNF-α production by T-cells; (d) increasing IL-2 secretion; (e) stimulating antibody responses; (f) inhibiting cancer cell growth; (g) promoting antigenic specific T cell immunity; (h) promoting $CD4^+$ and/or $CD8^+$ T cell activation; (i) alleviating T-cell suppression; (j) promoting NK cell activity; (k) promoting apoptosis or lysis of cancer cells; and/or (1) cytotoxic or cytostatic effect on cancer cells.

[0160] Also provided is the use of an immunostimulatory antibody, antigen-binding fragment or conjugate thereof according to at least some embodiments of the invention (optionally in a pharmaceutical composition) to antagonize at least one immune inhibitory effect of the PVRIG.

[0161] Such an antibody, antigen-binding fragment or conjugate thereof optionally and preferably mediates at least one of the following effects:
(i) increases in immune response, (ii) increases in activation of αβ and/or γδ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of αβ and/or γδ T-cell suppression, (vi) increases in pro-inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii)

increases in interferon-y production, (ix) increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs).

3. Assessment of Treatment

[0162] Generally the anti-PVRIG antibodies are administered to patients with cancer, and efficacy is assessed, in a number of ways as described herein. Thus, while standard assays of efficacy can be run, such as cancer load, size of tumor, evaluation of presence or extent of metastasis, etc., mmuno-oncology treatments can be assessed on the basis of immune status evaluations as well. This can be done in a number of ways, including both in vitro and in vivo assays. For example, evaluation of changes in immune status (e.g. presence of ICOS+ CD4+ T cells following ipi treatment) along with "old fashioned" measurements such as tumor burden, size, invasiveness, LN involvement, metastasis, etc. can be done. Thus, any or all of the following can be evaluated: the inhibitory effects of PVRIG on $CD4^+$ T cell activation or proliferation, $CD8^+$ T (CTL) cell activation or proliferation, $CD8^+$ T cell-mediated cytotoxic activity and/or CTL mediated cell depletion, NK cell activity and NK mediated cell depletion, the potentiating effects of PVRIG on Treg cell differentiation and proliferation and Treg- or myeloid derived suppressor cell (MDSC)-mediated immunosuppression or immune tolerance, and/or the effects of PVRIG on proinflammatory cytokine production by immune cells, e.g., IL-2, IFN-y or TNF-α production by T or other immune cells.

[0163] In some embodiments, assessment of treatment is done by evaluating immune cell proliferation, using for example, CFSE dilution method, Ki67 intracellular staining of immune effector cells, and 3H-Thymidine incorporation method,

[0164] In some embodiments, assessment of treatment is done by evaluating the increase in gene expression or increased protein levels of activation-associated markers, including one or more of: CD25, CD69, CD137, ICOS, PD1, GITR, OX40, and cell degranulation measured by surface expression of CD107A.

[0165] In general, gene expression assays are done as is known in the art. See for example Goodkind et al., Computers and Chem. Eng. 29(3):589 (2005), Han et al., Bioinform. Biol. Insights 11/15/15 9(Suppl. 1):29-46, Campo et al., Nod. Pathol. 2013 Jan; 26 suppl. 1:S97-S110, the gene expression measurement techniques of which are expressly incorporated by reference herein.

[0166] In general, protein expression measurements are also similarly done as is known in the art, see for example, Wang et al., Recent Advances in Capillary Electrophoresis-Based Proteomic Techniques for Biomarker Discovery, Methods. Mol. Biol. 2013:984:1-12; Taylor et al, BioMed Res. Volume 2014, Article ID 361590, 8 pages, Becerk et al., Mutat. Res 2011 June 17:722(2): 171-182, the measurement techniques of which are expressly incorporated herein by reference.

[0167] In some embodiments, assessment of treatment is done by assessing cytotoxic activity measured by target cell viability detection via estimating numerous cell parameters such as enzyme activity (including protease activity), cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Specific examples of these assays include, but are not limited to, Trypan Blue or PI staining, ^{51}Cr or ^{35}S release method, LDH activity, MTT and/or WST assays, Calcein-AM assay, Luminescent based assay, and others.

[0168] In some embodiments, assessment of treatment is done by assessing T cell activity measured by cytokine production, measure either intracellularly in culture supernatant using cytokines including, but not limited to, IPNγ, TNFα, GM-CSF, IL2, IL6, IL4, IL5, IL10, IL13 using well known techniques.

[0169] Accordingly, assessment of treatment can be done using assays that evaluate one or more of the following: (i) increases in immune response, (ii) increases in activation of αβ and/or γδ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of αβ and/or γδ T-cell suppression, (vi) increases in pro-inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon-y production, (ix) increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs.

Assays to measure efficacy

[0170] In some embodiments, T cell activation is assessed using a Mixed Lymphocyte Reaction (MLR) assay as is described in **EXAMPLE 23.** An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0171] In one embodiment, the signaling pathway assay measures increases or decreases in immune response as measured for an example by phosphorylation or dephosphorylation of different factors, or by measuring other post translational modifications. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0172] In one embodiment, the signaling pathway assay measures increases or decreases in activation of αβ and/or γδ T cells as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0173] In one embodiment, the signaling pathway assay measures increases or decreases in cytotoxic T cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0174] In one embodiment, the signaling pathway assay measures increases or decreases in NK and/or NKT cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0175] In one embodiment, the signaling pathway assay measures increases or decreases in αβ and/or γδ T-cell suppression, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0176] In one embodiment, the signaling pathway assay measures increases or decreases in pro-inflammatory cytokine secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0177] In one embodiment, the signaling pathway assay measures increases or decreases in IL-2 secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0178] In one embodiment, the signaling pathway assay measures increases or decreases in interferon-y production as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0179] In one embodiment, the signaling pathway assay measures increases or decreases in Th1 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0180] In one embodiment, the signaling pathway assay measures increases or decreases in Th2 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0181] In one embodiment, the signaling pathway assay measures increases or decreases cell number and/or activity of at least one of regulatory T cells (Tregs), as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0182] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophages cell numbers, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0183] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophage pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0184] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils increase, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0185] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0186] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of T cell activation, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0187] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of

CTL activation as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0188] In one embodiment, the signaling pathway assay measures increases or decreases in αβ and/or γδ T cell exhaustion as measured for an example by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[0189] In one embodiment, the signaling pathway assay measures increases or decreases αβ and/or γδ T cell response as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0190] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of antigen-specific memory responses as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD45RA, CCR7 etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below..

[0191] In one embodiment, the signaling pathway assay measures increases or decreases in apoptosis or lysis of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0192] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of cytotoxic or cytostatic effect on cancer cells. as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0193] In one embodiment, the signaling pathway assay measures increases or decreases direct killing of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0194] In one embodiment, the signaling pathway assay measures increases or decreases Th17 activity as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0195] In one embodiment, the signaling pathway assay measures increases or decreases in induction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[0196] In one embodiment, T cell activation is measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. For T-cells, increases in proliferation, cell surface markers of activation (e.g. CD25, CD69, CD137, PD1), cytotoxicity (ability to kill target cells), and cytokine production (e.g. IL-2, IL-4, IL-6, IPNγ, TNF-a, IL-10, IL-17A) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[0197] In one embodiment, NK cell activation is measured for example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. For NK cells, increases in proliferation, cytotoxicity (ability to kill target cells and increases CD107a, granzyme, and perforin expression), cytokine production (e.g. IFNγ and TNF), and cell surface receptor expression (e.g. CD25) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[0198] In one embodiment, γδ T cell activation is measured for example by cytokine secretion or by proliferation or by changes in expression of activation markers.

[0199] In one embodiment, Th1 cell activation is measured for example by cytokine secretion or by changes in expression of activation markers.

[0200] Appropriate increases in activity or response (or decreases, as appropriate as outlined above), are increases of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98 to 99% percent over the signal in either a reference sample or in control samples, for example test samples that do not contain an anti-PVRIG antibody of the invention. Similarly, increases of at least one-, two-, three-, four- or five-fold as compared to reference or control samples show efficacy.

EXAMPLES

Example 1: Expression Analysis of PVRIG Proteins

Example 1A:

[0201] The GDS3113 data set (http://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS3113) was analyzed to identify genes with a lymphoid organ specific pattern. PVRIG was identified as lymphocyte specific due to high expression in primary and secondary lymphoid organs, which include peripheral blood, bone marrow, spleen, lymph nodes, tonsil and thymus (Figure 2). Other tissue types were negative or showed expression at background levels. In order to investigate which specific cell types within the total population of immune cells express PVRIG, additional data sets form the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/GEO) were analyzed, as described in "methodology" section herein. The analysis was performed on immune cell populations derived from peripheral blood and bone marrow. PVRIG was expressed in lymphocytes both in the B-cell linage and the T-cell linage including CD8 T-cells naive, effector and memory (Figure 3). In addition, PVRIG was expressed in NK cells and had the highest expression in the iNKT population (Figure 4). The iNKT population of lymphocytes act as potent activators of antitumor immunity when stimulated with a synthetic agonist in experimental models. However, in some settings, iNKT cells can act as suppressors and regulators of antitumor immunity (Clin Dev Immunol. 2012;2012:720803). Furthermore, in early clinical trials of iNKT cell-based immunotherapy demonstrated that the infusion of ligand-pulsed antigen presenting cells treatment of and/or in vitro activated iNKT cells were safe and well tolerated in lung cancer and head and neck cancer (Clin Immunol. 2011 Aug;140(2):167-76.).

[0202] A key question in regards to PVRIG expression was whether Tumor Infiltrating Lymphocytes (TILs) retain expression of PVRIG in the tumor microenvironment. Analyzing expression data of TILs form follicular lymphoma, breast cancer and colon cancer showed clear expression of PVRIG in the TILs infiltrating the tumor. In the colon cancer example the specificity to the immune infiltrating cells was seen as the expression is found only in the CD45 positive population (leukocyte specific marker), and no expression is found in EPCAM positive population (epithelial specific marker) or in the CD45 negative EPCAM negative (stromal cell population). Although the CD45 is not a lymphocyte specific marker, the other expression description infers that it is expressed on the lymphocyte population (Figure 5 A colon cancer, Figure 5B breast cancer and Figure 5C follicular lymphoma).

[0203] The mRNA expression data shown herein indicates that PVRIG is expressed in lymphocytes and in tumor infiltrating lymphocytes (TILs). These results together with PVRIG inhibitory activity propose an inhibitory role of the molecule in T-cells, suggesting that inhibitory antibodies to PVRIG elevates PVRIG's suppressive role on the TILs and thus enable the TILs to induce an immune response against cancer. As the proposed mechanism of action is directed to the TILs infiltrating the tumor, rather than direct effect on the tumor cells, any cancer with immune infiltration is candidate for treatment using PVRIG inhibitory antibodies.

[0204] Methodology: Raw data is downloaded from the GEO site in SOFT format. In cases where the raw data was in MAS5 format, the data was taken without manipulation. If the data was in Log MAS5 then the data was converted to linear data. If the data was in RMA format CEL files (raw data) were downloaded and re-analyzed using MAS5. If raw CEL files were not available the RMA format was used.

[0205] Data was then normalized by multiplicative according to the 95th percentile for Affy data. Datasets analyzed: GSE49910, GSE47855, GSE39397, GSE36765, GSE27928.

Example 1B

[0206] A transcriptome reference was generated based on UCSC know genes models (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/knownGene.txt.gz). All RNA sequencing reads were aligned to the transcriptome sequences first. This alignment allowed for non-unique mapping because isoforms share many exons. Each read was then assigned genomic coordinates and exon junctions based on the transcriptome matching. The remaining unmapped reads were aligned directly to the genome by considering one or more exon junctions. Finally, read counts were normalized as described by Bo et al. (Bioinformatics 2010, 26 (4): 493-500) and converted to gene expression values as described by Trapnell et al (Nat Biotechnol. 2010 May;28(5):511-5).

[0207] As shown in Figure 6, based on Genotype-Tissue Expression (GTEx) data (http://www.nature.com/ng/journal/v45/n6/full/ng.2653.html; http://www.gtexportal.org/home/), PVRIG is expressed mainly in blood cells and to lesser extent in various normal tissues. The same results were observed in cancerous tissues from The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/) in which high expression are seen in blood cancers like B- cell lymphomas and AML (Figure 7). A gene expression signature was generated for a variety of cancers and normal tissues using GTEx and TCGA data by identifying genes with a highly correlated expression pattern to PVRIG.

[0208] The correlation analysis was conducted per tumor type and only correlations where both genes were expressed above 0 RPKM with at least 50 samples in the same tumor type, were considered. These gene expression signatures were tested for enrichment of interacting proteins, pathways and disease genes. Enrichment p-values were calculated for each tumor type and the mean -log(p-value) was used to rank the scoring gene sets. A clear signature of lymphocytes and T- cells was observed in a variety of cancers, as shown in Figure 8. For instance, the top scoring gene in protein interaction was IL2, meaning that genes known to interact with IL2 are more correlated with PVRIG than expected by chance across most cancers. Further analysis showed that PVRIG expression in cancer tissues are higher than normal. While in Figure 5 the median expression level of PVRIG is below 1 across most normal solid tissues, in Figure 6 it is clearly higher than 1 in many cancers. As an example, when compared side by side in Figure 7, melanoma PVRIG was expressed higher than normal skin (Figure 9). We further characterized the source of over-expression in cancer. PVRIG is highly expressed in T cells and is highly correlated to markers of T cells in cancer. In Figure 10, PVRIG correlation to CD3, CD4 and CD8 are shown as an example in three cancer types, namely, lung adenocarcinoma, colon adenocarcinoma and melanoma. In addition, PVRIG is highly correlated to PD1, a validated target for immunotherapy in cancer known to be expressed on T cells (Figure 10).

[0209] These gene expression signatures were tested for enrichment of interacting proteins, pathways and disease genes. A clear signature of lymphocytes and T- cells was observed in a variety of cancers, as shown in Figure 8. We further analyzed the correlation of PVRIG to PD1 and showed high correlation between their expression in various tumors including breast lung pancreas and kidney (Table 2). Both PD-1 and PVRIG are highly expressed on activated T cells. PVRIG showed high correlation with T cell markers in cancer, namely, CD8A, CD4 and CD3G (Figure 13). Taken together, these data demonstrate that cancer expression of PVRIG is associated with tumor infiltrating lymphocytes.

[0210] Methods: Genes correlation: FPKM values were transformed to log2 (FPKM+0.1). Samples with value that fulfills log2 (FPKM+0.1)< log2(0.1) for at least one of the genes, were omitted. Pearson Correlation Coefficient (PCC) and the Least Squared Estimators for the regression line were computed for the 2 lists (one list per gene). PCCs with lower value than 0.5 were omitted as well as PCCs that failed to show significant value when testing the linear correlation between the expression levels of the 2 genes.

[0211] Gene Enrichment analysis: Pathway, interaction and disease data were obtained from GeneGo Metacore (https://portal.genego.com), Reactome (http://www.reactome.org) and KEGG Pathways (http://www.genome.jp/kegg). To identify pathways and processes that were enriched within a given gene list, a hyper-geometric-based enrichment analysis was implemented. The hyper-geometric p-value was calculated using the R program (http://www.R-project.org) with the following command: phyper(x - 1, m, n-m, k and lower.tail = FALSE), where x is the number of genes from the gene list that are members of the pathway, m is the number of genes in the pathway, n is the total number of unique genes in all pathways, and k is the number of genes from the list that were present in at least one pathway. The resulting p-value is indicative of the likelihood of enriching for a specific pathway by chance given the size of the gene list. The same analytical procedure was applied to gene interactions where all genes interacting with a given gene were treated as a pathway; or genes associated with a disease where all associated genes were treated as a pathway. See Figure 64.

[0212] PVRIG expression was associated with exhausted T cells in cancer. Cancer samples from TCGA were chosen that have high (4th quartile) expression of the following 4 markers: CD8, PD-1, TIM-3 and TIGIT. Cancer samples were then divided to high, no change and low levels of the combined expression of the 4 markers. PVRIG was not detected in any of the low expressing markers (low or no exhausted T cells). The vast majority of tumors associated with high levels of exhausted T cells expressed high levels of

PVRIG (Figure 22).

Example 1C:

[0213] The expression of human and non-human primate PVRIG RNA and protein in cell lines and primary leukocytes was evaluated.

Protocols

[0214] FACS analysis of engineered over-expressing cells: The following cell lines were used to assess the specificity of anti-human PVRIG antibodies: HEK parental and HEK hPVRIG over-expressing cells. These cells were cultured in DMEM (Gibco) + 10% fetal calf serum (Gibco) + glutamax (Gibco). For the HEK hPVRIG over-expressing cells, 0.5ug/ml puromycin (Gibco) was also added to the media for positive selection. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (human IgG1, hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 30ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II (BD Biosciences) and analyzed using FlowJo (Treestar) and Prism (Graphpad) software.

[0215] FACS analysis of human cell lines: The following cell lines were used to assess the expression and specificity of anti-human PVRIG antibodies: Jurkat, CA46, NK-92, OV-90, HepG2, and NCI-H441. Jurkat, CA46, and NCI-H441 cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids (Gibco), sodium pyruvate (Gibco), and penicillin/streptomycin (Gibco). NK-92 cells were cultured in RPMI media + 25% fetal calf serum, glutamax, non-essential amino acids, sodium pyruvate, penicillin/streptomycin, and 500U/ml IL-2 (R&D systems). OV-90 cells were cultured in a 1:1 mixture of MCDB 105 media (Sigma) containing a final concentration of 1.5 g/L sodium bicarbonate (Life Technologies) and Media 199 (Sigma) containing a final concentration of 2.2 g/L sodium bicarbonate with a final concentration of 15% fetal calf serum. HepG2 cells were cultured in DMEM + 10% fetal calf serum + glutamax. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 30ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II and analyzed using FlowJo and Prism software.

[0216] FACS analysis of naive human primary leukocytes: Primary leukocytes were obtained by Ficoll (GE Healthcare) gradient isolation of peripheral blood (Stanford Blood Bank). Leukocytes as isolated peripheral blood mononuclear cells (PBMC) were frozen down in liquid nitrogen at a density between 1×107 and 5×107 cells/ml in a 10% DMSO (Sigma), 90% fetal calf serum mixture. To assess protein expression of PVRIG on PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or in some cases, as an 8 point titration series starting at 10 or 30ug/ml on ice for 30 mins-1 hr.

[0217] Briefly, antibody cocktail mixtures were added to resuscitated PBMC that were seeded at 5×10^5 - 1×10^6 cells/well upon prior Fc receptor blockade and live/dead staining (Aqua Live/Dead, Life Technologies). Antibody cocktails were incubated with PBMC for 30mins - 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using FlowJo and Prism software. Immune subsets that were analysed include CD56 dim NK cells, CD56 bright NK cells, CD4+ T cells, CD8+ T cells, non-conventional T cells (e.g. NKT cells and □□□T cells), B cells, and monocytes.

[0218] FACS analysis of activated human effector lymphocytes: In some cases, expression of PVRIG was assessed on activated effector lymphocyte subsets either isolated from whole PBMC or in whole PBMC preparations. Effector lymphocytes were stimulated with combinations of cytokines, combinations of antibodies and cytokines, or pathogenic products. FACS analysis of PVRIG expression on activated cells was performed analogous to that described above for naive primary leukocytes.

[0219] To study PVRIG expression on stimulated NK cells, CD56+ cells were isolated and cultured in various cocktails of cytokines for 1-3 days in NK cell media (RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, and beta-mercaptoethanol [Gibco]). NK cells were sorted either using anti-human CD56+ microbeads (Miltenyi Biotec) or the human NK cell isolation kit (Miltenyi Biotec) according to the manufacturer's instructions. Cocktails of cytokines used to simulate NK cells included IL-2, IL-12, IL-15, IL-2/IL-12, IL-2/IL-15, IL-12/IL-15 (R&D systems).

[0220] To study PVRIG expression on stimulated T cells, CD4+ or CD8+ T cells were isolated using CD4+ or CD8+ microbeads (Miltenyi Biotec). The isolated cells were cultured for 3 days in the presence of various activating conditions in T cell media (RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate). Conditions used to stimulate isolated T cells include human dynabead stimulation (beads coupled to CD3/CD28 antibodies, Life Technologies) with IL-2 or cytokine cocktails that drive T cells to certain phenotypes (e.g. Th1, Th2, Th17, and T regulatory phenotypes). Th1 driving cytokines are recombinant IL-12 (R&D systems) and an anti-IL-4 neutralizing antibody (Biolegend). Th2 driving conditions are recombinant IL-4 (R&D systems) and an anti-IFN-gamma neutralizing antibody (Biolegend). Th17 driving conditions are recombinant IL-6 (R&D systems), TGF-beta (R&D systems), IL-23 (R&D systems), and anti-IL-4 and anti- IFNγ neutralizing antibodies. T regulatory driving conditions are recombinant TGF-beta and IL-2, and anti-IL-4 and anti-IFNγ neutralizing antibodies.

[0221] Alternatively, activated T cells were also analyzed in whole stimulated PBMC cultures with staphylococcal enterotoxin B (SEB) antigen (List Biological Laboratories) for 3 days, or in a mixed lymphocyte reaction (MLR) where CD4+ T cells are co-cultured with allogeneic dendritic cells for 2 or 5 days.

[0222] FACS analysis of human polarized monocytes: PVRIG expression was assessed on dendritic cells derived from polarized monocytes. In this instance, CD14+ cells were enriched using RosetteSep human monocyte enrichment according to manufacturer's instructions. After CD14+ cell enrichment, monocytes were polarized to dendritic cells upon culture with GM-CSF (R&D systems) and IL-4 (R&D systems) for 4 days in RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, and beta-mercaptoethanol.

[0223] RNA expression analysis of human cell lines and leukocytes by qPCR: Cell lines that were assessed for RNA expression by qPCR were Jurkat, CA46, Daudi, Raji, and expi 293 cells. Jurkat, CA46, Raji, and Daudi cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids, sodium pyruvate, and penicillin/streptomycin. Expi 293 cells were cultured in DMEM + 10% FCS + glutamax. OV-90, HepG2, and NCI-H441 RNA was analysed by a bioinformatics screen of the cancer cell line atlas. For those cell lines that were assessed for RNA expression by qPCR, the cells were harvested in log phase growth and 1,000,000 cells were harvested, washed in PBS, and lysed in 350ul of RLT buffer (Qiagen). Lysed cells in RLT buffer were stored at -80oc until use.

[0224] Primary leukocytes that were assessed for RNA expression were CD56+ NK cells, CD4+ T cells, CD8+ T cells, and CD14+ monocytes. Cell populations were isolated using human CD56+, CD4+, CD8+, and CD14+ positive selection kits according to manufacturer's instructions (Miltenyi Biotec). After sorting, cells were lysed in 350ul of RLT buffer and stored at -80oc until use. In some instances, activated PBMC subsets (activation conditions outlined above) were harvested from culture and were lysed in 350ul of RLT buffer and stored at -80oc until use.

[0225] Upon day of use, RNA was generated from lysed cells using the Qiagen mini kit according to the manufacturer's instructions. cDNA was generated using Applied Biosystems high capacity cDNA reverse transcription kit. qPCR using cDNA was performed using Taqman primers (ThermoFisher) and Applied Biosystems Taqman fast advanced mastermix. The PVRIG primer set used was Taqman catalogue number: Hs04189293_g1. Beta-actin housekeeping primer set used was Taqman catalogue number: Hs01060665_g1. Expression of transcript was assessed by quantifying Ct values and relative expression was calculated by the 2(-ΔΔCt) method. Data was acquired on an Applied Biosystems Step One Plus instrument.

[0226] FACS analysis of cynomolgus PVRIG engineered over-expressing cells: The following cell lines were used to assess the cross-reactivity of anti-human PVRIG antibodies with cynomolgus PVRIG (cPVRIG): expi parental and expi cPVRIG over-expressing cells. These cells were cultured in DMEM + 10% fetal calf serum + glutamax. expi cPVRIG transient over-expressing cells were generated by electroporating cPVRIG DNA into parental expi cells using the Neon transfection system. For FACS analysis, expi cPVRIG cells were used between 1-3 days post transfection. Parental expi cells were harvested from log growth phase. 50,000-100,000 cells of per well of each type were seeded in 96 well plates. Anti-human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 100ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II and analyzed using FlowJo and Prism software.

[0227] FACS analysis of naive primary cynomolgus monkey leukocytes: Primary cynomolgus monkey (cyno) leukocytes were obtained from fresh blood which was drawn no longer than 24 hours prior to expression analysis. Blood was sourced from Bioreclamation. To assess protein expression of PVRIG on cyno PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml).

[0228] Briefly, antibody cocktail mixtures were added to PBMC that were seeded at 5×10^5 - 1×10^6 cells/well upon prior Fc receptor blockade and live/dead staining. Antibody cocktails were incubated with PBMC for 30mins - 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using Prism software. Immune subsets that were analysed include CD16+ lymphocytes, CD14+/CD56+ monocytes/myeloid cells, and CD3+ T cells.

[0229] RNA expression analysis of primary cynomolgus monkey leukocytes: Primary leukocytes that were assessed for RNA expression were CD56+, CD16+, and CD56-/CD16-subsets. Cell populations were isolated using non-human primate CD56 and CD16 positive selection kits according to manufacturer's instructions (Miltenyi Biotec). After sorting, cells were lysed in 350ul of RLT buffer and stored at -80oc until use.

[0230] Upon day of use, RNA was generated from lysed cells using the Qiagen mini kit according to the manufacturer's instructions. cDNA was generated using Applied Biosystems high capacity cDNA reverse transcription kit. qPCR using cDNA was performed using Taqman primers and Applied Biosystems Taqman fast advanced mastermix. Two sets of primers to detect cyno PVRIG were designed by Compugen USA, Inc and manufactured by Genscript. The sequence and primer codes are:

Primer set 1

Forward: CTTGTGTTCACCACCTCTGG

Reverse: TGTTCTCATCGCAGGAGGTC

Primer set 2

Forward: TTGGCTGTGGATACCTCCTT

Reverse: ATAAGGGTCGTGGAGAGCAG

[0231] Beta-actin primers were used for housekeeping and the primer set used was Taqman catalogue number: Mf04354341_g1. Expression of transcripts was assessed by quantifying Ct values and relative expression was calculated by the $2^{(-\Delta\Delta Ct)}$ method. Products generated with PVRIG primers and beta-actin primers were also size analysed by traditional RT-PCR using a 2.5% agarose gel. qPCR data was acquired using an Applied Biosystems Step One Plus instrument.

Results

[0232] PVRIG antibodies recognize PVRIG on overexpressing cells: To screen for antibodies that were specific for PVRIG, we assessed the ability of antibodies that were generated from a phage campaign to bind HEK cell lines that were engineered to overexpress PVRIG. The majority of antibodies from this campaign upon reformatting to human IgG1 bound to the HEK hPVRIG cells, albeit with varying affinity. Furthermore, the majority of these antibodies also showed low background binding to HEK parental cell lines indicating high specificity towards PVRIG. Figure 27 shows one example of the specificity of PVRIG antibodies. A summary of all binding characteristics of the antibodies towards HEK hPVRIG cells relative to control that were generated in this phage campaign are displayed in Figure 31.

[0233] Human PVRIG RNA is expressed in a range of cancer cell lines: To initially screen for cell lines that could be used to assess PVRIG protein expression by antibodies, we examined the cancer cell line atlas for cell lines that were high for PVRIG RNA as assessed by bioinformatics. We found four cell lines that were readily accessible commercially that were high expressors for PVRIG RNA that we chose to validate by qPCR analysis. These cell lines were Jurkat, CA46, Raji, and Daudi.

[0234] When qPCR analysis was conducted, we detected PVRIG RNA in all four cell lines consistent with the bioinformatics analysis (Figure 28). As a negative control we included expi cells that had relatively low PVRIG RNA expression.

[0235] Human PVRIG RNA is expressed in T cells and NK cells: To initially screen PBMC for subsets likely to be positive for PVRIG protein as detected by our antibodies, we sorted major PBMC subsets and examined PVRIG RNA expression by qPCR. Levels of PVRIG RNA in CD56+ NK cells, CD4+ T cells, CD8+

T cells, and CD14+ monocytes were compared to those in Jurkat, HEK parental, and HEK hPVRIG cell lines. As shown in Figure 29, PVRIG RNA was detected most highly and up to 50 fold higher in CD4+ T cells, CD8+ T cells, and CD56+ NK cells when normalized to HEK GFP cells. Similar to Figure 28, Jurkat cells also showed positive expression. In contrast, CD14+ monocytes did not show higher PVRIG expression relative to HEK GFP cells indicating very low PVRIG RNA expression.

[0236] In addition to analyzing naive PBMC, select populations (effector lymphocytes) were also activated under various stimulatory conditions and expression of PVRIG RNA was assessed. More specifically, NK cells were activated with various combinations of stimulatory cytokines, whereas T cells were polyclonally activated with human activator dynabeads or staphylococcus enterotoxin B (SEB) with or without polarizing cytokines (see protocol section for details). As shown in Figure 30A and B, PVRIG RNA expression generally increased in both NK cells and T cells upon various stimulation conditions, the extent of which depended on the individual donor. More specifically, Figure 30a shows PVRIG RNA expression in naive and activated CD4 T cells and NK cells. Figure 30b shows PVRIG RNA expression in naive and activated CD8 T cells.

[0237] PVRIG antibodies recognize PVRIG protein on NK cells most prominently in naive and activated primary immune subsets: Upon confirming the RNA expression pattern of PVRIG RNA expression in naive and activated PBMC subsets, we used our panel of PVRIG antibodies to assess protein expression. We first assessed PVRIG expression in naive PBMC subsets. The population which displayed the highest level of PVRIG was NK cells. CD4+ and CD8+ T cells showed low levels of PVRIG, while B cells and monocytes had no detectable expression. A summary of expression on NK cells and CD8+ T cells as detected by our antibodies is shown in Figure 32. Other minor subsets also displayed PVRIG expression and included non-conventional T cells such as NKT cells and γδ T cells. The expression pattern on PBMC subsets was very similar across all donors we sourced and analyzed.

[0238] When PVRIG protein was assessed after various stimulation conditions (including polyclonal simulation, cytokine stimulation, and MLR), there was no robust upregulation of PVRIG on any PBMC subsets, including NK cells and CD4+ and CD8+ T cells. Furthermore, monocytes which were polarized in vitro to dendritic cells with GM-CSF and IL-4 did not show detectable PVRIG expression consistent with that seen on non-polarized monocytes.

[0239] PVRIG is detected on cell lines by a proportion of PVRIG antibodies: In addition to screening PBMC for PVRIG protein expression, we wanted to understand whether it was also expressed on cancer cell lines. Using the positive cell lines identified by RNA expression (Figure 28), we chose to screen our antibodies on Jurkat and CA46 cells as they showed the lowest absolute Ct values relative to our housekeeping gene. We also chose a range of negative cell lines to further validate the specificity of our antibodies which included OV-90, NCI-H441, and HepG2. A proportion of our antibodies did detect PVRIG protein expression on Jurkat and CA46 cells (Figure 31), but not the negative cell lines. An example of PVRIG detection on Jurkat and CA46 is shown in Figure 33 with a representative antibody, CPA.7.021. The expression on Jurkat and CA46 was completely in accordance with each other and the intensity of expression was similar across the two cell lines.

[0240] PVRIG antibodies detect cynomolgus PVRIG transiently expressed on expi cells: In order to assess the pre-clinical suitability of our anti-human PVRIG antibodies for pharmacological studies in cynomolgus monkey, we wanted to understand whether our antibodies were able to cross-react with cynomolgus PVRIG (cPVRIG). A proportion of our antibodies were able to detect cPVRIG which was transiently transfected onto expi cells (Figure 29). An example of an antibody that yielded negative staining (CPA.7.021) and one that yielded positive staining (CPA.7.024) are shown in Figure 34.

[0241] PVRIG RNA is detected in cynomolgus PBMC: Prior to assessment of PVRIG protein on cyno PBMC, we firstly wanted to determine the PVRIG RNA expression profile in cyno PBMC subsets. As no cPVRIG primers set existed, we designed two sets that were directed at two distinct sites on the cPVRIG gene. One primer set was specific for the X2 variant of cPVRIG, while the other set was able to pick up both the X1 and X2 variant. As shown in Figure 35, both primer sets were able to detect cPVRIG RNA at a similar level when compared to each other. Furthermore, unlike human PBMC where there was a distinct PVRIG RNA signature in effector lymphocytes (NK and T cells) compared to monocytes, cPVRIG RNA was expressed at a similar level across all PBMC subsets from all donors assessed.

[0242] PVRIG protein expression on cynomolgus PBMC is very low or negative: Having established a cPVRIG RNA profile for cyno PBMC, we screened for the presence of cPVRIG protein on cyno PBMC using a select panel of anti-human PVRIG antibodies. The antibodies chosen to screen PBMC were based on their ability to bind cPVRIG transient cells and/or functional activity.. As shown in Figure 36, we were able to detect low level of expression of cPVRIG on the CD16+ lymphocyte subset (NK cells) from a range of antibodies, but not the CD3+ lymphocyte subset (T cells) nor the CD14+ CD56+ myeloid subset (monocytes). Despite this data, those antibodies that showed positive detection over control (as denoted by the solid black line) did not correlate to those that were able to bind the cPVRIG transient cells. For example, the level of staining by CPA.7.021 was more than CPA.7.024 despite the former not binding to cPVRIG transient cells (see Figure 36).

[0243] Summary and Conclusions: Using an antibody phage platform, we have been able to successfully generate monoclonal antibodies towards the human PVRIG antigen. Using engineered over-expressing cells as well as a suite of cancer cell lines, we showed that our antibodies are highly specific to the PVRIG antigen, and are able to detect protein expression which correlated with RNA expression. Upon analysis of human PBMC subsets, we showed that the PVRIG protein is most highly expressed on NK cells, with low expression on conventional CD3+ T cells, and not detectable on B cells and myeloid cells. The expression did not robustly change upon exposing these cell types to various stimulation conditions. We also showed that a panel of our antibodies are cross-reactive with the cynomolgus monkey (cyno) PVRIG antigen through assessing their binding to over-expressing cells. However, the combination of the low level of binding of this panel of antibodies to cyno PBMC, the lack of protein correlation with RNA, and the discordance of their ability to bind to over-expressing cells (compared to PBMC) indicates that the PVRIG antigen on cyno PBMC may be very low/negative, or it is expressed in a different/more complex form compared to the over-expressing cells.

Example 1D:

[0244] Expression of PVRIG in PBMC subsets from healthy donors: The expression of PVRIG in PBMC subsets from healthy donors was tested (gating strategy is shown in Figure 1a). In the tested samples, PVRIG was shown to express on CD8+ T cells (data not shown), CD8α+ γδ T cell (data not shown), double-negative γδ T cells (data not shown) and to a milder extent also on CD4+ T cells (data not shown) of healthy donors PBMCs (n=5).

Example 1E

[0245] Co-expression of PVRIG with PD1, TIGIT and HLA-DR in Ovarian Cancer ascites, PBLs of MSS, CRC, and in resting and allo-activated healthy PBMCs: PVRIG is co-expressed with TIGIT on CD8+ T cells in ovarian cancer ascites (data not shown) . In this sample, a mixed level of PVRIG expression was observed, that overlapped with that of PD-1 expression. Low level of HLA-DR correlated with low level of PVRIG expression. Very low level of PVRIG was observed on CD4+ T cells is in this specific sample, indicating no correlation with PD1, TIGIT and HLA-DR.

[0246] In PBLs of MSS CRC patients, PVRIG is co-expressed with TIGIT on CD8+ T cells (data not shown). Low expression levels of PVRIG were observed in this sample which was in correlation with the low levels of TIGIT and HLA-DR. TILs from this patient had small CD8+ population that stained positive for surface PVRIG, which was also positive for PD1 and TIGIT (data not shown). Intracellular stain reveled prominent PVRIG stain that mirrored the expression pattern of PD-1, showing two distinct populations that are PD1-PVRIG- and PD1+PVRIG+ (data not shown). Intracellular PVRIG+ CD8+ T cells seem to better correlate with the HLA-DR+ and TIGIT+. PVRIG was not detectable on the surface of CD4+ T cells and only minority of the CD4+ cells showed positive intracellular PVRIG stain in the PD1+ population. Due to the very small intracellular PVRIG+ population, it is difficult to determine if PVRIG is co-expressed with TIGIT and HLA-DR.

[0247] In healthy PBMCs, PVRIG stain on CD8 T cells mirrored the expression pattern of PD-1 and TIGIT, showing distinct PD1-PVRIG- and PD1+PVRIG+ populations and distinct TIGIT-PVRIG- and TIGIT+PVRIG+ populations (data not shown). PVRIG was not detected on CD4+ cells. Interestingly, following allo-activation, co-expression of PVRIG and PD-1 was observed on CD4+ (but no on CD8+) (data not shown).

[0248] In summary, PVRIG was shown to co-express with TIGIT in CD8+ T cells from ovarian cancer ascites, MSS CRC patient's PBLs and with PD-1 healthy donor's PBMCs and with PD1 in CD4+ T cells of allo activated PBMCs from healthy donor.

Example 1F

[0249] Expression of PVRIG on lymphocyte populations from Healthy PBMCs Urachal cancer, colorectal cancer, ovarian cancer ascites and lung cancer: Results: The expression of PVRIG on CD4+ and CD8+ T cells, NK cells and on CD4+ and CD8+ NKT cells was analyzed in healthy donors' PBMCs and tonsils and in TILs from urachal cancer, colorectal cancer, ovarian cancer ascites, lung cancer and melanoma.

[0250] In healthy donors' PBMCs (n=5) and in ovarian cancer ascites TILs (n=1) high levels of PVRIG expression was detected on NK cells (data not shown) and CD8+NKT cells (data not shown) and to a lower extent also on CD8+ T cells (data not shown) and CD4+ NKT (data not shown). CD4+ T cells also stained positively for PVRIG in some of the PBMCs, however the level of expression was quite low (data not shown).

[0251] In addition, PVRIG expression was detected on CD4+ T cells from two out of 6 colorectal cancer TILs tested, and in lung cancer TILs (n=3) (data not shown) and on NK cells from urachal cancer TILs (n=1).

[0252] No PVRIG expression was detected in melanoma TILs due to absence of TILs in the tested sample.

Example 1G

[0253] Additional evaluations were done to identify addition tissues that over express PVRIG in human and mouse cell lines.

[0254] Reagents: Human PVRIG TaqMan probes (Life technologies) Hs04189293_g1, Cat. # 4331182, TaqMan probe for Housekeeping gene (HSKG) (Life technologies) human RPL19 Mm 01577060_gH, human HPRT1 Hs02800695_m1, human SDHA Hs00417200_m1, human PBGD Hs00609296_g1, and human TATA Box Hs00375874_g1. Mouse PVRIG TaqMan probes (Life technologies) CC70L8H, CC6RN19 Custom TaqMan probes. TaqMan probes for Housekeeping gene (HSKG) (Life technologies) mouse RPL19: Mm02601633_g1. ABI TaqMan Fast Advanced Master mix, part no. 4444557, Applied Biosystem. Commercial Human and Mouse cancer cell lines from American Type Culture Collection (ATCC) and CLS (Cell line service) are detailed in Table 1. RNA extraction from human and mouse cell lines was performed with RNAeasy Mini Kit (Qiagen cat # 74014). cDNA was produced using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems cat#4368814. Commercial mouse polyclonal Anti-PVRIG Ab MaxPab (B01), Abnova, Cat#H00079037-B01, diluted 1:200. Mouse IgG1, Life Technologies, Cat#MG100, diluted 1:200. Commercial mouse polyclonal Anti-PVRIG Ab, Sigma, Cat#SAB 1407935, 10ug/ml. Chrom pure Mouse IgG, whole molecule, Jackson, Cat#015-000-003, 10ug/ml. Goat Anti Mouse-PE, Jackson, Cat#115-116-146, diluted 1:100. Custom polyclonal Rat-Anti mouse PVRIG, Batch#20153456C.1, Aldevron, 10ug/ml. Custom Rat total IgG, Batch#GV20884.1, Aldevron, 10ug/ml. Goat Anti Rat-PE, Jackson, cat# 112-116-143, diluted 1:100. Anti-human PVRIG-CPA.7.024 mIgG1 conjugated to AF647, 10ug/ml. Anti-human PVRIG-CPA.7.050 mIgG1 conjugated to AF647, 10ug/ml. Anti-human PVRIG-CPA.7.005 mIgG1 conjugated to AF647, 10ug/ml. Anti-human PVRIG-CPA.7.002 mIgG1 conjugated to AF647, 10ug/ml. Synagis IgG1 conjugated to A647, 10ug/ml. Anti-human PVRIG-CPA.7.021 mIgG1 conjugated to AF647, 10ug/ml. Synagis IgG2 conjugated to A647, 10ug/ml. Rabbit polyclonal anti PVRIG Ab, Sigma, Cat#HPA047497, diluted 1:300. Goat Anti Rabbit-HRP, Jackson, Cat# 111-035-003, diluted 1:100. VioBlue, Fixable viability stain 450, BD Bioscience, cat # 562247, diluted 1:1000. Human Trustain FcX, Biolegend, Cat#422302 . Rat anti mouse CD16/CD32 Fc block, BD, Cat#553142 . Ingenio Electroporation solution, Mirus, Cat#MIR50114. ON-TARGETplus Human PVRIG siRNA - SMARTpool, Dharmacon, Cat# L-032703-02. ON TARGET plus non targeting siRNA, Dharmacon, Cat# D-001810-01-05. The human cell lines used in the study are shown in Figure 54.

[0255] Transcript expression. Quantitative RT-PCR (qRT-PCR): RNA (1-5ug) extraction of human and mouse cell lines (detailed above in Tables 1 and 2) was preformed according to manufactures protocols. cDNA was prepared according to manufactures protocols (1ug RNA diluted in 20ul cDNA mix reaction). cDNA, prepared as described above, diluted 1:10 (representing 25ng RNA per reaction), was used as a template for qRT-PCR reactions, using a gene specific TaqMan probes (detailed in materials & methods 1.11-4) Detection was performed using QuantStudio 12k device. The cycle in which the reactions achieved a threshold level of fluorescence (Ct= Threshold Cycle) was registered and was used to calculate the relative transcript quantity in the RT reactions. The absolute quantity was calculated by using the equation Q=2 ^-Ct. The resulting relative quantities were normalized to a relative quantities of housekeeping gene, mRPL19 or hRPL19.

[0256] Protein expression detection by Western Blot (WB): The expression of human PVRIG in human cell lines was analyzed by WB using whole cell extracts (45ug for the cancer cell lines, and 30ug for the over expressing cell line and negative control cell line). Commercial rabbit polyclonal anti-human PVRIG pAb, Sigma, cat # HPA047497, diluted 1:300 in 5% BSA/TBST followed by secondary Ab goat anti-Rabbit - Peroxidase conjugated (Jackson, cat # 111-035-003), diluted 1:20,000 in 5% milk TBST.

[0257] Protein expression analysis by Flow Cytometry (FACS): The cell surface expression of PVRIG protein was analyzed by FACS. Human or mouse cell lines were stained with VioBlue reagent diluted 1:1000 in PBS. Cells were incubated 15 min at R.T. and then washed once with PBS. Cell lines for endogenous protein analysis were pre-incubated with the Fc receptor blocking solutions listed above in material section (2.5 µl/reaction of human blocker and 1µl/reaction of mouse blocker was used according to the manufactures procedures). To detect the human PVRIG protein, cells were stained with a commercial polyclonal anti human PVRIG or by a custom monoclonal anti-human PVRIG mAbs (Inc production, detailed in materials & methods section above) diluted to a concentration of 10ug/ml or 1:200 (for Sigma Ab and for mAb or for Abnova Ab respectively) or IgG1 Isotype control at the same concentration followed by Goat anti mouse PE conjugated Ab.

[0258] To detect the mouse PVRIG protein, cells were stained with a Custom rat polyclonal anti-mouse PVRIG pAb (Aldevron,) diluted to a concentration of 10ug/ml or rat IgG whole molecule as isotypes control at the same concentration followed by Donkey anti Rat-PE conjugated Ab diluted 1:100.

[0259] PVRIG knock down: Knock down of endogenous human PVRIG was carried out by transient transfection of siRNA. Transfection of 100 pmol PVRIG siRNA pool or scrambled siRNA performed by electroporation using Amaxa nucleofector device and MIRUS Ingenio electroporation solution, as listed above in materials & methods and according to the manufacture procedure. 48 hours post transfection, cells were collected for further analysis by qRT-PCR and FACS.

Results: Endogenous expression of the PVRIG transcript in human and mouse cell lines by qRT-PCR

[0260] Human cell lines: In order to verify the presence of the PVRIG transcript in human cell lines (listed in Figure 54), qRT-PCR was performed using a specific TaqMan probe as describe above in Material & Methods. As shown in Figure 56. human PVRIG transcript is observed using TaqMan probe Hs04189293_g1 with relatively high levels in Jurkat (A, B), HUT78 (A, B) and HL60 (B) cell lines. Lower transcript level is observed in THP1, RPMI8226 (B) cell lines. All other cell lines show very low to no transcript.

[0261] Endogenous expression of the PVRIG transcript in mouse cell lines by qRT-PCR: In order to verify the presence of the PVRIG transcript in mouse cell lines (listed in Figure 55), qRT-PCR was performed using a specific TaqMan probe as describe above in Material & Methods. As shown in Figure 57 mouse PVRIG transcript is observed using TaqMan probe CC70L8H with relatively high levels in NIH/3T3, Renca, Sal/N and J774A.1 (A), cell lines. Lower transcript level is observed in CT26 (A) and B-104-1-1(B) cell lines. All other cell lines show very low transcript.

[0262] Endogenous expression of the PVRIG proteins in human cell lines by WB: WB analysis for endogenous expression of PVRIG protein was carried out on various human cancer cell lines lysates as detailed in Figure 54 using commercial anti human PVRIG pAb (Sigma, HPA047497) as described in Materials & Methods above. As a positive control, whole cell extract of stable HEK293 cell pool over-expressing PVRIG was used while cells transfected with an empty vector served as the negative control. As shown in Figure 58. a protein band corresponding to ~35kD was detected in the positive control HEK293 over expressing cells (lane 2), as well as in the Jurkat cell line (lane 3). No expression of human PVRIG was detected in the empty vector cells (lane 1) which served as a negative control nor in ZR75-1 human cell line (lane 4).

Endogenous expression of the PVRIG proteins in human and mouse cell lines by FACS:

[0263] Human cell line: To verify the cell-surface endogenous expression of human PVRIG, various human cell lines (detailed in Figure 54) were tested as described in Material & Methods above. The cell lines were stained with the commercial Ab (Abnova) or with Isotype control followed by a secondary goat anti mouse PE Ab. Analysis was performed by FACS. Binding of Abnova antibody was observed in Jurkat human cancer cell line as compared to isotype control binding. No binding of Abnova Ab was observed in the other tested cell lines: For Capan2 and ZR75-1 as compared to isotype control binding, additional FACS analysis was done using Sigma commercial Ab on a various human cell lines (Jurkat, HUT78, Karpas299 and NK-YTS), binding was observed in Jurkat cells only but no binding was observed to other cell lines (data not shown).

[0264] Further analysis for endogenous confirmation of human PVRIG in Jurkat cell line, was done by testing binding of various monoclonal antibodies of the invention. Jurkat cell line was stained with five anti-human PVRIG custom mAbs (CPA.7.024, CPA.7.050, CPA.7.005, CPA.7.002 and CPA.7.021) conjugated to AF647 or with relative Isotype control Ab conjugated to AF647 Analysis was performed by FACS. The expression of human PVRIG in Jurkat human cell line was observed by CPA.7.021 and CPA.7.050 only, as compared to isotype control expression. No binding for human PVRIG was observed in Jurkat cell line by using the other three mAbs.

[0265] Mouse cell line: to verify the cell-surface endogenous expression of mouse PVRIG, various mouse cell lines: J774A.1, NIH/3T3, SaI/N and Renca (detailed in Figure 55), were tested as described in Material & Methods above. The cell lines were stained with the custom polyclonal rat anti mouse PVRIG Ab (Aldevron), or with Isotype control (Aldevron) followed by a secondary goat anti rat PE Ab. Analysis was performed by FACS. No binding for mouse PVRIG protein was observed in either of the tested mouse cell lines by Aldevron polyclonal Ab (data not shown).

[0266] Knock down of human PVRIG in human cell lines: In order to further confirm endogenous expression of PVRIG protein in Jurkat cell line, human PVRIG siRNA pool was used for knock down as described in Material & Methods. 48 hours post siRNA transfection, cells were harvested for further analysis by qRT-PCR and by FACS.

[0267] Knock down of human PVRIG in human cell lines tested by qPCR: As shown in Figure 59 human PVRIG transcript level in Jurkat cells transfected with human PVRIG siRNA pool is significantly reduced (right histogram bar) as compared to cells transfected with scrambled siRNA (left histogram bar)analyzed by qRT-PCR as described in Material & Methods..

[0268] Knock down of human PVRIG in human cell lines tested by FACS: Further analysis of human PVRIG membrane expression in the same siRNA transfected cells was performed by FACS. As shown in Figure 60 membrane expressions of human PVRIG protein is reduced in cells transfected with PVRIG siRNA (green for CPA.7.021mAb or red for Sigma Ab) as compared to cells transfected with scrambled siRNA (orange). The fold change (anti PVRIG vs, Isotype control) in Jurkat cell line is decreased from 8 fold to 3.3 fold by using Sigma Ab, or from 15.3 fold to 2.8 fold by using CPA.7.021 mAb.

[0269] This report includes preliminary data on PVRIG endogenous expression in cell lines both at the RNA level and the protein level in human and mouse cell lines.

[0270] Various human cancer cell lines were tested by qRT-PCR, WB and FACS for endogenous expression of PVRIG.

[0271] Cell surface expression of human PVRIG was observed in Jurkat cell line by using the commercial polyclonal Abs (Sigma and Abnova) and the mouse monoclonal Abs (Inc), as shown in Figure 4A and 4B respectively. These observations are in correlation to RNA transcript levels as shown in FigureIA & B, and to WB results as shown in Figure 3.

[0272] Additional confirmation of endogenous human PVRIG in Jurkat cell lines was done by knock down experiment confirming clear reduction in the RNA transcript following PVRIG siRNA transfection, as shown in Figure 5, and also reduction was observed in the protein cell surface expression in Jurkat cell lines as shown in Figure 6 by commercial Ab and by monoclonal Ab.

[0273] Various mouse cell lines were tested by qRT-PCR and FACS for endogenous expression of PVRIG. In the transcript level, presence of PVRIG was observed in J774A.1, NIH/3T3, Sal/N and Renca cell lines as shown in Figure 2A & B. Although no membrane expression of mouse PVRIG was observed in these tested cell lines detected by polyclonal Ab (Aldevron) (data not shown). Figure 61 and Figure 62 indicate the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down.

Example 1H

[0274] The aim of this experiments is to evaluate the expression of PVRIG protein on resting or activated human (Tumor infiltrating lymphocytes) TILs isolated from human melanoma samples and propagated in the presence of melanoma specific antigens and IL2. Human mAb were produced directed against the extracellular domain (ECD) of human PVRIG. These Abs were directly labeled with Alexa flour 647 in order to examine the expression of PVRIG on cells by FACS analysis.

Materials and Methods

[0275] TILs: In this experiments series three different Tumor-infiltrating lymphocyte (TIL) from resected metastases of three melanoma patients were used: 1) TIL-412- HLA-A2-Mart1 specific; 2) TIL-F4- HLA-A2-gp100 specific, and 3) TIL-209- HLA-A2-gp100 specific. Human TILs (>90% CD8+), were thawed 24h prior to beginning of experiment. Cells were thawed in 12 ml of TIL medium (IMDM + 10% human serum +1% Glutamax + 1% Na-Pyruvate + 1% non-essential amino acids + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover from freezing for 24 hours.

[0276] Assay conditions: After recovery, TILs were tested in four different conditions: 1) Resting - with 300U/ml of IL2 (Biolegend cat-589106), 2) With polyclonal activation of T cells, using 1 (µg/ml of plate bound anti CD3 antibody (eBioscience clone OKT3, cat-16-0037-85) + 2 µg/ml of anti CD28 ab (eBioscience clone CD28.2 cat-16-0289-85) + 300 U/ml of IL2. 3) Co-cultured (1:1) with Mel888 (LIMS ID: CL-216) melanoma cells (HLA-A2 negative) and 4) Co-cultured (1:1) with Mel624 (LIMS ID CL-218) melanoma cells (HLA-A2 + Mart1/gp100 positive).

[0277] After 12 hours of resting / activation / co-culture, cells were tested by FACS for PVRIG expression as well as the expression of other members of PVRIG pathway and other surface markers.

[0278] Staining cells: Cells were harvested after 12 hours and washed twice with PBS. Cells were stained in room temp for 20 minutes with PBS supplemented with 1/1000 of fixable viability stain efluor 450 (BD horizon cat-562247). After staining, cells were washed twice with PBS and stained for 15 minutes on ice with FACS buffer (PBS + 0.5% BSA + 2 mM EDTA + 0.05% Azide) supplemented with 1/25 of human Truestain FC-Block (Biolegend, 422302). After FC-blocking, cells were stained on ice for 30 minutes with the Abs and concentrations that are listed in table 1.

Antibodies	**Isotype**	**Conjugated to**	**Manufacturer**	**Catalog number**	**concentration (ug/ul)**	**Staining concentration**
Anti-human PVRIG - CPA.7.021	Human IgG2	AF-647	Compugen - iNC	CPA.7.021	0.2	5 ug/ml
Human IgG2 isotype control	Human IgG2	AF-647	Compugen - iNC		0.2	5 ug/ml
CD96	mIgG1	APC	Biolegend	338410	0.2	4 ug/ml
PVR	mIgG1	APC	Biolegend	337618	0.05	1 ug/ml
PVRL2	mIgG1	APC	Biolegend	337412	0.1	2 ug/ml
TIGIT	mIgG1	APC	eBioscience	17-9500-42	0.025	0.5 ug/ml
DNAM1	mIgG1	APC	Biolegend	338312	0.1	2 ug/ml
PD1	mIgG1	AF647	Biolegend	329910	0.1	2 ug/ml
CD8	mIgG1	FITC	Biolegend	300906	0.15	3 ug/ml

[0279] After staining, cells were washed once and re-suspended in FACS buffer for analysis. Compensation calibration was done using compensation beads (BD, 552843). One drop of beads were stained for 30 minutes with above antibodies. Beads staining was done with same concentrations as cell staining. After beads staining, compensation was performed on MacsQuant FACS machine according to standard procedure. All samples were acquired on a MACSQuant analyzer (Miltenyi) and data was analyzed using Tree Star FlowJo software (v10.0.8).

[0280] PVRIG is expressed on human resting TILs: Resting TILs, cultured for 12 hours with 300 U/ml of IL2 only, were stained for PVRIG expression and analyzed by FACS. Gating strategy for TILs: Lymphocytes were gated first according to size and granularity in FCS:SSC graph, than single cells were gated according to FSC-H and FSC-A, than live cells were gated according to viability Dye staining in Vioblue:FSC graph, than CD8$^+$ cells were gated according to CD8 staining in CD8:FSC graph. Expression levels of PVRIG was than plotted according to PVRIG staining in histograms.

[0281] PVRIG expression on human TILs is downregulated upon activation with anti CD3 + anti CD28 abs: Human TILs, cultured for 12 hours with anti CD3 + anti CD28 abs + IL2 were stained for PVRIG expression and analyzed by FACS. PVRIG expression on surface of all three TILs examined is downregulated upon activation, comparing to resting TILs (data not shown).

[0282] PVRIG expression on human TILs is slightly downregulated upon co-culture with Mel888: Human TILs, co-cultured for 12 hours with Mel888 cells were stained for PVRIG expression and analyzed by FACS. PVRIG expression on surface of all three TILs examined is slightly downregulated upon co-culture with Mel888 comparing to resting TILs.

[0283] PVRIG expression on human TILs is downregulated upon co-culture with Mel624: Human TILs, co-cultured for 12 hours with Mel624 cells were stained for PVRIG expression and analyzed by FACS.PVRIG expression on surface of all three TILs examined is slightly downregulated upon co-culture with Mel624 comparing to resting TILs.

[0284] Expression of other pathway members on resting TILs: Human TILs, co-cultured for 12 hours with IL2 only were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. CD96, TIGIT and DNAM1 is expressed on all three examined TILs. PVR is expressed on the surface of all three TILs as well but to relatively low levels. PVRL2 is not detected on any of the TILs.

[0285] Expression of other pathway members on TILs activated with anti CD3 and anti CD28 abs: Human TILs, cultured for 12 hours with anti CD3 and anti CD28 abs were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Upon activation with anti CD3 + anti CD28 abs, CD96 is downregulated, PVR is slightly upregulated, TIGIT is slightly upregulated and DNAM1 is upregulated as well.

[0286] Expression of other pathway members on TILs Co-cultured with Mel888: Human TILs, co-cultured for 12 hours with Mel888 cells were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Upon co-culture with Me1888, CD96 is downregulated, PVR is highly upregulated, TIGIT and DNAM1 is downregulated, PVRL2 is slightly induced as well.

[0287] Expression of other pathway members on TILs Co-cultured with Mel624: Human TILs, co-cultured for 12 hours with Mel624 cells were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Gating strategy was done according to figure 1. Upon co-culture with Me1624, CD96 is downregulated, PVR is highly upregulated, TIGIT is stable or slightly upregulated, DNAM1 is downregulated and PVRL2 is slightly induced.

[0288] Expression of PD1 on TILs: Human TILs, cultured for 12 hours with IL2 only or activated with anti CD3 + anti CD28 abs or co-cultured with Mel888 or with Mel624 cells were stained for the expression of PD1 and analyzed by FACS. As can be seen in figure 16 and figures 17, PD1 is expressed on resting TIL412 only. No change in PD1 expression is noticed upon co-culture with Me1888, But, PD1 is upregulated in all three TILs upon co-culture with Mel624 or upon activation with anti CD3 + anti CD28 abs.

[0289] Summary and conclusions: For all TILs that were tested:

- Anti PVRIG - CPA.7.021 ab stains TILs (up to 2.6 fold)
- PVRIG expression is downregulated upon activation of 12 hours with anti CD3 + anti CD28 abs or upon co-culture with Mel624 (almost to background level).
- Resting TILs express CD96, TIGIT and DNAM1(up to 35, 12 and 79 fold respectively)
- CD96 expression is downregulated upon activation (from up to 35 to ~11 fold) or co-culture with irrelevant (HLA-A2-) melanoma
- DNAM1 expression is upregulated upon activation with αCD3/CD28 abs (from up to 79 to 102 fold) but strongly downregulated upon co-culture of TILs with Mels (down to 8 fold).
- TIGIT expression is slightly downregulated upon co-culture of TILs with mel888 cell line, and was stable with a slight upregulation upon co-culture with Mel624 or activation with anti CD3 + anti CD28 abs.
- PD1 expression is upregulated upon activation (from 0 up to 18 fold)
- High levels of PVR were detected following TILs co-culture with melanomas (from <2 up to 18 fold).

[0290] Resting TIL-412 show positive staining for PD1. TIL-F4 is also slightly positive for PD1 whereas TIL-209 is negative. Summary of changes in expression levels of all parameters tested, in the different conditions can be seen in Table 2.

Table 2:

	+IL2	+αCD3+αCD28+IL2	+Mel888	+ Mel624
PVRIG	1.4 - 2.6	0-12	1.3 - 1.7	0-1.2
CD96	23 - 35	12.7 - 16	11.7 - 17.6	11.1-16.6
TIGIT	5.7 - 12.6	7.8 - 12.5	4-7.3	6.1 - 12.5
DNAM1	43 - 79	56 - 100	14-20	17-25
PVR	1.6 - 1.8	2.6 - 3.2	13.6 - 18	11-17
PVRL2	0	0	1.4-2.3	1.2 - 1.8
PD1	0-4.5	2.3 - 18.4	0 - 4.6	2 - 9.3

Example 1I: EXPRESSION OF PVRIG ON RESTING AND ACTIVATED HUMAN T CELLS AND TILS

[0291] The aim of this example was to evaluate the expression of PVRIG protein on resting and activated human isolated primary CD4+ and CD8+ T cells, as well as TILs (Tumor Infiltrating Lymphocytes) isolated from human melanoma samples and propagated in the presence of melanoma specific antigens and IL2. Human mAbs were produced against the extracellular domain (ECD) of human PVRIG. These Abs were directly labeled with Alexa flour 647 in order to examine the expression of PVRIG on cells by FACS analysis.

Materials and Methods

[0292] TILs: In this series of experiments, two different TILs, from resected metastases of three melanoma patients, were used:

TIL-Mart1- HLA-A2-Mart1 specific

TIL-209- HLA-A2-gp100 specific

Human TILs (>95% CD8+), were thawed 24h prior to beginning of experiment. Cells were thawed in 12 ml of TIL medium (IMDM + 10% human serum +1% Glutamax + 1% Na-Pyruvate + 1% non-essential amino acids + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover for 24 hours.

[0293] Primary T cell: In this series of experiments two different donors were used:

CD4+ and CD8+ from donor #147

CD4+ and CD8+ from donor #186

Human primary cells (>95% purity), were thawed 24h prior to beginning of experiment. Cells were thawed in RPMI complete medium (RPMI + 10% FBS + 1% Glutamax + 1% Na-Pyruvate + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover for 24 hours.

[0294] Assay conditions: After recovery, cells were activated using a polyclonal activation of T cells, with 1 µg/ml of plate bound anti CD3 antibody (BD-pharmingen clone Ucht-1, cat-555329), 2 µg/ml of anti CD28 ab (eBioscience clone CD28.2 cat-16-0289-85) and 300 U/ml of IL2.

[0295] Activation was carried out for 24h, 48h, 72h and 144h.

[0296] Staining cells: Cells were harvested and washed with PBS. Cells were stained at room temprature for 10 minutes with PBS supplemented with 1/1000 of fixable viability stain efluor 450 (BD horizon cat-562247). After staining, cells were washed twice with PBS and stained with the Abs at the concentrations listed in

[0297] Figure 65 for 30 minutes on ice in FACS buffer (PBS + 0.5% BSA + 2 mM EDTA + 0.05% Azide) and concentrations that are listed in

[0298] Figure 65. After staining, cells were washed once and re-suspended in FACS buffer for analysis.

[0299] **<u>Results</u>:** Human T cells from two different donors and TILs were left untreated (resting) or polyclonal stimulated for various timepoints as described in Materials and Methods. Cell activation state was evaluated by detection of surface expression of CD137 and PD-1 at each time point compared to isotype control (FMO), as shown for activated CD8+, CD4+ T cells and TILs (Figure 70A, B & C respectively). As expected, PD-1 and CD137 expression was detected and elevated upon activation (Figure 70A, B & C).

[0300] PVRIG expression was observed on both resting CD4+ and CD8+ T cells, with higher expression on CD8+ cells (6-8 fold) as compared to CD4+ cells (3 folds), and diminished upon activation (Figure 71A, B & C). On days 3-6 of activation, PVRIG expression was increased on CD8+ (4-5 fold) and CD4+ (2-3 fold) T cells, as can be seen in Figure 71.

[0301] In addition, PVRIG expression was also observed on Mart1 and 209 resting TILs, and expression was decreased apon activation (Figure 72A, B & C). On day 3-6 of activation PVRIG expression was increased, as can be seen in Figure 72, compared to day 1-2 of activation.

Example 2: Generation and Characterization of PVRIG-Expressing Stable Transfectant Cell Pools

[0302] Recombinant stable pools of cell lines overexpressing PVRIG human and mouse proteins were generated, for use in determining the effects of PVRIG on immunity, for PVRIG characterization and for identifying immunoregulatory PVRIG based therapeutic agents.

[0303] Materials & Methods:

Reagents: DNA constructs:

Human PVRIG flag pUC57

Human PVRIG flag pCDNA3.1

Human PVRIG flag pMSCV

Recombinant cells:

HEK293 pCDNA3.1 Human PVRIG flag

HEK293 pMSCV Human PVRIG flag

Commercial antibodies:

Anti PVRIG, Sigma cat. HPA047497 - Rabbit polyclonal

Anti-PVRIG, Abnova cat. H00079037-B01 -Mouse polyclonal

Full length validation of mouse PVRIG was done using PCR reactions and sequencing of the PCR products.

[0304] Three couples of primers were used (Table 3).

Table 3: Sequence of primers used for mouse full length validation

Primer name	Sequence :
200-554_mPVRIG_F	CCACCAACCTCTCGTCTTTC
200-553_mPVRIG_R	TCATGCCAGAGCATACAG
200-571_mPVRIG _F	CAGTGCCTCTAACTGCTGAC
200-572_mPVRIG _R	TCACTGTTACCAGGGAGATGAG
200-549_mPVRIG _F	CACAGGCTGCCCATGCAAC
200-551_mPVRIG _R	TGCCTGGGTGCTAGTGAGAG
200-554_mPVRIG _F	CCACCAACCTCTCGTCTTTC
200-546_mPVRIG _R	GACCCTGTTACCTGTCATTG

[0305] As a templet for the PCR reaction, cDNA of NIH 3T3 cell line or a mix of three commercial cDNA panels were used:

1. 1. cDNA panel I, Mouse, Biochain, Cat no. C8334501 (Heart, Brain, Kidney,Liver).
2. 2. cDNA panel II, Mouse, Biochain, Cat no. C8334502 (Lung, Pancreas, Spleen, Skeletal Muscle).
3. 3. cDNA, Clontech, Cat no. 637301, (Brain, Heart, day 7 Embrio, Testis, Spleen).

Expression constructs

[0306] Full length cloning of human and mouse PVRIG-flag was performed by gene synthesis (GenScript) using codon optimized sequence in pUC57 vector for human transcript and non optimized for mouse transcript and subcloned into a mammalian expression vector, pcDNA3.1 or to pMSCV, to create the expression plasmid.

[0307] Human PVRIG sequence that was subcloned into pcDNA3.1 initiate from the second methionine of human PVRIG protein, whereas the human PVRIG sequence that was subcloned into pMSCV initiate from the first methionine of human PVRIG protein.

-Construct encoding the Human PVRIG-flag.

[0308] Full length human PVRIG gene, synthesis by GenScript was subcloned into using pcDNA3.1 using BamI and NheI restriction enzymes.

[0309] Constructs encoding the mouse PVRIG proteins:
Four contracts encoding the mouse sequence were synthesize by GenScript as following:

1. First Methionine no tag
2. First Methionine with Flag
3. Second Methionine no tag
4. Second Methionine with Flag

The synthesize gene were subcloned into pCDNA3.1

[0310] Generation of stable transfectants over expressing PVRIG proteins

[0311] The resulting expression construct was verified by sequence and subsequently used for transfections and stable pool generation as described below. The protein sequences encoded by the expression constructs are as set forth in Figure 103.

Generation of stable transfectant pools expressing human PVRIG-flag protein

[0312] HEK293 (ATCC, catalog number: CRL-1573) cells were transfected with pCDNA3.1+ human PVRIG-flag plasmid or with empty vector (pCDNA3.1+ as negative control), using FUGENE 6 Reagent (Roch, catalog number 11-988-387). Geneticin, G418 (Gibco, catalog number: 11811-031) resistant colonies were selected for stable pool generation.

[0313] GP2-293 packaging cell line (Clontech cat#631458) was transfected with pMSCV-human PVRIG or with pMSCV empty vector using Lipofectamine 2000 transfection reagent (Invitrogen, catalog number 11668019). 48 hours post transfection supernatants containing virions were collected, and directly used for infection of the human cell line as follows:

[0314] HEK-293 (ATCC, CRL- CRL-1573) cells was infected with virions expressing human PVRIG or with pMSCV empty vector virions as negative control, Puromycin (Invivogen, catalog number: 58-58-2) resistant colonies were selected for stable pool generation.

Expression validation

Expression validation by Western blot

[0315] Whole cell extracts of cell pool (30ug of total protein) were analyzed by western blot. As negative control, whole cell extracts of stable cell pools transfected with the empty vector were used. For the human PVRIG-flag detection, anti-flag and anti PVRIG antibodies were used as follow:

- Mouse anti Flag M2-Peroxidase, Sigma, cat. A8592 diluted 1:1000 in TTBS/5% BSA;
- Anti PVRIG, Sigma cat. HPA047497- Rabbit polyclonal, diluted 1:200 in TTBS/5% BSA. Followed by Goat Anti Rabbit-HRP, Jackson, Cat: 111-035-003 diluted 1:20,000 in 5%milk/TTBS solution.

Expression validation by Flow Cytometry (FACS)

[0316] In order to validate the cell surface expression of the human PVRIG protein in the recombinant stable pools, 1×10^5 cells were stained with Fixable viability stain 450 (BD, 562247) diluted 1:1000 in PBS, for 10 min at R.T. Mouse polyclonal anti PVRIG, (Abnova, Cat.H00079037-B01) diluted 1:200 or with mouse IgG1 isotype control (Life Technologies), were then added to cells followed by staining with Goat Anti Mouse-PE (Jackson, cat.115-116-146).

Results Expression validation of HEK293 Stable pool cells over expressing the Human PVRIG-Flag protein

[0317] To verify expression of the PVRIG protein in the stably transfected HEK293 cells pools, whole cell extracts were analyzed by western blot using anti-flag antibody or anti PVRIG antibodies (Abnova), as described in Material and Methods. The results, shown in Figure 24, demonstrate a band corresponding to the expected protein size of ~33kDa in the extracts of HEK293 cell pools expressing human PVRIG, but not in the cells transfected with the empty vector.

[0318] In order to verify cell surface expression of the PVRIG protein, HEK293 stably transfected cells over-expressing the PVRIG- flag pCDNA3.1 vector were analyzed by FACS using mouse anti-PVRIG pAb (Abnova) as described in Material and Methods. The results presented in Figure 25 show that the binding of mouse anti-PVRIG pAb to cells stably expressing the human PVRIG- flag (gray) is higher than that observed with cells transfected with the empty vector (light gray).

Example 3: PVRIG-ECD Ig fusion protein production

[0319] PVRIG mECD-mIg fusion protein (see Figure 103), composed of the ECD of mouse PVRIG fused to the Fc of mouse IgG2a, was produced at ProBioGen (Germany) in CHO-DG44 cells by culturing stable cell pools for 12 days, followed by Protein A purification of cell harvest and preparative SEC purification for aggregate removal. The final product was formulated in 5mM Na citrate, 5mM Na/K phosphate, 140mM NaCl, 0.01% Tween pH5.5.

[0320] Expression vector used was ProBioGen's PBG-GPEX6. PVRIG gene is driven by CMV/EF1 hybrid promoter followed by polyadenylation signal pA-1. The vector contains puromycin N-acetyl-transferase gene that allows selection of transfected cells using puromycin, as well as dehydrofolate reductase gene that allows selection of transfected cells using methotrexate (MTX).

[0321] PVRIG hECD-hIg fusion protein (see Figure 103), composed of the ECD of human PVRIG fused to the Fc of human IgG1 bearing C220, C226 and C229 to S mutations at the hinge, was produced at GenScript (China) by transient transfection in CHO-3E7 cells which were cultured for 6 days, followed by protein A purification of cell harvest. The final product was formulated in PBS pH 7.2.

[0322] Expression vector used was Mammalian Expression Vector pTT5, in which PVRIG gene is driven by CMV promoter.

Example 4: Expression of PVRIG on human PBLs and binding of PVRIG-Fc to melanoma cell lines

[0323] PVRIG is a novel immune checkpoint protein, which without wishing to be limited by a single theory functions as a CD28 like receptor on T cells. In this study, the expression of PVRIG on human peripheral blood lymphocytes and the binding of PVRIG-ECD-Ig (composed of the extra-cellular domain of human PVRIG fused to human IgG1) to melanoma cell lines was evaluated.

Materials and Methods

[0324] Three human melanoma cell lines which present the MART-1 antigen in HLA-A2 context (SK-MEL-23, Mel-624 and Mel-624.38) were used as targets for CTLs. Mel-888 which does not express HLA-A2, served as a negative control.

[0325] Buffy coats from human healthy donors were obtained from Tel Hashomer Blood Bank. Peripheral blood mononuclear cells were stimulated with PHA and cultured for 3 days, and subsequently transduced with MSCV-based retroviral vector (pMSGV1). Following transduction, cells were further grown in lymphocyte medium (Bio target medium, fetal bovine serum (10%), L Glutamine Penicillin/ Streptomicyn (100 units/ml), IL-2 300 IU) for additional 5 days.

[0326] To evaluate PVRIG expression on PBLs, cells were stained with a specific antibody for PVRIG (mouse poly clonal) at 5ug/ml for 30min at 4 degrees. Following washing, cells were stained with FITC conjugated Goat anti mouse mAb (1:250) (Invitrogen, Cat# A10667) in FACS buffer in the dark for 30 minutes at 4 degrees. Following two washes in FACS buffer, samples were read on a BD Bioscience FACS Calibur with a Cytek HTS.

[0327] To evaluate binding of PVRIG-Ig to the melanoma cell lines, SK-MEL-23, Mel-624, Mel-624.38 and mel-888, cells were co-cultured with F4 transduced or un-transduced (designated w/o) PBLs and subsequently stained with 20ug/ml of the fusion protein PVRIG-Ig HH batch #125. Following two washes in FACS buffer, samples were stained with a secondary goat anti-human PE (Jackson, cat# 109-116-098).

Results

[0328] To evaluate the endogenous expression of PVRIG on primary human leukocytes, PBLs were stimulated with PHA and subsequently transduced with an empty vector and stained with an anti-PVRIG specific antibody. As shown in Figure 11, in two different donors staining with anti-PVRIG is observed relative to an isotype matched control.

[0329] To evaluate the endogenous expression of PVRIG on melanoma cell lines and to determine whether the endogenous expression is affected by co-culture with antigen specific T cells, 4 different melanoma cell lines (SK-MEL-23, Mel-624, Mel-624.38 and mel-888) cu-cultured with PBLs either expressing or not expressing the F4 (gp100 specific TCR). Cells were subsequently stained with the fusion protein composed of the extra-cellular domain of human PVRIG fused the Fc portion of human IgG1. As shown in Figure 12, all 4 tested human melanoma cell lines exhibit binding to PVRIG-Ig. Binding intensity is not affected by T cell dependent activation following co-culture with melanoma reactive engineered T cells.

[0330] Summary: The results presented herein suggest that PVRIG is expressed on PHA activated human primary peripheral blood leukocytes (PBLs). In addition, 4 melanoma cell lines that were tested in this study bind to the fusion protein composed of the extra-cellular domain of human PVRIG fused the Fc portion of human IgG1 suggesting that these cell lines express the counterpart for PVRIG.

Example 5: ReceptorLigand identification and Validation

[0331] A first validation study was performed using a cell microarray technology was used to screen for interactions of PVRIG to 3559 full-length human plasma membrane proteins, which were individually expressed in human HEK293 cells.

[0332] Human HEK293 cells were grown over slides spotted with expression vectors encoding 3559 full-length human membrane proteins. An expression vector (pIRES-hEGFR-IRES-ZsGreenl) was spotted in quadruplicate on every slide, and was used to ensure that a minimal threshold of transfection efficiency had been achieved or exceeded on every slide. Human HEK293 cells were used for reverse transfection/expression. A fusion protein composed of the ECD of PVRIG fused to a human IgG1 was added at 20ug/ml to each slide following cell fixation. Detection of binding was performed by using an appropriate fluorescent secondary antibody. Two replicate slide-sets were screened. Fluorescent images were analyzed and quantitated (for transfection efficiency) using ImageQuant software (GE).

[0333] A protein 'hit' was defined as a duplicate spot showing a raised signal compared to background levels. This was achieved by visual inspection using the images gridded on the ImageQuant software. Hits were classified as 'strong, medium, weak or very weak', depending on the intensity of the duplicate spots. To confirm the hits, all vectors encoding the hits identified in the primary screen were arrayed on new slides. Confirmation/Specificity screen and analyses was carried out as for primary screening (n=2 replicate slides per sample), except that identical slides were also probed with appropriate negative controls. Additionally, all the vectors encoding the hits were sequenced. Vectors encoding every primary hit was sequenced confirming its identity.

[0334] Background screen showed negligible binding to untransfected HEK293 cells at 2, 5 and 20 ug/ml (Figure 13). Based upon the background data, 20 ug/ml was chosen for full profiling. Primary screen resulted in multiple duplicate hits (clones), with the majority being weak or very weak intensity. All primary hits identified, and a control EGFR-ZsGreen1 vector, were spotted and re-expressed in duplicate and probed with PVRIG at 20ug/ml for the Confirmation/Specificity screen.

[0335] A single specific hit, PVRL2, with strong intensity, was identified (Figure 14). Another weak hit, MAG, was later shown to bind also other fusion proteins tested (data not shown), thus suggesting that it is not specific. These results are consistent with the recently published abstract https://www.yumpu.com/en/document/view/7263720/sunday-december-4-late-abstracts-1-molecular-biology-of-the-/133 by G. Quinones in New Technologies & Frontlers. PVRL2 is known to play a role as a ligand for TIGIT and DNAM1, which are both modulators of T cell and NK cell activation. TIGIT has been recently reported to be a key player in the inhibition of the immune response directed against tumor cells (Noa Stanietsky, journal of immunology, vol. 106 no. 42, 17858-17863; Robert J Johnston, Cancer cell, Volume 26, Issue 6, p923-937, 8 December 2014). Results presented in Example 5, showing interaction of PVRIG with the same counterpart as TIGIT, suggests an involvement of PVRIG in an important regulatory pathway that regulates cancer immune surveillance and thus positions PVRIG as a potential target for cancer treatment.

Additional Validation Study 2

Materials and Methods

Materials

[0336] Fc fusion proteins, His-tagged proteins and control Ig: The Fc fusion protein PVRIG-Fc M:M was used for binding studies. Mouse IgG2a was used as isotype control. Other commercial mouse proteins used in the study were PVRL2-his (R&D, 3869-N2), and PVRL2-his (Sino Biological, 50318-M08H).

[0337] Cells: HEK293 over-expressing (OX) mouse PVRIG and PVRIG-FLAG were generated (RC-287 and RC-286, respectively) and binding of PVRL2 to these cells was compared to HEK293 cells expressing empty vector (EV) (RC-83). HEK293 OX mouse PVRL2 splice variants 1 and 2 (sv1 and sv2) were generated (RC-334 and RC-335, respectively) and binding of PVRIG to these cells was compared to HEK293 cells expressing EV. B16-F10 cells (CL-161, mouse skin melanoma cells endogenously expressing mPVRL2) were also used to study the interaction between PVRIG and PVRL2.

[0338] Antibodies: Anti-mouse PVRL2-PE Ab (R&D, FAB3869P, 25µg/ml, 1:100) was used for detection of PVRL2. Rat IgG2A-PE (R&D, IC006P, 25µg/ml, 1:100) was used as isotype control. Anti-mouse-PE (Jackson Immunoresearch, 115-115-206, 0.5mg/ml, 1:200) and anti-his Ab (Abcam, ab72467, 0.1mg/ml, 1:300) were used to detect binding of recombinant proteins. Anti-DYKDDDDK Tag (anti-FLAG) Ab (BioLegend, 637302, 0.5mg/ml, 1:300) was used for detection of PVRIG expression on HEK293 OX mouse PVRIG-FLAG.For PVRIG labeling, Alexa Fluor® 647 Antibody Labeling Kit (Molecular Probes, A-20186) was used according to manufacturer's protocol. For biotinylation of PVRIG, DSB-X™ Biotin Protein Labeling Kit (Molecular Probes, D-20655) was used according to manufacturer's protocol. Biotinylated PVRIG was detected by streptavidin-PE (SA-PE) (Jackson Immunoresearch, 016-110-084, 0.5mg/ml, 1:300).

Methods

[0339] FACS analysis of mouse PVRIG-Fc binding to stable HEK293 cells over-expressing (OX) mouse PVRL2 or to B16-F10 cells: HEK293 cells OX PVRL2 (sv1 or sv2) or B16-F10 cells were suspended to 10^6 cells/ml in PBS. For each 1ml of cells, 1µl of viability stain stock solution (BD Horizon Fixable Viability Stain 450, cat. 562247, BD Bioscience) was added. Cells were incubated for 10min protected from light at room temperature. The cells were then washed twice with PBS and suspended to 3×10^6 cells/ml in the presence of 1:50 human TruStain FcXTM (BioLegend 422302) in FACS buffer (PBS supplemented with 2% FBS and 0.5mM EDTA) at room temperature for 15min for blocking of Fcγ-receptors. Without washing, 1×10^5 cells/well were then plated in 96-well V-shaped plates (Costar #3357). Expression of PVRL2 was examined by anti-PVRL2 antibody (see above). Binding of PVRIG-Fc to cells was examined with various batches (see above), generally at 60µg/ml or with several concentrations. Cells were incubated with antibodies or PVRIG-Fc for 40min at room temperature, then washed once. Secondary antibody (anti-mouse-PE) was added for 15min at room temperature, cells were washed twice and were taken for analysis by MACSQuant® FACS analyzers (Miltenyi Biotec), followed by data analysis using Flow-Jo 10 software.

[0340] FACS analysis of mouse PVRL2-his binding to stable HEK293 cells OX mouse PVRIG: PVRIG levels were examined with anti-FLAG antibody. PVRL2-his binding was monitored by anti-his antibody. FACS analysis was performed as described above.

[0341] Biophysical SPR analysis of mouse PVRIG / PVRL2 interaction by Biacore: The interaction between mouse PVRIG and PVRL2 was analyzed in a Biacore T100 SPR biomolecular interaction analyzer at Bar-Ilan University. Proteins were diluted to 100nM in acetate buffer pH 4.0, and were covalently coupled to a unique flow cell of a CM5 Series S Biacore chip using standard amine coupling chemistry. Surfaces were activated with EDC-NHS, and later blocked by injection of 1M ethanolamine (pH 8.5). Running buffer was 10mM Hepes pH 7.3, 150mM NaCl, 3mM EDTA and 0.05% Tween-20 (HBS-EP+). Final immobilization levels were ~1000RU. Proteins used as analytes were diluted to 2500nM, 500nM and 100nM. In each run one tube contained running buffer only for reference. After each run a regeneration step with 4M MgCl2 for 30 sec at 20µl/sec was performed.

Results

[0342] Binding of mouse PVRIG to HEK293 cells OX PVRL2 sv1: In order to validate the interaction between mouse PVRIG and mouse PVRL2 we first tested the binding of PVRIG-Fc to cells over-expressing (OX) PVRL2. The level of PVRL2 expression on HEK293 OX PVRL2 sv1 was determined using specific anti-mouse PVRL2 antibodies. Mouse PVRL2 expression was 10-fold higher compared to HEK293 cells expressing empty vector (data not shown). Four batches of PVRIG-Fc were examined for binding to PVRL2 OX cells. All PVRIG-Fc batches showed 6-11-fold binding to cells OX PVRL2 compared to empty vector cells (data not shown). Binding of PVRIG-Fc to PVRL2 OX cells was also examined using biotinylated and fluorescently labelled (Alexa Fluor 647) PVRIG proteins. While the biotinylated proteins displayed slightly stronger binding to PVRL2 OX cells compared to untagged PVRIG-Fc (data not shown), fluorescently labelled PVRIG demonstrated much lower binding (data not shown). These results show that PVLR2 is detected on the membrane of HEK293 cells OC PVRL2; binding of mouse PVRIG-Fc to PVLR2 OX cells is detected by anti-mouse IgG2A antibodies; binding of biotinylated mouse PVRIG-Fc to PVLR2 OX cells is detected by streptavidin-PE, and binding of Alexa Fluor 647-labeled PVRIG-Fc to PVLR2 OX cells.

[0343] Binding of mouse PVRL2 to HEK293 cells OX PVRIG : To further validate the interaction between mouse PVRIG and mouse PVRL2 we tested the binding of PVRL2 to cells OX PVRIG with or without a FLAG-tag. Membrane expression of mouse PVRIG on HEK293 cells OX PVRIG with a FLAG-tag was confirmed using an anti-FLAG antibody (data not shown). As expected, HEK293 cells OX PVRIG without a FLAG-tag showed no expression using an anti-FLAG antibody. Using anti-PVRIG supernatants (Aldeveron), these cells demonstrated lower expression of PVRIG compared to cells OX PVRIG with a FLAG-tag. Commercial mouse PVRL2 recombinant protein was available only as a His-tagged protein. Therefore, extensive calibrations were required to obtain an appropriate anti-His antibody and conditions for detection. His-tagged PVRL2, from two different sources, were tested for binding to PVRIG OX cells at 60μg/ml and demonstrated 2-fold (data not shown) and 3-4 fold (data not shown) binding compared to HEK293 cells expressing empty vector. That is, his-tagged mouse PVLR2 binds HEK293 OX mouse PVRIG, and mouse PVRIG is expressed on membranes of HEK293 cells OX PVRIG.

[0344] Study of mouse PVRIG and mouse PVRL2 interaction using SPR-Biacore: In order to assess the interaction between mouse PVRIG-Fc and mouse His-tagged PVRL2, both proteins were immobilized to a Biacore chip. Following immobilization, both proteins, as well as PVRIG-Fc (data not shown) were run as analytes at three concentrations: 2500, 500 and 100nM (PVRIG batch #480 and PVRL2 were run twice as analytes). Interaction between the two proteins was detected in both directions and with both batches of PVRIG (data not shown). Due to complex kinetics, an exact KD could not be determined from the Biacore results.

[0345] Dose response binding of mouse PVRIG to HEK293 cells OX PVRL2 sv2 and B16-F10 cells: As shown above, mouse PVRL2 binding to mouse PVRIG OX cells was relatively low. In order to establish a method for screening anti-mouse PVRIG antibodies capable of blocking the interaction between mouse PVRIG and mouse PVRL2, the binding of PVRIG-Fc to PVRL2 OX cells was selected. First, a dose response binding curve of mouse IgG2A and mouse PVRIG-Fc to cells OX mouse PVRL2 was generated and compared to cells expressing empty vector (EV). The dose response was performed in two-fold serial dilutions (1:2) from 50μg/ml to 0.1μg/ml. While no difference in mouse IgG2A binding was observed (data not shown), PVRIG-Fc demonstrated saturation of binding at 12.5μg/ml and reduced binding in correlation with the decrease in protein concentration (data not shown). Similar results were obtained also with PVRIG-Fc (data not shown). These results suggest that this binding assay can be considered for screening of blocking antibodies.

[0346] In order to consider also an endogenous system for screening of anti-mouse PVRIG antibodies, the expression of PVRL2 on B16-F10 cells was assessed using an anti-PVRL2 antibody. Results show that PVRL2 is highly expressed on B16-F10 cells (data not shown). Therefore, a similar dose response binding curve was produced also for binding of mouse IgG2A and mouse PVRIG-Fc to B16-F10 cells. Similarly to the results obtained with HEK293 cells OX PVRL2, mouse PVRIG-Fc demonstrated dose response binding to B16-F10 cells reaching saturation at 12.5μg/ml, while no change in binding of mouse IgG2A was detected (data not shown).

[0347] Discussion and Conclusions: Human PVRIG interaction with human PVRL2 was identified using Cell Microarray Technology at Retrogenix. To validate this interaction also in mouse, several approaches were taken. Among them the use of PVRIG or PVRL2 OX cells, and biophysical measurements using SPR-Biacore. All approaches indicated that mouse PVRIG interacts with mouse PVRL2. However, the binding of mouse PVRL2 to cells OX PVRIG was relatively low compared to the binding of PVRIG to cells OX PVRL2. The reason for this could be the fact that commercial PVRL2 is available only as a monomer His-tagged protein and not as an Fc-fused protein (as for PVRIG). To this end, a custom Fc-fused mouse PVRL2 was produced at GenScript. However, from preliminary data, only a minor increase in binding was observed with this protein (-5-fold compared to 2-3 fold with the PVRL2-his). Therefore, some other factors might influence this relatively low binding.

[0348] Due to the low PVRL2 binding to cells OX PVRIG, it was decided to establish an anti-PVRIG antibody blocking assay using PVRIG-Fc binding to cells OX PVRL2. According to the observed dose response curves we suggested three working concentrations: 0.1, 0.2 and 0.4 μg/ml. Following similar results obtained with binding of PVRIG to PVRL2 endogenously expressing B16-F10 cells, we suggested to perform the antibody blocking assay also on these cells at the following concentrations: 0.2, 0.4, 0.8 μg/ml.

[0349] PVRIG is a presumed receptor, therefore, preferably the antibody blocking assay should be performed with PVRL2 as a soluble protein and PVRIG expressed on the cells. Thus, it should be considered to examine anti-mouse PVRIG antibodies that demonstrate blocking activity in the current format also in this system.

Additional Validation Study 3

[0350] The objective of this study is to confirm the binding partners of PVRIG, a novel immuno-oncology target. Preliminary studies indicate that one of these ligands is PVRL2. In this study, binding of the recombinant PVRIG protein to several potential ligands in the PVRIG axis has been investigated by ELISA.

Protocols

[0351] List of reagents: Current literature on the PVRIG proteins suggests that there are three potential ligands: PVR (CD155), PVRL2 (CD112), and PVRL3 (CD113). To investigate their ability to bind the PVRIG receptor, these three ligands were sourced commercially, as follows: PVR and PVRL3 from Sino Biologicals Inc. and PVRL2 from R&D Systems and Sino Biologicals Inc. The human PVRIG recombinant protein was generated at Compugen as the PVRIG extra-cellular domain (ECD) fused to a human IgG1 Fc domain (PVRIGHH).

[0352] ELISA to determine receptor-ligand interaction: Commercially sourced His-tagged ligands, PVR, PVRL2, and PVRL3, were coated on the wells of a high binding EIA/RIA plate (Costar 9018) overnight at 4°C. An irrelevant His-tagged protein was included as a negative control. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound ligands were incubated with varying concentrations of PVRIGHH in solution (linear range of 0.1 µg/mL to 4 µg/mL in a 50 µL/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well of a HRP-conjugated secondary antibody was added (Human IgG Fc domain specific, Jackson ImmunoResearch). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer. The data were exported to Excel (Microsoft) and plotted in GraphPad Prism (GraphPad Software, Inc.).

[0353] Results: PVRIG preferably binds to PVRL2: The human PVRIG Fc-fusion protein was assayed for binding to PVR, PVRL2 and PVRL3, which were immobilized on an EIA/RIA plate. Varying concentrations of the receptor PVRIG in solution phase were incubated with the immobilized ligand. The data clearly show dose-dependent binding of PVRIGHH to PVRL2, but no binding to ligands PVR, PVRL3 or the negative control protein (data not shown). The ELISA A450 signal was plotted as a function of the receptor concentration using a one-site binding equation, revealing an equilibrium binding constant (KD) of 13 ± 1 nM.

[0354] Summary and Conclusions: PVRIG is a novel immuno-oncology target for which the biology is not fully understood. In an effort to shed more light on this biology, we examined its binding to several potential ligands. PVRL2 was clearly identified as the binding partner of PVRIG. Quantitative analysis suggests that this interaction is very strong, with a KD of 13±1 nM. Our results also suggest that human PVRIG either does not bind the human PVR and PVRL3, or the binding is too weak to detect by ELISA.

Additional Validation Study 4:

[0355] In this example, PVRIG expression on PBMC cell subsets was evaluated pre and post allo-activation. Following allo-activation the expression of PVRIG was upregulated on CD4+ T cells as well as on CD8+ T cells and double negative gamma delta T cells. This upregulation was observed in PBMCs of one out of two donors tested (see Figure 52).

Example 6 SURFACE PLASMON RESONANCE STUDIES OF PVR, PVRL2, AND PVRL3 BINDING TO PVRIG, DNAM, AND TIGIT

Materials and Methods

[0356] All experiments were performed using a ProteOn XPR 36 instrument at 22°C.

[0357] Step 1: A high density goat anti-human fc polyclonal antibody surface (Invitrogen H10500) was prepared over all six lanes of a GLC chip using a ProteOn XPR 36 biosensor. The activation step for the anti-human fc surface occurred in the horizontal flow direction while the immobilization step for the high density pAb occurred in the vertical flow direction. The blocking step occurred in both the vertical and horizontal positions so that the horizontal "interspots" could be used as reference surfaces. An average of ~4400 RU of goat anti-human pAb was immobilized on each lane.

[0358] Step 2: For each cycle, three different lots of human PVRIG fusion protein (human fc, GenScript lots 451, 448, 125), human DNAM-1 fusion protein (human fc, R&D Systems), human TIGIT fusion protein (human fc, R&D Systems), and a control human IgG (Synagis) were each captured over a different vertical lane for two minutes at a concentration of 2 µg/mL. PVR, two lots of PVRL2, and PVRL3 were each injected in the horizontal flow direction at six different concentrations over all six captured ligands at different ligand capture cycles. The injections were two minutes followed by 10 minutes of dissociation at a flow rate of 50µL/min. The PVR concentration range was 1.4nM-332nM in a 3-fold dilution series, both lots of PVRL2 were injected at a concentration range of 1.3nM-322nM in a 3-fold dilution series, and PVRL3 was injected at a concentration range of 1.4nM-334nM in a 3-fold dilution series. All protein reagents were prepared in running buffer which was degassed PBS buffer with 0.05% Tween 20 and 0.01% BSA added. The anti-human fc capture surfaces were regenerated with two 30-second pulses of 146 mM phosphoric acid after each cycle.

[0359] Step 3: Sensorgram data of the analytes binding to each captured ligand were processed and double-referenced using ProteOn Manager version 3.1.0.6 making use of interspot referencing and a pre-blank injection identical to the analyte injections.

Results

[0360]

1. **a) PVR:** Binds weakly to captured DNAM-1 and TIGIT and shows no binding to all three lots of

PVRIG and the control IgG. Not enough information was generated to estimate the K_D of the PVR interactions with DNAM-1 and TIGIT (data not shown).

2. **b) PVRL2:** Both lots of PVRL2 showed binding to all three lots of PVRIG and to DNAM-1 but minimal or no binding to TIGIT and no binding to the control IgG. Sensorgrams showed complex kinetics, therefore binding constants could not be estimated (data not shown).
3. **c) PVRL3:** Showed minimal binding to TIGIT and did not bind the other proteins (data not shown).

Example 7: IN-VITRO IMMUNOMODULATORY ACTIVITIES OF PVRIG ECD-IG ON MOUSE T CELLS

[0361] In these experiments the immunomodulatory activities of the recombinant fused protein PVRIG-ECD-Ig was investigated on mouse T cell activation. The effect of PVRIG-ECD-Ig on activation of mouse CD4 T cells was investigated using a number of in-vitro T cell activation readouts: cell activation markers, cytokine secretion and proliferation.

[0362] In order to evaluate the activity of pvrig protein on t cell activation, recombinant protein was produced comprising the mouse extracellular domain (ecd) of the mouse pvrig fused to the fc of mouse igg2a (designated pvrig-ecd ig m:m) (seq id no:29). The effect of the fc fused protein co-immobilized with anti-cd3 on mouse cd4 t cell functions, as manifested by activation markers and cytokines secretion was investigated.

Materials and Methods

[0363] Fc fusion protein and control Ig : Fc fusion protein, PVRIG-ECD-Ig (batch #198) was tested. Mouse IgG2a (clone MOPC-173; Biolegend or C1.18.4; BioXcell) was used as isotype control.

[0364] Mouse CD4 T cells isolation: Untouched CD4+CD25- T cells were isolated from pools of spleens of BALB/C mice using a T cell isolation Kit (Miltenyi Cat# 130-093-227) according to the manufacturer's instructions. The purity obtained was >90%.

[0365] Activation of mouse CD4 T cells: Anti-mouse CD3-ε mAb (clone 145-2C11; BD Biosciences) at 2µg/ml together with PVRIG-ECD-Ig protein or control Ig at various concentrations (1, 3 or 10□g/ml), were co-immobilized for 3hr at 37°C, on 96-well flat bottom tissue culture plates (Sigma, Cat. # Z707910). Control Ig was added to each well in order to complete a total protein concentration of 12µg/ml per well. Wells were washed 3 times with PBS and plated with 1×10^5 purified CD4+CD25- T cells per well and kept in a humidified, 5% CO2, 37°C incubator. In some experiments, soluble anti-CD28 (clone: 37.51; eBioscience; 1µg/ml) was added. Culture supernatants were collected at the indicated times post stimulation and analyzed for mouse IFNγ or IL-2 secretion by ELISA kits (R&D Systems). The effect of PVRIG-ECD-Ig protein (see Figure 103) on the expression of the activation marker CD69 on mouse CD4+ T cells was analyzed by flow cytometry. Cells were stained 48h post stimulation with a cocktail of antibodies including PerCP-anti-CD4 (clone G41.5; Biolegend), FITC or PE-anti-CD69 (clone H1.2F3; Biolegend), in the presence of anti-CD16/32 (clone 2.4g2; BD Biosciences) for blocking of Fcy-receptors. Cells were evaluated using MACSQuant analyzer 9 (Miltenyi) and data analyzed using BD CellQuest or by MACSQuantify TM Software. Data was analyzed using Excel or Prism4 software.

Results and Summary

[0366] Effect of PVRIG-ECD Ig M:M (see Figure 103) on mouse CD4+ T cells function: Figure 15 shows in-vitro immunomodulatory activities of PVRIG-ECD-Ig (see Figure 103)on isolated mouse splenic T cells (CD4+, >95%purity) stimulated with microplates co-immobilized with anti-CD3 (2ug/ml) alone or co-immobilized with control Ig (mIgG2a) or PVRIG-ECD-Ig (see Figure 103)) (10 ug/ml) in the presence of soluble anti-CD28 (1ug/ml). PVRIG-ECD-Ig (see Figure 103) suppressed mouse CD4 T cell activation in a dose dependent manner, as manifested by reduced CD69 up-regulation (Figure 15A, D), and reduction in TCR-induced cytokines (IL-2 and IFNy) secretion (Figure 15B-C, E). The magnitude of the inhibitory effect of PVRIG-ECD-Ig ((see Figure 103)) was in the range of 30-100%. Inhibitory effect of PVRIG-ECD-Ig ((see Figure 103)) on IFNγ secretion was observed in concentrations as low as 3ug/ml (-60% inhibition vs. control Ig).

[0367] PVRIG-ECD-Ig (see Figure 103) inhibits T cell activation in a concentration-dependent manner when the Fc fusion protein is co-immobilized with anti-CD3 on plates. Maximal inhibitory effect was observed at 10ug/ml of PVRIG-ECD-Ig (see Figure 103).

[0368] The results demonstrate the inhibitory effect of PVRIG-ECD-Ig on mouse T cells activation, manifested by reduced cytokine secretion, and suppression of activation marker CD69 upregulation. This inhibition of T cell activation, supports the therapeutic potential of immunoinhibitory PVRIG proteins (PVRIG polypeptides and fusion proteins) according to the present invention in treating T cell-driven autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease, as well as for other immune related diseases and/or for reducing the undesirable immune activation that follows gene therapy. In addition, these results also support the therapeutic potential of immunostimulatory PVRIG proteins (PVRIG polypeptides and fusion proteins) that reduce the inhibitory activity of PVRIG for treating conditions which should benefit from enhanced immune responses, in particular enhanced CTL immunity and proinflammatory cytokines such as cancer, infectious diseases, particularly chronic infections and sepsis wherein T cell-mediated depletion of diseased cells is therapeutically advantageous.

Example 8: IN-VITRO IMMUNOMODULATORY ACTIVITIES OF PVRIG ON HUMAN CYTOTOXIC T CELLS (CTLs)

[0369] The experiments described in this example evaluated the effect of ectopic expression of human PVRIG on different melanoma cell lines on their ability to activate CTLs (cytotoxic T lymphocytes) and serve as targets for killing by these cells.

MATERIALS & METHODS:

[0370] Three human melanoma cell lines which present the MART-1 antigen in HLA-A2 context (SK-MEL-23, Mel-624 and Mel-624.38) were used as targets for CTLs. Mel-888 which does not express HLA-A2, served as a negative control.

[0371] Ectopic expression of human PVRIG on cytotoxic T lymphocytes (CTLs): In order to express human PVRIG in peripheral blood leukocyte (PBL) cultures, the cDNA encoding for PVRIG was amplified using specific primers and cloned into an MSCV-based retroviral vector (pMSGV1) or in tripartite vectors: the CD8-dependent F4 TCR □- and □-chains were linked with a P2A sequence and cloned into pMSGV1 vector, either followed by an internal ribosome entry site (IRES) and PVRIG. The retroviral vector encoding for NGFR1, as negative control or in tripartite vectors: the CD8-dependent F4 TCR □- and □-chains were linked with a P2A sequence and cloned into pMSGV1 vector, either followed by an internal ribosome entry site (IRES) and NGFR .Verification of the cloning was done first using restriction enzyme digestion and subsequently by sequencing. Upon sequence confirmation, large amounts of the retroviral vector (Maxi-prep) were produced for subsequent use.

[0372] Peripheral blood leukocytes of healthy human donors were transduced with the retroviral constructs encoding PVRIG or with the retroviral vectors encoding for NGFR1 or an empty vector, as negative control. Transduction was carried out using a retronectin-based protocol; briefly, retroviral supernatant was produced in 293GP cells (a retroviral packaging cell line) following transfection with the retroviral vector and an amphotropic envelop gene (VSV-G). The retroviral supernatant was plated on retronectin-coated plates prior to the transduction to enable the binding of virions to the plate, and the PBLs were added to the plate for 6 hours. After that, the cells were replenished in a new culture vessel. Transduction efficiency and expression of the protein was determined by staining the transduced PBLs with commercial PVRIG specific rabbit polyclonal antibody or with commercial anti-NGFR (Cat.No 345108; BioLegend). Rabbit IgG (Sigma Cat. No. 15006) was used as isotype control, and as secondary antibody we used APC-conjugated anti-rabbit IgG (Jackson, Cat. No. 711-136-152).

[0373] Ectopic expression of the F4 T cell receptor on cytotoxic T lymphocytes (CTLs): In order to obtain effector lymphocytes that express the MART-1-specific F4 TCR, specifically recognizing MART-126-35-/HLA-A2 peptide-MHC complex, freshly isolated human PBLs previously transduced to express either with PVRIG, NGFR or an empty vector were stimulated with PHA and cultured for 5-10 days, and subsequently transduced with in vitro-transcribed mRNA encoding both α and β chains from the MART-1-specific F4 TCR. The transduced lymphocytes were cultured in lymphocyte medium (Bio target medium, fetal bovine serum (10%), L Glutamine Penicillin/ Streptomicyn (100 units/ml), IL-2 300 IU), replenished every 2-3 days. F4 TCR expression levels were verified by FACS staining using a specific monoclonal antibody that recognizes the extra-cellular domain of the beta-chain from the transduced specific TCR. (TCR-Vb12-PE, (Cat.No IM2291; Beckman Coulter).

[0374] Cytokine secretion from PVRIG, NGFR or an empty vector and F4-TCR transduced lymphocytes upon co-culture with melanoma cells: PBLs expressing PVRIG or NGFR along with F4-TCR were co-cultured with un-manipulated melanoma cells. 10^5 transduced PBLs were co-cultured with 10^5 melanoma target cells for 16 hours. In order to assess the response of the effector CD8 T cells to the different tumor cell lines, cytokine secretion (IFNγ, IL-2 and TNF-α) was measured by ELISA in culture supernatants (IFNγ (Cat.No DY285E), IL-2 (Cat.No DY202E), TNF-α (Cat.No DY210E) R&D SYSTEMS), diluted to be in the linear range of the ELISA assay.

[0375] Cell mediated cytotoxicity assay: This assay was performed in order to asses target cell killing upon co-culture. PVRIG and F4 were expressed in PBLs using a bi-cystronic vector and co-cultured with CFSE labeled melanoma Target cells (labeled with 2 mM CFSE (eBioscience) for 6 min), at 37°C for 18hr, at E:T ratio of 3:1. Cells were collected after 18hr and and 1 mM propidium iodide (Sigma-Aldrich) was added for assigning the ratio of cell death. Samples were run on a CyAn-ADP flow cytometer (Beckman Coulter).

Results:

[0376] General design of the experimental system: In the experimental system described herein, PVRIG is over expressed on human PBLs which are next manipulated to express the MART1-specific and HLA-A2 restricted F4 TCR. Over expressing cells are then co-cultured with HLA-A2 positive (name them) and HLA-

A2 negative (names) melanoma cell lines (reference). The F4 TCR was recently used in clinical trials in terminally-ill melanoma patients to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus encoding the TCR (Morgan et al, 2006 Science, 314:126-129). The effect of PVRIG expression on antigen-specific activation of CD8 T cells by co-culture with cognate melanoma cells was assessed by cytokine secretion.

[0377] Over expression of PVRIG on human PBLs - experiment 1: Human PBLs were transduced with a retroviral vector encoding the PVRIG or an empty vector as negative control, as described in Materials & Methods. The levels of PVRIG were assessed by flow cytometry at 48hrs after transduction, and compared to cells transduced with an empty vector. The percentage of the transgene-expressing cells was 62.4% as shown in Figure 16.

[0378] Over expression of PVRIG on human PBLs - experiment 2: Human PBLs were transduced with a retroviral vector encoding the PVRIG or NGFR or an empty vector as negative controls, as described in Materials & Methods. The levels of PVRIG were assessed by flow cytometry at 48hrs after transduction, and compared to cells transduced with an empty vector. The percentage of the PVRIG-expressing cells was in the range of 20%. The expression of NGFR was of 63% as shown in Figure 17. A few additional attempts to over express PVRIG on PBLs were un-successful. One possibility is that the difficulty in expressing PVRIG in primary PBLs stems from a basal endogenous expression level in these cells.

[0379] Over expression of F4 TCR on human PBLs : To perform functional assays with human CTLs, we used PBLs engineered to express the F4 TCR, which recognizes HLA-A2+/MART1+ melanoma cells, as described in Materials & Methods. Figure 18A shows levels of F4 TCR expression obtained upon TCR transduction of leukocytes used in experiment 1, Figure 18B shows levels of F4 TCR expression obtained upon TCR transduction of leukocytes used in experiment 2.

[0380] Effect of PVRIG expression on IFNγ secretion - experiment 1: PVRIG or Empty-vector and F4-transduced PBLs were co-cultured with melanoma cell lines. The levels of IFNγ secretion were measured at 16-hours of co-culture. As shown in Figure 19, the magnitude of inhibition of IFNγ secretion due to PVRIG over-expression was more than 90%. Co-culture with the HLA-A2 negative cell line Mel-888 which served as a negative control, caused only a minor activation dependent IFNγ secretion from F4-transduced lymphocytes. PBLs not expressing the F4 TCR (designated W/O) serve as an additional negative control.

[0381] Effect of PVRIG expression on IFNγ secretion - experiment 2: PVRIG, NGFR or Empty-vector and F4 were transduced into PBLs in co-transduction (Figure 20A) or using a bi-cystronic vector (Figure 20B). Transduced PBLs were co-cultured with melanoma cell lines. The levels of IFNγ secretion were measured at 16-hours of co-culture. As shown in Figure 20A, the magnitude of inhibition of cytokine secretion due to PVRIG over-expression was in the range of 30%. Co-culture with the HLA-A2 negative cell line Mel-888 which served as a negative control, caused only a minor activation dependent IFNγ secretion from F4-transduced lymphocytes. PBLs not expressing the F4 TCR (designated W/O) serve as an additional negative control. As shown in Figure 20B, when PVRIG is co-transduced with the F4 TCR, no inhibition of IFNγ was observed.

[0382] Effect of PVRIG on CTL mediated killing activity - experiment 2: PVRIG or NGFR and F4 were transduced to PBLs using a bi-cystronic vector and co-cultured with CFSE labeled melanoma cell lines. As shown in Figure 21, the percentage of propidium Iodide positive events (reflecting intensity of killing activity) was decreased by ~50% by the expression of PVRIG relative to negative control NGFR transduced cells. Killing activity of PVRIG expressing cells is similar to that of co-culture between melanoma and PBLs not expressing the F4 TCR (designated W/O).

[0383] Summary: Without wishing to be limited by a single hypothesis, the results presented herein indicate that overexpression on primary lymphocytes results in reduced cytokine secretion by CTLs, suggesting that PVRIG has an inhibitory effect on CTLs.

Example 9: HUMAN ANTI-PVRIG ANTIBODIES

[0384] The objective of this study was to isolate human antibodies that bind to the PVRIG immuno-oncology target with high affinity and specificity, and block the interaction of PVRIG with its binding partner, PVRL2. This was achieved by panning a human fab fragment phage display library against a recombinant protein comprising the human PVRIG extracellular domain (ECD) fused to the human IgG1 Fc region, and screening the resulting antibodies for their ability to block the PVRIG interaction with PVRL2.

Protocols

[0385] Functional QC of reagents: The purity of the panning reagent, PVRIG ECD fused to human IgG1 Fc domain (PVRIGHH), was determined by Microfluidics Capillary Electrophoresis using a LabChip System (PerkinElmer). Activity of the panning reagent was validated by its ability to bind its ligand PVRL2.

[0386] ELISA to detect protein-protein interaction: His-tagged PVRL2 recombinant protein was diluted to 2 µg/mL in phosphate buffered saline (PBS) and 50 µL aliquots were coated on the wells of a high binding EIA/RIA plate (Costar) overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound PVRL2 was incubated with varying concentrations of PVRIGHH in solution (linear range of 0.1 µg/mL to 4 µg/mL in a 50 µL/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well of a HRP-conjugated secondary antibody was added (Human IgG Fc domain specific). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer.

[0387] Preparation of biotinylated PVRIG: To facilitate phage panning in solution using streptavidin-coated magnetic beads, PVRIGHH and an irrelevant human IgG1 Fc isotype control were biotinylated using Lightning-Link® Biotin kit (Innova Biosciences). Biotinylation reactions were performed following the manufacturer's protocol and the biotinylated reagents were stored at 4°C for further QC and biopanning. The purity and activity of the biotin-labeled proteins was assessed by LabChip and functional ELISA, as described in Section 2.1. In addition, the degree of biotinylation was assessed by ELISA using two approaches: 1) the biotinylated reagents were adsorbed on a high binding EIA/RIA plate and the proteins were detected using HRP-conjugated streptavidin, and 2) the biotinylated proteins were incubated on EIA/RIA plate pre-coated with streptavidin and the binding was detected using a HRP-conjugated human IgG Fc domain specific secondary antibody.

[0388] Phage panning of human antibody library: Panning reactions were carried out in solution using streptavidin-coated magnetic beads to capture the biotinylated antigens. Note that all washing and elution steps were conducted using a magnetic rack to capture the beads (Promega). All incubation steps were conducted at room temperature with gentle mixing on a tube rotator (BioExpress). Four panning sub-campaigns were conducted, each with a different combination of antigen concentrations, washes and Fc-binder depletion steps (Table 1).

[0389] All the panning sub-campaigns were carried out using the biotinylated PVRIGHH antigen. For each round of panning, the phage libraries were depleted against 100 pmol of an irrelevant human IgG1 Fc protein in two successive steps. Following depletion, sub-campaigns A and B involved panning against 50 nM of the antigen in each round, under low and high stringency wash conditions, respectively. Sub-campaigns C and D were identical to sub-campaign B, except that in campaign C the library was blocked with 10-fold excess of the irrelevant IgG1 Fc protein in panning rounds 2 and 3. Sub-campaign D differed in that 5 nM antigen was used in round 3.

Table 1: Antigen and washing conditions used for phage panning against PVRIGHH.

	Sub-campaign Round Antigen Concentration			Washes	Fc
Depletion					
	A	1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
		2	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
		3	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
	B	1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
		2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol	
		3	50 nM 6x PBS-T + 6x PBS	2X 100 pmol	
	C	1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
		2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol + block with 1	
nmol					
		3	50 nM 6x PBS-T + 6x PBS	2X 100 pmol	
		block with 1 nmol			
	D	1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol	
		2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol	
		3	5 nM 6x PBS-T + 6x PBS	2X 100 pmol	

[0390] 2.4. Preparation of phage library for panning: All phage panning experiments used the XOMA031 human fab antibody phage display library (XOMA Corporation, Berkeley, CA). Sufficient phage for a 50-fold over-representation of the library were blocked by mixing 1:1 with 10% skim milk powder in PBS (final skim milk concentration 5%) and incubating for 1hr.

[0391] 2.4.1. Antigen coupling to streptavidin beads: For each sub-campaign, three 100 µL aliquots of Dynal streptavidin-coated magnetic beads (Life Technologies) were blocked by suspension in 1 mL of blocking buffer (5% skim milk powder in PBS) and incubated for 30 mins. One blocked bead aliquot was mixed with 100 pmols of biotinylated PVRIGHH. The other two aliquots were mixed with 100 pmols of the irrelevant antigen for depletion of Fc-only binders. Biotin-labeled antigens were coupled to the beads for 30 mins at RT. Bead suspensions were washed twice with PBS to remove free antigen and re-suspended in 100 µL blocking buffer.

[0392] 2.4.2. Depletion of human IgG1 Fc and streptavidin bead binders from the phage library: It was necessary to remove unwanted binders to streptavidin beads and the Fc region of PVRIGHH before phage panning could commence. To achieve this, blocked phage was mixed with one 100 µL aliquot of uncoupled streptavidin beads and incubated for 45 mins. The beads (and presumably unwanted bead and human IgG1 Fc-binders) were discarded. This step was repeated once and depleted phage library supernatants were reserved for panning.

[0393] 2.5. Phage panning round 1: The blocked and depleted phage library was mixed with biotinylated PVRIGHH coupled to magnetic beads described above. This suspension was incubated for 1hr at RT with gentle rotation to allow binding of PVRIGHH specific phage. Non-specific binders were removed by washing according to the protocol in Table 1. After washing, bound phage were eluted by incubation with 500 µL of 100 mM triethylamine (TEA) (EMD) for 15 mins at RT. The eluate was neutralized by adding 500 µL of 1 M Tris-HCl pH 8.0 (Teknova).

[0394] 2.5.1. Determination of phage titer: 10 µL of the initial phage library (input titer) or panning eluate (output titer) was serially diluted (10-fold) in PBS. A 90 µL aliquot of each phage dilution was mixed with 500 µL of TG1 E. coli cells grown to an optical density of -0.5 at 600 nm (OD 600nm). Phage were allowed to infect the cells by stationary incubation for 30 mins, then shaking incubation (250 rpm) for 30 mins, all at 37°C. A 10 µL aliquot of each infected cell culture was spotted on a 2YT agar plate supplemented with 2% glucose and 100 µg/mL carbenicillin (2YTCG, Teknova). Plates were incubated overnight at 30°C. Colonies growing from each 10 µL spot were counted and used to calculate input and output titers.

[0395] 2.5.2. Phage rescue: The remaining phage eluate (~1 mL) was mixed with 10 mL of TG1 E. coli cells grown to an OD 600 nm of 0.5. Phage were infected into cells as detailed in section 2.5.1. Infected cells were pelleted by centrifugation at 2500xG, re-suspended in 750 µL 2YT medium (Teknova) and spread on 2YTCG agar plates. These were incubated overnight at 37°C and the resulting E. coli lawns were scraped and re-suspended in -20 mL liquid 2YTCG (Teknova). A small aliquot of re-suspended cells was inoculated into 50 mL 2YTCG to achieve an OD 600nm of 0.05, and then grown at 37°C with 250 rpm shaking until the OD reached 0.5. The resulting culture was infected with M13K07 helper phage (New England Biolabs) and incubated overnight at 25°C with shaking to allow phage packaging. The culture supernatant containing rescued phage particles was cleared by centrifugation at 2500xG and 1 mL was carried over for either a) a subsequent round of panning or b) fab binding screens.

[0396] Phage panning rounds 2-3: Second and third rounds of panning were conducted as per the steps above, except that the rescued phage supernatant from the previous round was used in place of the phage library. The washing conditions, depletion and the antigen concentrations used are listed in Table 1.

2.6. Binding screens using fabs prepared from periplasmic extracts

[0397] 2.6.1. Fab expression vectors: The XOMA031 library is based on phagemid constructs that also function as fab expression vectors. These vectors contain fab heavy chain and light chain expression cassettes, a lac promoter to drive expression of the antibody genes, and an ampicillin resistance gene. The antibody chains are appended with N-terminal signal peptides to drive their secretion into the periplasmic space. The C-terminal of the heavy chain carries a truncated gene III protein sequence for incorporation into phage particles. The heavy chain also carries hexa-histidine, c-myc and V5 affinity tags. Transformation of these vectors into E. coli and induction with isopropyl β-D-1-thiogalactopyranoside (IPTG) results in periplasmic expression of soluble fab molecules.

[0398] 2.6.2. Fab PPE production: Eluted phage pools from panning round 3 were diluted and infected into TG1 E. coli cells (Lucigen) so that single colonies were generated when spread on a 2YTCG agar plate. This resulted in each colony carrying single fab clone. Individual clones were inoculated into 1 mL 2YTCG starter cultures in 96-well deep well blocks (VWR) using a Qpix2 instrument (Molecular Devices). These starter cultures were grown overnight in a Multitron 3mm incubator (Infors) at 37°C with 700 rpm shaking. For fab expression, 20 µL of 1 mL starter cultures were transferred into a second set of deep well plates containing 1 mL 2YT with 0.1% glucose and 100 µg/mL ampicillin. Cultures were grown until the average OD 600nm was 0.5-1.0 and protein expression was induced by adding IPTG (Teknova) to a final concentration of 1 mM. Expression cultures were incubated overnight in the Multitron instrument at 25°C with 700 rpm shaking.

[0399] Fab proteins secreted into the E. coli periplasm were extracted for analysis. Cells were harvested by centrifugation at 2500xG, the supernatants were discarded and pellets were re-suspended in 75 µL ice-cold PPB buffer (Teknova). Extracts were incubated for 10 mins at 4°C with 1000 rpm shaking, and 225 µL ice-cold ddH2O was added and incubated for a further 1hr. The resulting periplasmic extract (PPE) was cleared by centrifugation at 2500xG and transferred to separate plates or tubes for ELISA and FACS analysis. All extraction buffers contained EDTA-free Complete Protease Inhibitors (Roche).

[0400] Each plate of samples also included duplicate "blank PPE" wells to serve as negative controls. These were created by intentionally leaving two 1 mL cultures un-inoculated and then processing them in the same way as the fab PPEs, thereby creating a sample with no bacterial growth and therefore no fab expression.

[0401] 2.6.3. Primary screen by ELISA: Two 96-well plates of PPE extracts per sub-campaign were tested for binding to PVRIGHH by ELISA. Note that a non-biotinylated version of the protein was used for the ELISA screen to avoid the selection of biotin or streptavidin-binders. PVRIGHH recombinant protein was diluted to 2 µg/mL in phosphate buffered saline (PBS) and 50 µL aliquots were coated on the wells of a high binding EIA/RIA plate (Costar) overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound PVRIG was incubated with the PPEs, pre-blocked with 3% skim milk, at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well HRP-conjugated, anti-human Fab secondary antibody (Jackson ImmunoResearch) was added at a 1:2000 dilution in 5% milk in PBS. This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer. Wells that showed signal over background (blank PPE) ratio > 3 were selected as positive hits.

[0402] 2.6.4. Sequence analysis of ELISA positive fabs: The positive hits from the ELISA screen were selected and re-arrayed into a new 96-well plate. The clones were grown overnight at 37°C and the plasmid DNA was sequenced using heavy chain and light chain-specific primers. The sequences were assembled and analyzed using Xabtracker (XOMA) software. The clones were deemed sequence-unique if there were more than one non-conservative differences in the heavy chain CDR3. Clones with same or similar heavy chain but significantly different light chains were labeled as siblings of the original clone.

[0403] 2.6.5. FACS screening of fabs as PPEs: The sequence-unique ELISA-positive fab clones were selected and analyzed for their ability to bind PVRIG over-expressing cells by fluorescence-activated cell sorting (FACS). Analyses were conducted using HEK293 cells over-expressing the human PVRIG antigen. In a parallel experiment, un-transfected HEK293 cells were used as a negative control for each fab sample.

[0404] The PPEs for the sequence-unique ELISA-positive fab clones were generated as described above. All the assays were conducted using FACS buffer (1% BSA and 0.1% sodium azide in PBS). The human PVRIG and un-transfected HEK293 cells were harvested, washed twiceand re-suspended at a density of 2×10^6 cells/ml. A 25 µl aliquot of cells was mixed with 25 µl of each PPE sample and incubated for 1 hr at 4°C with gentle shaking. Two blank PPE controls were also included in the analysis. Plates were washed one time in 200 µl of FACS buffer and 50 µL of a 2 µg/mL dilution of a mouse anti-C-myc antibody (Roche) was added to each well. After incubation for 30 mins at 4°C, cells were washed again and 25 µl of a 5 µg/mL dilution of goat anti mouse fab-AF647 (Jackson Immunoresearch) was added to each PPE and negative control well. All secondary antibodies were incubated for 30 min at 4°C. After two washes, cells were re-suspended in a final volume of 50 µl of fixation buffer (2% paraformaldehyde in FACS buffer). Samples were read on an Intellicyt HTFC screening system, recording approximately 5000 events per well in a designated live gate. Data was analyzed using FlowJo (De Novo Software, CA, USA) and exported to Excel. Ratio of Mean Fluorescence Intensity (MFI) for the human PVRIG over-expressing HEK cells and the un-transfected 293 cells was calculated using Xabtracker software (XOMA). Positive hits on each plate were identified as those giving an MFI ratio 5-fold greater than the averaged blank PPE control signal.

[0405] Re-formatting of fab hits and production as human IgG molecules: Potential PVRIG binding fabs were converted to full length human IgGs for further characterization. Protein expression constructs were derived by PCR-amplification of variable heavy, lambda and kappa domain genes, which were sub-cloned into pFUSE-CHIg-hG1 (human IgG1 heavy chain), pFUSE2-CLIg-hK (human kappa light chain) or pFUSE2-CLIg-hL2 (human lambda 2 light chain) vectors, respectively (all expression vectors were sourced from Invivogen).

[0406] Expi293 cells (Life Technologies) were seeded at 6×10^5 cells/ml in Expi293 medium (Life Technologies) and incubated for 72 hrs at 37°C in a humidified atmosphere of 8% CO2 with shaking at 125 rpm. This cell stock was used to seed expression cultures at 2.0×10^6 cells/ml in Expi293 medium. These cultures were incubated as above for 24 hrs with shaking at 135 rpm.

[0407] For transfection, cells were diluted again to 2.5×10^6 cells/ml in Expi293 medium. The protein expression constructs for antibody heavy chain and light chain were mixed at a ratio of 1:2. For every 30 mL of expression culture volume, 30 µg of DNA and 81 µL of Expifectamine (Life Technologies) were each diluted separately to 1.5 mL with Opti-MEM (Life Technologies) and incubated for five minutes. Diluted DNA and Expifectamine were then mixed and incubated at RT for 20 mins. This was then added to the expression culture in a shaker flask and incubated as described above, with shaking at 125 rpm.

[0408] Approximately 20 hrs post-transfection, 150µL of ExpiFectamine 293 transfection Enhancer 1 and 1.5mL of ExpiFectamine 293 Transfection Enhancer 2 was added to each flask. Cultures were incubated for a further five days (six days post-transfection in total) and supernatants were harvested by centrifugation. IgGs were purified from the supernatants using an AKTA Pure FPLC (GE Healthcare Bio-Sciences) and HiTrap MabSelect Sure affinity columns (GE Healthcare Bio-Sciences) according to manufacturer's instructions.

[0409] FACS screening of reformatted IgG1 antibodies: FACS screening of the reformatted antibodies was done similarly to the PPE based screen described herein, except that a dose-dependent titration of the purified antibodies was performed. The human PVRIG over-expressing HEK293 cells, or the un-transfected HEK293 cells, were incubated with varying concentrations (0 - 10 µg/ml) of the anti PVRIG antibodies or isotype controls in FACS buffer at 4°C for 60 mins. Cells were washed once in FACS buffer, re-suspended in 50 µl of Alexa Fluor 647 conjugated anti-human IgG (Fab fragment specific) diluted 1:200 and incubated for 30 mins at 4°C in the dark. Cells were washed twice and re-suspended in a final volume of 80 µl of FACS buffer and Propidium Iodide (Biolegend cat# 421301) diluted 1:1000. Samples were analyzed using an Intellicyt HTFC screening system (Intellicyt). Data was analyzed using FlowJo (DeNovo), exported to Excel (Microsoft) and plotted in GraphPad Prism (GraphPad Software, Inc.).

Results

[0410] Functional QC of the PVRIGHH recombinant protein: The purity of the PVRIGHH protein was assessed by microfluidics capillary electrophoresis using a LabChip system. Under reducing conditions, the recombinant protein migrated at 80 kDa, consistent with its calculated molecular weight of 80.4 kDa, and showed 99% purity (data not shown). Under non-reducing conditions, one additional peak was observed which likely resulted from the presence of a dimeric form of the protein due to Fc-Fc interaction.

[0411] The functional integrity of the recombinant protein was assessed by evaluating its binding to PVRL2 (a known ligand for PVRIG) in ELISA. A dose-dependent response was observed for the binding of PVRIGHH to PVRL2 (data not shown). In comparison, no binding was observed for a irrelevant human IgG1 Fc control. Taken together, this indicated that the PVRIGHH recombinant protein is of high purity and is functionally active, and thus is suitable for biopanning.

[0412] QC of the biotinylated PVRIGHH recombinant protein: The purity of the biotinylated PVRIGHH protein was assessed by microfluidics capillary electrophoresis using LabChip system. No significant differences were observed between the non-biotinylated and the biotinylated recombinant proteins (data not shown). Note that an additional 44.3 kDa peak observed in the biotinylated protein sample. This peak may result from the monomeric form of the PVRIGHH protein or maybe an artifact of the quenching reaction of the biotinylation kit.

[0413] Successful biotinylation was confirmed by incubating the biotinylated protein on a streptavidin-coated EIA plate and detecting the bound protein using a HRP-conjugated anti human IgG1 Fc secondary antibody. The binding of biotinylated PVRIGHH to the streptavidin-coated EIA plate was comparable to a commercially sourced irrelevant biotinylated protein (data not shown).

[0414] Phage panning: The biotinylated PVRIGHH protein was used for phage panning against the XOMA031 human fab antibody phage display library (XOMA Corporation, Berkeley, CA). Three rounds of biopannings were performed, under 4 different combinations of washing stringency, antigen concentration, and depletion of Fc binders (sub-campaigns A - D). The success of each round was estimated using the phage output titers. Qualitative guidelines were used to define the success of the panning sub-campaigns, such as significant reduction in phage titers after round 1, increase or maintenance of phage titers after rounds 2 and 3, and decrease in phage titers upon increasing wash stringency or decreasing antigen concentration. All 4 sub-campaigns resulted in phage titers in the expected range that were consistent among the sub-campaigns (data not shown).

[0415] Screening of phage output as fab PPEs: Two 96-well plates of fab clones (as PPEs) for each of the four sub-campaigns were screened to evaluate the success of biopanning. The results are summarized in table 3 and are discussed in further detail below. Overall, all 4 sub-campaigns yielded significant numbers of PVRIGHH specific fabs. A total of 49 target-specific unique fabs were identified. The sub-campaigns B and D showed the highest ELISA hit rates and FACS correlation and were selected for an extended screen.

[0416] Table 3: Summary of pilot screen of fab PPEs. For each sub-campaign, the total number of clones screened, ELISA hits, FACS hits and sequence uniqueness are listed. Open reading frames (ORFs) represent the clones that were successfully sequenced as a full-length fab. Specificity is based on the lack of non-specific binding to irrelevant proteins in ELISA. FACS correlation represents the percent of ELISA hits that were also FACS positive (specifically bound to PVRIG over-expressing HEK293 cells).

	Sub A	Sub B	Sub C	Sub D	Overall				
Clones screened			182	182	182	182	728		
ELISA positive (>3 S/N)				48	51	44	68	211	
ELISA Hit rate			26%	28%	24%	37%	29%		
ORFs	36 (75%)		45 (88%)		35 (80%)		63 (93%)		179 (85%)
Unique sequences			25	21	17	31	73		
Diversity		69%	47%	49%	49%	41%			
Specificity by ELISA*				100%	100%	100%	100%	100%	
FACS Binders (>5 S/N)				14	17	14	24	49**	
FACS correlation			56%	81%	82%	77%	67%		
*No non-specific binding to irrelevant Fc conjugates or PVRL2; **35 unique HCs, 14 siblings									

[0417] Primary fab screen (ELISA): Two 96-well plates (182 fab clones) of PPEs for each sub-campaign were screened by ELISA against the PVRIGHH recombinant protein. Note that although biotinylated protein was used for panning, the non-biotinylated version was used for the ELISA screen, which avoided detection of biotin or streptavidin-specific binders. The 4 sub-campaigns resulted in ELISA hit rates ranging from 24 - 37% when the threshold for a 'positive' signal was set at a 3-fold ratio of target-specific binding: blank PPE control signal.

[0418] Secondary screen (DNA sequence analysis, ELISA and FACS) fabs: The ELISA positive clones were sequenced to select non-redundant fabs. Seventy-three sequence-unique fab clones were identified. 19 clones were unique to sub-campaign A, 13 clones were unique to sub-campaign B, 10 clones were unique to sub-campaign C, 18 clones were unique to sub-campaign D, while the remaining 23 clones were shared between the campaigns. Sequence-unique, ELISA-positive fab clones were re-expressed as PPEs and screened for specific binding by FACS. A total of 49 out of 73 unique clones were identified as PVRIG specific ELISA and FACS binders (following the criteria established in 2.6.5). The 49 FACS binders corresponded to 35 antibodies with unique heavy chains and 14 siblings that have unique light chains but share the heavy chain with one of the unique clones. A summary of FACS binding data is presented in Table 4.

[0419] The sequence unique fabs were also tested for non-specific binding. All the fab PPEs analyzed bound to the PVRIGHH recombinant protein with an assay signal greater than 3-fold over the blank PPE control. In a parallel assay, fab PPEs were tested for binding to two irrelevant proteins with the same IgG1 Fc region, as well as the PVRL2 recombinant protein. None of the clones showed significant non-specific binding to the controls, suggesting that the selected fabs are specific for PVRIG.

Table 4: FACS binding summary for PVRIG fabs. All unique ELISA positive fabs were analyzed by FACS. The mean fluorescence intensity (MFI) was measured for the PVRIG over-expressing HEK293 cells as well as the un-transfected HEK293 cells. The MFI ratio for the target-specific vs off-target binding was calculated. Clones with MFI ratio > 5 were selected as hits and are listed below.

fab clone	MFI ratio	fab clone		MFI ratio
CPA.7.001	11	CPA.7.026	5.3	
CPA.7.002	8.9	CPA.7.027	9.2	
CPA.7.003	9.5	CPA.7.028	17	
CPA.7.004	9.3	CPA.7.029	6.7	
CPA.7.005	6.5	CPA.7.030	15	
CPA.7.006	9.6	CPA.7.031	8.5	
CPA.7.007	14	CPA.7.032	7.6	
CPA.7.008	14	CPA.7.033	22	
CPA.7.009	10	CPA.7.034	7.7	
CPA.7.010	7.6	CPA.7.035	14	
CPA.7.011	10	CPA.7.036	5	
CPA.7.012	19	CPA.7.037	5.3	
CPA.7.013	12	CPA.7.038	6.3	
CPA.7.014	14	CPA.7.039	12	
CPA.7.015	15	CPA.7.040	12	
CPA.7.016	7.6	CPA.7.041	7.6	
CPA.7.017	13	CPA.7.042	5.4	
CPA.7.018	7.8	CPA.7.043	13	
CPA.7.019	16	CPA.7.044	7.9	
CPA.7.020	6.9	CPA.7.045	7.8	
CPA.7.021	15	CPA.7.046	10	
CPA.7.022	7.5	CPA.7.047	8.4	
CPA.7.023	12	CPA.7.049	10	
CPA.7.024	9.8	CPA.7.050	22	
CPA.7.025	6			

[0420] Reformatting of the ELISA and FACS positive fabs into hIsG1: All unique ELISA and FACS binders were reformatted for expression as human IgG1 molecules in Expi293 cells. Out of the original 49 antibodies, 44 were successfully expressed as full-length antibodies. These reformatted antibodies were tested for retained binding to PVRIG over-expressing HEK293 cells alongside an irrelevant human IgG1 isotype control. All antibodies were also tested against un-transfected HEK293 cells. The resulting binding results were used to demonstrate the specificity of the antibodies and also plotted to calculate the equilibrium binding constant (KD). Nine out of the remaining 44 antibodies showed weak binding or significant non-specific binding. The remaining 35 antibodies were selected for further analysis in cell-based functional assays. The FACS-based KD of these antibodies are listed in Table 6. The KD values range from 0.30 nM to 96 nM, with a median of 9.4 nM, suggesting that most antibodies obtained from the panning campaign are very specific and bind to PVRIG with high affinity.

Table 5: Expression and binding summary of reformatted antibodies. All unique ELISA and FACS positive fabs were reformatted into the human IgG1 backbone. FACS KD values were determined by dose titration against the PVRIG over-expressing HEK293 cells. Off-target binding was determined by dose titration against the un-transfected HEK293 cells.

Antibody	FACS KD (nM)	Antibody	FACS KD (nM)
CPA.7.001	No-expression	CPA.7.026	Non-binder
CPA.7.002	44.35	CPA.7.027	Non-binder
CPA.7.003	Non-specific binding	CPA.7.028	7.14
CPA.7.004	21.71	CPA.7.029	Weak binding
CPA.7.005	95.56	CPA.7.030	No-expression
CPA.7.006	No-expression	CPA.7.031	Non-binder
CPA.7.007	0.73	CPA.7.032	8.78
CPA.7.008	No-expression	CPA.7.033	12.8
CPA.7.009	33.00	CPA.7.034	14.2
CPA.7.010	21.89	CPA.7.035	Non-binder
CPA.7.011	66.02	CPA.7.036	6.0
CPA.7.012	0.30	CPA.7.037	Non-specific binding
CPA.7.013	No-expression	CPA.7.038	20.26
CPA.7.014	2.04	CPA.7.039	3.76
CPA.7.015	1.34	CPA.7.040	0.79
CPA.7.016	22.02	CPA.7.041	52.2
CPA.7.017	1.82	CPA.7.042	24.26
CPA.7.018	9.29	CPA.7.043	13.2
CPA.7.019	0.45	CPA.7.044	9.4
CPA.7.020	86.97	CPA.7.045	3.73
CPA.7.021	11.22	CPA.7.046	Non-specific binding
CPA.7.022	4.17	CPA.7.047	5.36
CPA.7.023	4.08	CPA.7.049	19.9
CPA.7.024	9.08	CPA.7.050	68.3
CPA.7.025	Non-binder		

Summary and Conclusions

[0421] A phage display antibody discovery campaign was conducted to isolate binders against the immuno-oncology target PVRIG using a recombinant Fc-tagged version of the antigen. Quality control analysis showed that the panning antigen was pure and functionally active. The panning effort yielded 49 unique fab clones that specifically bound to the PVRIG target, both as a recombinant protein and on the cell surface. Of these, 35 were successfully produced as human IgG1 antibodies and were shown to retain specific binding to the PVRIG. This pool of antibodies displayed high affinities in a FACS assays, with 18 out of 35 antibodies binding with a KD < 10 nM.

Example 10 Demonstration of the ability of the anti-human PVRIG fabs to block the interaction between PVRIG and PVRL2 by ELISA.

[0422] **Method:** The human PVRL2-His (Catalog #2229-N2-050/CF, R&D Systems), was coated on the ELISA plate. Fab periplasmic extracts (PPEs), diluted 1:1 in 5% skim milk, were preincubated with 1 ug/ml (final concentration) of the human PVRIG-Fc, for 15 min at RT. The fab-receptor mixture was allowed to bind the PVRL2-His coated on the ELISA plate. The PVRIG-Fc/ PVRL2-His interaction was probed using anti-human Fc antibody, conjugated to HRP (Jackson Immuno Research catalog #709-035-098). In the absence of PPE (negative wells), a strong positive signal was expected. For blocking fabs, the signal would be significantly reduced. The fab clones with >5-fold lower signal than the negative wells (>80% blocking) could be selected as blocking fabs.

Protocol:

[0423] ELISA plates (Costar 9018) were coated with 50 ul of 2 ug/ml antigen and were stored at 4 C overnight. The antigen-coated plates were washed 3 times with 1× PBS. The plate was blocked with 200 µl of 5% skim milk in PBS and incubated 1 hr at RT (room temperature). Next the plate was washed with 1× PB.

[0424] After adding 50 µl/well of Fab PPEs (diluted in 5% skim milk), the plate was preincubated with 1 ug/ml of the human PVRIG-Fc that was added to the respective wells. The "no fab" control was performed with 2 wells.

[0425] The plate was incubated 1 hr at RT.

[0426] The plates were washed 3 times with 1× PBST and 3 times with 1× PBS.

[0427] After adding 50 µl/well of the HRP-conjugated secondary antibody (Jackson Immuno Research, 709-035-098), diluted in 5% milk in PBS, the plate was incubated 1 hr at RT.

[0428] The plates were washed 3 times with 1× PBST and 3 times with 1× PBS.

[0429] After adding 50 µl/well of the TMB substrate and waiting until the color develops, the reaction was stopped by adding 50 µl/well of 2N H_2SO_4. Absorbance was measured at 450 nm.

Results

[0430] Figure 52 shows the results of testing anti-PVRIG antibodies for their ability to block at least 80% of PVRL2 binding to PVRIG. As shown, a large number of such antibodies were able to successfully block at least 80% of the binding. Specifically the antibodies which blocked successfully are designated as follows:

[0431] CPA.7.001, CPA.7.003, CPA.7.004, CPA.7.006, CPA.7.008, CPA.7.009, CPA.7.010, CPA.7.011, CPA.7.012, CPA.7.013, CPA.7.014, CPA.7.015, CPA.7.017, CPA.7.018, CPA.7.019, CPA.7.021, CPA.7.022, CPA.7.023, CPA.7.024, CPA.7.033, CPA.7.034, CPA.7.036, CPA.7.040, CPA.7.046, CPA.7.047, CPA.7.049, CPA.7.050,

Example 11: Surface Plasmon Resonance Study of Epitope Binning of 37 Anti PVRIG IgG Antibodies binding to human PVRIG fusion protein

Materials and Methods

[0432] Experiments were performed using a ProteOn XPR 36 instrument at 22 □C with all samples kept at 4 □C during the experiment.

[0433] Step 1: The following anti-PVRIG mAbs were each diluted to ~10µg/mL in 10mM sodium acetate, pH 5.0 and covalently immobilized on independent spots on a ProteOn GLC biosensor chip using standard amine coupling:

CPA.7.002
CPA.7.003
CPA.7.004
CPA.7.005
CPA.7.007
CPA.7.009
CPA.7.010
CPA.7.011
CPA.7.012
CPA.7.014
CPA.7.015
CPA.7.016
CPA.7.017
CPA.7.018
CPA.7.019
CPA.7.020
CPA.7.021
CPA.7.022
CPA.7.023
CPA.7.024
CPA.7.026
CPA.7.028
CPA.7.029
CPA.7.032
CPA.7.033
CPA.7.034
CPA.7.036
CPA.7.037
CPA.7.038
CPA.7.039
CPA.7.040
CPA.7.043
CPA.7.045
CPA.7.046
CPA.7.047
CPA.7.050

[0434] The activation step occurred in the horizontal flow direction for five minutes while the immobilization step occurred in the vertical flow direction. MAbs were injected for four minutes after surface activation. The blocking step occurred in both the vertical and horizontal positions at five minutes each so that the horizontal "interspots" could be used as reference surfaces. MAbs were immobilized at a range of ~450RU-5000RU. An additional mAb CPA.7.041 was also binned in this study, but only as an analyte in solution. See below.

[0435] Step 2: Preliminary experiments involved several cycles of injecting -20 nM PVRIG antigen (PVRIGHH-2-1-1 #448, GenScript) over all immobilized mAbs for three minutes at a flow rate of 25µL/min followed by regeneration with a 30-second pulse of 10 mM glycine-HCl, at either pH 2.0 or pH 2.5, depending on the horizontal row of mAbs in the GLC chip array. Antigen samples were prepared in degassed PBST (PBS with 0.05% Tween 20) running buffer with 100 µg/mL BSA. These preliminary experiments showed that clones CPA.7.026 and CPA.7.029 did not bind to the antigen and were therefore not binned. The remaining mAbs on the ProteOn array showed reproducible binding to the antigen.

[0436] Step 3: A "pre-mix" epitope binning protocol was performed because of the bivalency of the fc-fusion PVRIG antigen. In this protocol each mAb listed in Step 1, plus mAb CPA.7.041, was pre-mixed with PVRIG antigen and then injected for three minutes over all immobilized mAbs. The molar binding site concentration of each mAb was in excess of the molar antigen binding site concentration. The final binding site concentration of each mAb was ~400nM and the final binding site concentration of the antigen was ~20nM. An antigen-only control cycle was performed after very eight mAb injection cycles to monitor the activity of the immobilized mAbs throughout the experiment. Buffer blank injections were also performed after about every eight mAb injection cycles for double-referencing. Additional controls included each mAb injected alone over all immobilized mAbs at concentrations identical to the pre-mix injection cycles. All surfaces were regenerated with a 30 second pulse of 10 mM glycine-HCl at either pH 2.0 or pH 2.5 depending on which row of mAbs in the array was being regenerated, and all cycles were run at a flow rate of 25 µL/min. MAb and antigen samples were prepared in degassed PBST running buffer with 100 µg/mL BSA.

[0437] Step 4: Sensorgram data were processed and referenced using ProteOn Manager Version 3.1.0.6 using interspots and buffer blanks for double-referencing. The mAb-only control injections were used as the injection references where significant binding with the mAb-only injections was observed. An antibody pair was classified as having a shared antigen binding epitope (designated as a red "0" in the matrix inFigure 43) if no binding was observed from the injection of mixed mAb and antigen over the immobilized mAb, or if binding was significantly reduced as compared to the antigen-only control injection over the same immobilized mAb. An antibody pair was classified as binding to different antigen epitopes, or "sandwiching" the antigen (designated as a green "1" in the matrix inFigure 43) if the injection of mixed mAb and antigen showed binding to the immobilized mAb similar to or greater than the antigen-only control over the same immobilized mAb.

[0438] Step 5: The blocking pattern for mAb CPA.7.041 (#37) was studied only as an analyte because the GLC chip array has only 36 spots. Therefore for consistency, hierarchical clustering of the binding patterns in the binary matrix for each mAb pre-mixed with antigen (vertical patterns inFigure 42) was performed using JMP software version 11.0.0. The blocking patterns of the immobilized mAbs (horizontal patterns inFigure 42) were also clustered as a comparison to the blocking patterns of the mAbs pre-mixed in solution (data not shown, see Results for discussion).

[0439] Results: Figure 42 shows the binary matrix of the blocking ("0") or sandwiching ("1") between each mAb pair where the mAbs are listed in identical order both vertically (mAbs on the surface - "ligands") and horizontally (mAbs in solution - "analytes"). Identical "bins" of blocking patterns for all mAbs as analytes are highlighted in Figure 42 with a black box around each group of similar vertical patterns. Figure 43 shows the dendrogram of the vertical (analyte) blocking patterns in the matrix in Figure 42. For the strictest definition of an epitope "bin" where only those mAbs which show identical blocking patterns technically bin together, there are a total of 4 discrete bins. Specifically, 33 of the 35 mAbs that were binned comprise two bins where the only difference between these two bins is whether a mAb sandwiches (Bin 2, see Figure 42 and Figure 43) with or blocks (Bin 1, see Figure 42 and Figure 43) binding to CPA.7.039. This means that CPA.7.039 is in its own separate bin. The fourth bin consists only of mAb CPA.7.050 which is unable to block antigen binding to any of the other 34 mAbs. Hierarchical clustering of the blocking patterns of the mAbs as ligands (horizontal patterns in Figure 42) showed mAb CPA.7.016 sandwiching antigen with mAb CPA.7.039 whereas as an analyte it blocks antigen binding to immobilized CPA.7.039. Hence clone CPA.7.016 would be placed in bin 2 rather than in bin 1. The mAbs in each bind are listed in Figure 43. Processed sensorgram data representative of each bin are shown in Figure 44 to Figure 47.

[0440] Summary: 35 anti-PVRIG IgG mAbs were binned using SPR according to their pair-wise blocking patterns with fc fusion human PVRIG. By the strictest definition of an epitope bin, there are a total of four discrete bins. 33 of the 35 mAbs comprise two bins which differ only by whether their respective component mAbs block or sandwich antigen with clone CPA.7.039.

Example 12 SURFACE PLASMON RESONANCE KINETIC SCREEN OF 50 ANTI-PVRIG HUMAN FABS PREPARED IN PERIPLASMIC EXTRACTS

Materials and Methods

[0441] All experiments were performed using a Biacore 3000 instrument and a ProteOn XPR 36 instrument at 22°C.

[0442] Step 1: The molar concentration of all 52 fabs in periplasmic extract supernatant were quantitated using a Biacore 3000 instrument at 22°C. Each fab was diluted 20-fold and then injected for 2 minutes at 5μL/min over high density anti-human fab (GE Healthcare 28-9583-25) surfaces prepared using standard amine coupling with a CM5 Biacore chip (GE Healthcare). A standard human fab at a known concentration (Bethyl P80-115) was then injected over the anti-fab surface with the same conditions as the fab supernatants. Samples were prepared in the running buffer which was degassed HBSP (0.01 M HEPES, 0.15 M NaCl, 0.005% P20, pH 7.4) with 0.01% BSA added. The association slopes of each SPR sensorgram from each fab supernatant was fit against the SPR association slope of the standard human fab of known concentration using CLAMP 3.40 software to estimate the molar concentrations of each fab in supernatant.

[0443] Step 2: A high density goat anti-human fc polyclonal antibody surface (Invitrogen H10500) was prepared using standard amine coupling over two lanes of a GLC chip using a ProteOn XPR 36 biosensor. A high density anti-mouse fc polyclonal antibody surface (GE Healthcare BR-1008-38) was prepared using standard amine coupling over two different lanes of the same GLC chip. The activation and blocking steps for all four capture surfaces occurred in the vertical flow direction. Each fab in supernatant was then injected at three concentrations over fc-fusion human PVRIG (PVRIG-HH-2-1-1 #448, GenScript) and fc-fusion mouse PVRIG (PVRIG-MM-2-1-1 #198, GenScript) which were captured to one high density anti-human fc surface and one anti-mouse fc surface (respectively) at an average of ~200RU and ~290RU per cycle, respectively. Each fab concentration series was injected for two minutes followed by 10 minutes of dissociation at a flow rate of 50 μL/min. The starting concentration range (as determined in Step 1) was ~20nM - ~400nM with two threefold dilutions of the highest concentration for each fab. Fabs were diluted into the running buffer which was degassed PBS with 0.05% Tween 20 and 0.01% BSA added. The anti-human fc capture surfaces were regenerated with two 30-second pulses of 146 mM phosphoric acid after each cycle and the anti-mouse fc surfaces were regenerated with two 30-second pulses of 10mM glycine, pH 1.7 after each cycle.

[0444] Step 3: Sensorgram data of fabs in supernatant binding to captured PVRIG were processed and double-referenced using ProteOn Manager version 3.1.0.6. The sensorgrams were double-referenced using the corresponding anti-species capture surfaces with no captured PVRIG as reference surfaces and a blank injection over the captured PVRIG under identical conditions as the injections of the fabs. Where possible, the sensorgrams for the three different concentrations of each fab were then globally fit to a 1:1 kinetic model (with a term for mass transport) to estimate the association and dissociation rate constants.

Sensorgrams which did not show simple 1:1 binding were not fit with the kinetic model and therefore were not assigned estimates for ka and kd.

Results

[0445] None of the fabs included in this study showed binding activity to mouse PVRIG (data not shown). Sensorgrams for 17 of the 50 fabs screened against the human PVRIG could be fit for reliable estimates of their rate constants. Twenty eight clones showed complex kinetics, five of the fabs did not show any binding to the captured human PVRIG fusion protein (CPA.7.025, CPA.7.026, CPA.7.027, CPA.7.029, CPA.7.035) and one clone (CPA.7.035) showed no titer when performing the concentration determination in Step 1. The rate constants and their corresponding sensorgrams are shown below in Figure 49 and Figure 50. The clones listed below showed complex kinetics. Figure 51 shows some examples of these data.

CPA.7.001	CPA.7.006	CPA.7.013	CPA.7.045
CPA.7.030	CPA.7.036	CPA.7.014	CPA.7.046
CPA.7.031	CPA.7.037	CPA.7.041	CPA.7.017
CPA.7.032	CPA.7.009	CPA.7.042	CPA.7.018
CPA.7.033	CPA.7.038	CPA.7.043	CPA.7.047
CPA.7.034	CPA.7.039	CPA.7.016	CPA.7.023
CPA.7.003	CPA.7.011	CPA.7.044	CPA.7.024

EXAMPLE 13 MEASURING THE BINDING AFFINITY OF IGG CLONE CPA.7.021 TO PVRIG EXPRESSED ON HEK CELLS USING FLOW CYTOMETRY

Materials and Methods

[0446] Flow cytometry was used to measure the affinity of CPA.7.021 IgG binding to human PVRIG expressed on HEK 293 cells. CPA.7.021 conjugated with Alexa 647 was added in duplicate at a binding site concentration range of 3 pM - 101 nM in a 2-fold serial dilution to a constant number of cells (100,000 cells/well) over 17 wells in a 96-well plate. One well contained cells without any added IgG to serve as a blank well. The cells were equilibrated for 4 hours with IgG at 4°C. Cells were washed twice and then the Mean Fluorescence Intensity (MFI) was recorded over approximately 10,000 "events" using an Intellicyte flow cytometer. The resulting MFI values as a function of the CPA.7.021 IgG binding site concentration are shown below. The KD of CPA.7.021 binding to HEK 293 cells expressing human PVRIG was estimated by fitting the MFI vs. the IgG binding site concentration curve with a 1:1 equilibrium model as detailed in Drake and Klakamp, Journal of Immunol Methods, 318 (2007) 147-152.

[0447] Results: Alexa647 labelled CPA.7.021 IgG was titrated with HEK 293 cells expressing human PVRIG and the binding signal was measured using flow cytometry. The resulting binding isotherm, showing MFI in duplicate vs. the binding site concentration of CPA.7.021, is presented below. The red line is a 1:1 equilibrium fit of the curve that allows for a KD estimate of 2.5 nM ± 0.5 nM (95% confidence interval of the fit, N=1).

Example 14 Effect of PVRIG Knock down (KD) and anti-PVRIG antibody on human Melanoma specific TILs function

[0448] The aim of these assays is to evaluate the functional capacity of PVRIG in human derived TILs, as measured by activation markers and cytokine secretion, upon co-culture with melanoma target cells. PD1 was used as a benchmark immune-checkpoint for the knock down (siRNA) studies. The effect of anti-PVRIG antibody (CPA.7.21), which has been shown to block the interaction of PVRIG and PVRL2, alone or in combination with other antibodies (e.g aTIGIT, DNAM1) was evaluated.

Materials and Methods

TILs

[0449] Tumor-infiltrating lymphocytes (TILs) from three melanoma patients were used:

□ TIL-412- HLA-A2-Mart1 specific

□ TIL-F4- HLA-A2-gp100 specific

□ TIL-209- HLA-A2-gp100 specific

[0450] TILs were thawed in IMDM (BI, 01-058-1A) full medium supplemented with 10% human serum (Sigma, H3667) + 1% Glutamax (Life technologies, 35050-038) + 1% Na-Pyruvate (Biological Industries, 03-042-1B) + 1% non-essential amino acids (Biological Industries, 01-340-1B) + 1% Pen-Strep (Biological Industries, 03-031-1B) + 300 U/ml of rhIL2 (Biolegend, 509129).

[0451] Tumor cell lines: Human melanoma cells Mel-624 express MART-1 and gp-100 antigens in the context of MHC-I haplotype HLA-A2. Cells were cultured in complete DMEM medium (Biological Industries, 01-055-1A) supplemented with 10% FBS (BI, 04-127-1A), 25 mM HEPES buffer (BI, 03-025-1B), 1% Glutamax (Life technologies, 35050-038), and 1% Pen-Strep (Biological Industries, 03-031-1B).

[0452] Knock down in TILs: Knock-down (KD) of human PVRIG and human PD1 in TILs was done using 100pmol of Dharmacon ON-TARGETplus human PVRIG siRNA - SMARTpool (L-032703-02) or Human PD1 siRNA - SMARTpool (L-004435) or non-targeting siRNA (D-001810-01-05). siRNA were electroporated to TILs (AMAXA, program X-005). Electroporation was done on resting TILs cultured in full IMDM supplemented with IL-2 24hr post thawing. After the electroporation TILs were seeded in 96 well TC plate to recover for 24hr. After 24 hr, cells were harvested and stained with viability dye (BD Horizon; Cat# 562247, BD biosciences), washed with PBS and stained with anti-human PVRIG - CPA.7.021 (CPA.7.021 IgG2 A647, 7.5ug/ml) or with anti-human PD-1 (Biolegend, #329910 AF647, 5ug/ml) in room temperature for 30min. isotype control used are synagis (IgG2 A647, 7.5ug/ml) and mouse IgG1 (Biolegend #400130 A647, 5ug/ml) respectively. All samples were run on a MACSQuant analyzer (Miltenyi) and data was analyzed using FlowJo software (v10.0.8).

[0453] Co-culture of TILs with 624 melanoma cells: siRNA electroporated TILs were harvested and seeded in 96 TC plate 5×104/well. Mel-624 cells were harvested as well and seeded in 1:1 / 1:3 E:T ratios in co-culture. The plate was incubated overnight (18hr) in 37°C, 5% CO2.

[0454] To assess the effect of anti-PVRIG antibody (CPA.7.021), anti-TIGIT (Clone 10A7) and anti-DNAM1 (clone DX11) on melanoma specific TIL activity, TILs (1×10^5 cells/well) were pre-incubated with tested antibodies or relevant isotype controls in mono-treatment (10µg/mL) or in combination-treatment (final 10µg/mL for each) prior to the addition of 624 Melanoma target cells at a 1:1 Effector:target ratio. The plate was incubated overnight (18hr) in 37°C, 5% CO2.

[0455] Assessment of TILs activation: 16 hours post co-culture, cells were stained with viability dye (BD Horizon; Cat# 562247, BD biosciences), washed with PBS and exposed to Fc blocking solution (cat# 309804, Biolegend), followed by surface staining with anti-CD8a (Cat #301048, Biolegend) and anti-CD 137 (Cat #309804, Biolegend) in 4°C for 30min. All samples were run on a MACSQuant analyzer (Miltenyi) and data was analyzed using FlowJo software (v10.0.8). Culture supernatants were collected and analyzed for cytokine secretion by CBA kit (Cat #560484, BD).

Results

[0456] PVRIG Knock-Down in TILs: TIL MART-1 and TIL F4 were cultured 24 hr with IL-2. 100 pmol of ON-TARGETplus human PVRIG siRNA - SMART pool (L-032703-02) or Human PD1 siRNA - SMARTpool (L-004435) or non-targeting siRNA (D-001810-01-05) were electroporated to TILs (AMAXA, program X-005). Detection of PVRIG or PD-1 was performed 24 hr post electroporation (and prior to co-culture). Cells were stained for viability dye followed by 30min RT incubation with anti PVRIG or anti PD-1. The percentage of KD population is indicated in Figure 82.

[0457] Functional assay using knocked down TILs: Human TILs, cultured for 24 hours with IL2 were electroporated with siRNA encoding for human PVRIG or PD-1 or scrambled sequence as control. TILs were tested for PVRIG and PD-1 expression 24 hr post electroporation. ~80% knock down of PVRIG and ~50% knock down of PD-1 compared to scrambled-electroporated TILs was observed (Figure 82).

[0458] KD TILs were cultured with Mel-624 cells in 1:1 or 1:3 E:T for 18hr and were stained for the expression of CD137. Elevated levels of activation marker CD137 were shown in TIL MART-1 electroporated with PVRIG siRNA, similarly to TILs that were electroporated with PD-1 siRNA, compared to control scrambled siRNA (Figure 83A). Co-culture supernatant was collected and tested for the presence of secreted cytokines. TILs that were electroporated with PVRIG siRNA show a significant increase in IFNγ and TNF levels compared to control SCR siRNA. A similar effect was shown in TILs that were electroporated with PD-1 siRNA (Figure 83B-C).

[0459] The same trend of increase in activation levels was observed in TIL F4. Co-culture of PVRIG siRNA electroporated TIL F4 with Mel-624 in 1:3 E:T led to increased levels of CD137 surface expression (Figure 84A) as well as increased secretion of IFNγ in co-culture supernatant (Figure 84B). Similar trends were observed in TILs that were electroporated with PD-1 siRNA.

Functional assay using blocking Abs:

[0460] In vitro monotherapy and combo therapy of anti-PVRIG and anti-TIGIT: 209 TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. Co-culture supernatant was collected and tested for the presence of secreted cytokines. Treatment with anti TIGIT did not affect IFNγ or TNF secretion levels. However, an increase in IFNγ and TNF levels was observed when anti TIGIT and anti PVRIG were added to co-culture in combination (Figure 85A-B).

[0461] In vitro monotherapy and combo therapy of anti-PVRIG and anti-TIGIT: 209 TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. TILs were stained for surface expression of activation marker CD137 and showed reduced level of expression upon treatment with anti DNAM-1. Co-culture supernatant was collected and tested for presence of secreted cytokines. Treatment of anti DNAM-1 mediated a trend to increase secreted cytokines IFNγ and TNF. Treatment with anti DNAM-1 and anti PVRIG in combination partially reversed the effect on CD137 expression (Figure 86C) and enhanced the effect on cytokine secretion IFNγ and TNF (Fig. 5A-B). MART-1 TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. Co-culture supernatant was collected and tested for the presence of secreted cytokines. Treatment with anti DNAM-1 reduced CD137 surface expression on TILs and also the secreted cytokines IFNγ and TNF. Treatment with anti DNAM-1 and anti PVRIG in combination partially reversed these effects (Figure 86D-F).

Summary and conclusions

[0462] PD1 KD improved TIL activity, as measured by IFNγ and secretion in F4 and MART-1 TILs. An increase (~20%) of IFNγ and TNF secretion was observed upon PVRIG KD in MART-1 TILs compared to control siRNA. The same trend was observed in CD137 expression upon co-culture with 624 Melanoma cells on F4 TILs.

[0463] Treatment of anti-TIGIT did not affect IFNγ or TNF secretion levels from TILs co-cultured with 624 Mels, however, an increase in IFNγ and TNF levels was observed when anti TIGIT and anti PVRIG (CPA.7.021) were added to co-culture in combination.

[0464] Anti DNAM-1 treatment reduced TIL-MART-1 activation manifested by reduced CD137 and cytokine secretion and anti-PVRIG (CPA.7.21) could partially reverse this effect in combo treatment with DNAM-1 Ab. In TIL 209, IFNγ and TNF secretion levels were slightly elevated (-10%) with anti DNAM-1, and an increase in IFNγ and TNF levels (~40% and 30%, respectively) was observed when anti DNAM1 and anti PVRIG (CPA.7.021) were added to co-culture in combination. Collectively, our results suggest that PVRIG is a new co-inhibitory receptor for PVRL2.

EXAMPLE 15 EFFECT OF ANTI-PVRIG ANTIBODY ON HUMAN MELANOMA SPECIFIC TILS FUNCTION IN COMBINATION WITH ANTI-TIGIT AND ANTI-PD1 ANTIBODIES

Materials and Methods

[0465] TILs: Tumor-infiltrating lymphocytes (TILs) from three melanoma patients were used:

□ TIL-412- HLA-A2-Mart1 specific

□ TIL-F4- HLA-A2-gp100 specific

□ TIL-209- HLA-A2-gp100 specific

[0466] TILs were thawed in IMDM (BI, 01-058-1A) full medium supplemented with 10% human serum (Sigma, H3667) + 1% Glutamax (Life technologies, 35050-038) + 1% Na-Pyruvate (Biological Industries, 03-042-1B) + 1% non-essential amino acids (Biological Industries, 01-340-1B) + 1% Pen-Strep (Biological Industries, 03-031-1B) + 300 U/ml of rhIL2 (Biolegend, 509129).

[0467] Tumor cell lines: Human melanoma cells Mel-624 express MART-1 and gp-100 antigens in the context of MHC-I haplotype HLA-A2. Cells were cultured in complete DMEM medium (Biological Industries, 01-055-1A) supplemented with 10% FBS (BI, 04-127-1A), 25 mM HEPES buffer (BI, 03-025-1B), 1% Glutamax (Life technologies, 35050-038), and 1% Pen-Strep (Biological Industries, 03-031-1B).

[0468] Co-culture of TILs with 624 melanoma cells in the presense of anti-PVRIG, anti-TIGIT and PD1 blocking antibodies: To assess the effect of anti-PVRIG antibody (CPA.7.021), anti-TIGIT (Clone 10A7) and anti-PD1 (mAb 1B8, Merck) on melanoma specific TIL activity, TILs (3×104cells/well) were pre-incubated with tested antibodies or relevant isotype controls in mono-treatment (10µg/mL) or in combination-treatment (final 10µg/mL for each) prior to addition of 624 Melanoma target cells at 1:3 Effector:target ratio. Plate was incubated overnight (18hr) in 37°C, 5% CO2.

[0469] Assessment of TILs activation: Culture supernatants were collected and analyzed for cytokine secretion by CBA kit (Cat #560484, BD).

[0470] In vitro monotherapy anti-PVRIG and combo-therapy of with anti-TIGIT and PD1 blocking antibodies: F4 TILs (gp100 sepecific) were cultured with Mel-624 cells in 1:3 E:T for 18hr. Co-culture supernatant was collected and tested for presence of secreted cytokines. Treatment of anti-TIGIT or anti-PDI did not affect IFNγ or TNF secretion levels. However, an increase in IFNγ and TNF levels was observed when anti TIGIT or anti-PDI in combination with anti PVRIG were added to co-culture in combination (Figure 87A-B).

[0471] Treatment of anti-PVRIG, anti-TIGIT and PD1 alone did not affect IFNγ or TNF secretion levels from TILs co-culture with 624 Mels, however, an increase in IFNγ and TNF levels was observed when anti-TIGIT or anti-PDI antibodies were added in combination with anti PVRIG (CPA.7.021). The presented data suggest that there is synergestic effect for combinatory therapy with anti-TIGIT or anti-PD1 antibodies.

EXAMPLE 16:EFFECT OF ANTI-PVRIG ANTIBODIES ON TCR SIGNALING USING REPORTER GENE ASSAY

[0472] A reporter assay system for TCR signaling, such as the Jurkat-NFAT-Luc cell line, is used to test the effect of anti-PVRIG antibodies on TCR mediated signaling. This Jurkat cell line derivative expresses the luciferase reporter gene under the control of the NFAT response element. These cells are transfected with a vector encoding full length human PVRIG. As negative control, cells transfected with empty vector are used. Transfectants with vectors encoding for costimulatory or coinhibitory reference molecules, such as CD28 and PD-1, serve as positive control. Transfectants are stimulated by the addition of anti-human CD3 (e.g. OKT3) in the absence or presence of anti-PVRIG antibodies. Isotype control serves as negative control. Known functional antibodies against the reference molecules serve as positive controls. A functional agonistic crosslinking antibody is expected to show an inhibitory effect on the luciferase activity.

EXAMPLE 17 EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION USING PVRL2-FC

[0473] A plate bound assay is used to test the effect of anti-PVRIG antibodies on T cell activation, proliferation and cytokine secretion. Purified human bulk T cells are stimulated using 1 ug/ml plate bound anti-human CD3 (e.g. OKT3) and 5 ug/ml PVRL2-Fc (recombinant fused protein composed of the ECD of PVRL2, the counterpart of PVRIG) or negative control. T cell activation is evaluated by expression of activation markers, e.g. CD137, or by cell division as evaluated by dilution of CFSE dye (T cells are labeled with CFSE prior to their stimulation). Cytokine production (e.g. IFNg, IL-2) is also assessed as additional readout of T cell activation. T cell subtype markers are used to distinguish specific effects on CD4 or CD8 T cells. The co-immobilized PVRL2-Fc could have a basal stimulatory effect on T cell activation, mediated through endogenous DNAM1 - a known costimulatory counterpart receptor of PVRL2 on T cells. In the presence of antagonistic anti-PVRIG Abs, this stimulatory basal effect of PVRL2-Fc is expected to be further enhanced, due to their blocking of the inhibitory influence of endogenous PVRIG on T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of T cell activation.

EXAMPLE 18: Effect of anti-PVRIG antibodies on T cell activation using PVRL2 ectopic expressing cells

[0474] A cell based assay is used to test the effect of anti-PVRIG antibodies on T cell activation, proliferation and cytokine secretion. Purified human bulk or CD4 or CD8 T cells are stimulated upon co-culture with CHO stimulator cells (CHO cells expressing membrane-bound anti-CD3) ectopically expressing PVRL2 or empty vector. T cell activation is evaluated by expression of activation markers, e.g. CD137, or by cell division as evaluated by dilution of CFSE dye (T cells are labeled with CFSE prior to their stimulation). Cytokine production (e.g. IFNγ, IL-2) is also assessed as additional readout of T cell activation. T cell subtype markers are used to distinguish specific effects on CD4 or CD8 T cells. The PVRL2-expressing CHO stimulators are expected to have a basal sExample 19timulatory effect on T cell activation, mediated through endogenous DNAM1 - a known costimulatory counterpart receptor of PVRL2 on T cells. In the presence of antagonistic anti-PVRIG Abs, this stimulatory basal effect of surface expressed PVRL2 is expected to be further enhanced, due to their blocking of the inhibitory influence of endogenous PVRIG on T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of T cell activation.

EXAMPLE 20 EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION USING THE SEB ASSAY

[0475] Anti-PVRIG antibodies are tested for their effect on T cell activity using blood cells from healthy volunteers and SEB (Staphylococcus enterotoxin B) superantigen to engage and activate all T cells expressing the Vβ3 and Vβ8 T cell receptor chain. Human PBMCs are cultured in 96-well round-bottom plates and pre-incubated for 30-60 min with the tested antibodies. SEB is then added at various concentrations ranging from 10 ng/mL to 10 µg/mL. Supernatants are collected after 2 to 4 days of culture and the amount of cytokine (e.g. IL-2, IFNγ) produced is quantified by ELISA or using standard CBA kit. SEB stimulates cytokine production by whole-blood cells in a dose dependent manner.. The effect of anti-PVRIG mAbs on cytokine production is tested at several Ab doses. Blocking anti-PVRIG mAbs are expected to enhance IL-2 production over control IgG. In addition to IL-2, the effect of the Abs on the levels of additional cytokines such as TNFα, IL-17, IL-7, IL-6 and IFNγ can be tested in this assay using a CBA kit.

EXAMPLE 21 Effect of anti-PVRIG antibodies in Ag-specific assays

[0476] An assay that is used to profile the functional effect of anti-human PVRIG antibodies on Ag specific stimulation of pre-existing memory T cells in healthy donor blood is the tetanus toxoid (TT) assay. To this end, freshly prepared PBMC (2 x 10^5 cells) are plated in 96 well round-bottom plates in complete RPMI 1640 medium (containing 5% heat inactivated human serum), pre-incubated with tested antibodies at varying concentration and stimulated with TT (Astarte Biologics) at a concentration of 100 ng/mL The cells are incubated for 3-7 days at 37°C, after which supernatants are harvested. Cytokine concentrations (e.g. IL-2, IFN-y) are determined by ELISA and/or CBA kit. Blocking anti-PVRIG Abs are expected to enhance T cell proliferation and cytokine production compared to that obtained with TT antigen alone.

[0477] Similarly to the method described above, which uses TT to stimulate human memory T cells, we can test the effect of anti-PVRIG Abs on T cell activation upon recall responses to additional antigens such as CMV, EBV, influenza HIV, mumps, and TB, using a similar experimental setup as described above. This can also be used to test the effect of anti-PVRIG antibodies on stimulation of naive cells using neo-antigens such as KLH.

[0478] In addition, the effect of anti-PVRIG Abs is tested on the antigen specific responses of tetramer-sorted Ag-specific CD8 T cells from peripheral blood of patients suffering from viral infections such as HCV and HIV. Tetramer sorted CD8 T cells are co-cultured with peptide-loaded autologous PBMCs for 5 days. Proliferation of CD8 Ag-specific T cells and secretion of cytokines (e.g. IFNγ, IL2, TNF-α) are evaluated. We expect anti-PVRIG antibodies to enhance proliferation and cytokine production, compared to antigen alone.

EXAMPLE 22 BINDING AND FUNCTIONAL ANALYSIS OF HYBRIDOMA-DERIVED ANTIBODIES AGAINST PVRIG

[0479] This example shows the characterization of binding of hybridoma-derived antibodies (the CHA antibodies) to human and cynomolgus PVRIG protein in cell lines and primary leukocytes, as well as the characterization of the capacity of hybridoma-derived antibodies to block the interaction between PVRIG and PVRL2.

Protocols

[0480] FACS analysis of hPVRIG over-expressing cells: The following cell lines were used to assess the specificity of anti-human PVRIG antibodies: HEK parental and HEK hPVRIG over-expressing cells. These cells were cultured in DMEM (Gibco) + 10% fetal calf serum (Gibco) + glutamax (Gibco). For the HEK hPVRIG over-expressing cells, 0.5ug/ml puromycin (Gibco) was also added to the media for positive selection. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II (BD Biosciences) or IntelliCyt (IntelliCyt Corporation) and analyzed using FlowJo (Treestar) and Prism (Graphpad) software.

[0481] FACS analysis of human cell lines for hPVRIG: The following cell lines were used to assess the expression and specificity of anti-human PVRIG antibodies: Jurkat and HepG2. Jurkat cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids (Gibco), sodium pyruvate

(Gibco), and penicillin/streptomycin (Gibco). HepG2 cells were cultured in DMEM + 10% fetal calf serum + glutamax. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II or IntelliCyte and analyzed using FlowJo and Prism software.

[0482] FACS analysis of naive human primary leukocytes for hPVRIG: Primary leukocytes were obtained by Ficoll (GE Healthcare) gradient isolation of peripheral blood (Stanford Blood Bank). Leukocytes as isolated peripheral blood mononuclear cells (PBMC) were frozen down in liquid nitrogen at a density between 1×10^7 and 5×10^7 cells/ml in a 10% DMSO (Sigma), 90% fetal calf serum mixture. To assess protein expression of PVRIG on PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or in some cases, as a 4 point titration series starting at 10ug/ml on ice for 30 mins-1 hr.

[0483] Briefly, antibody cocktail mixtures were added to resuscitated PBMC that were seeded at 5×10^5 - 1×10^6 cells/well upon prior Fc receptor blockade and live/dead staining (Aqua Live/Dead, Life Technologies). Antibody cocktails were incubated with PBMC for 30mins - 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using FlowJo and Prism software. Immune subsets that were analysed include CD56 dim NK cells, CD56 bright NK cells, CD4+ T cells, CD8+ T cells, non-conventional T cells (e.g. NKT cells and γδ T cells), B cells, and monocytes.

[0484] FACS analysis of cynomolgus PVRIG engineered over-expressing cells: The following cell lines were used to assess the cross-reactivity of anti-human PVRIG antibodies with cynomolgus PVRIG (cPVRIG): expi parental and expi cPVRIG over-expressing cells. These cells were cultured in DMEM + 10% fetal calf serum + glutamax. expi cPVRIG transient over-expressing cells were generated by electroporating cPVRIG DNA into parental expi cells using the Neon transfection system. For FACS analysis, expi cPVRIG cells were used between 1-3 days post transfection. Parental expi cells were harvested from log growth phase. 50,000-100,000 cells of per well of each type were seeded in 96 well plates. Anti-human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II or IntelliCyte and analyzed using FlowJo and Prism software.

[0485] FACS analysis of naive primary cynomolgus monkey leukocytes: Primary cynomolgus monkey (cyno) leukocytes were obtained from fresh blood which was drawn no longer than 24 hours prior to expression analysis. Blood was sourced from Bioreclamation. To assess protein expression of PVRIG on cyno PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr.

[0486] Briefly, antibody cocktail mixtures were added to PBMC that were seeded at 5×10^5 - 1×10^6 cells/well upon prior Fc receptor blockade and live/dead staining. Antibody cocktails were incubated with PBMC for 30mins - 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using Prism software. Immune subsets that were analysed include CD16+ lymphocytes, CD14+/CD56+ monocytes/myeloid cells, and CD3+ T cells.

[0487] Cellular-based competition assays: The ability of PVRIG antibodies to inhibit the interaction of PVRIG with its ligand PVRL2 was assessed in a cellular competition assay. In this assay, the ligand PVRL2 is endogenously expressed on un-manipulated HEK cells and soluble Fc-tagged PVRIG (manufactured on demand by Genscript) is added. In this case, the ability of PVRIG antibodies to block soluble PVRIG binding to HEK cells were assessed through the concomitant addition of 33nM of soluble PVRIG protein and PVRIG antibodies (0.066-66 nM) to 100,000 HEK cells and incubated for 1 hour on ice. The extent of PVRIG Fc binding was detected by addition of anti- human Fc Alexa 647 (Jackson Laboratories) for 20-30 minutes on ice. Cells were washed twice in PBS for acquisition using a FACS Canto II. Data was analyzed using FlowJo (Treestar), Excel (Microsoft) and Prism (GraphPad).

Results

[0488] Hybridoma PVRIG antibodies recognize PVRIG on overexpressing cells: To screen for antibodies that were specific for PVRIG, we assessed the ability of antibodies that were generated from two hybridoma campaigns to bind HEK cell lines that were engineered to overexpress human PVRIG. The majority of antibodies from these campaigns bound to the HEK hPVRIG cells, albeit with varying affinity. Furthermore, the majority of these antibodies also showed low background binding to HEK parental cell lines indicating high specificity towards PVRIG. Figure 77 shows one example of the specificity of the PVRIG antibodies. A summary of all binding characteristics of the antibodies towards HEK hPVRIG cells relative to control that were generated in the hybridoma campaigns are displayed in Figure 79.

[0489] PVRIG antibodies recognize PVRIG protein on naive NK and T cells: The populations which displayed the highest level of PVRIG on naive PBMC subsets were NK and CD8 T cells, and the absolute level of expression between these two cell subsets was similar (gMFI). CD4 T cells showed lower levels of PVRIG, while B cells and monocytes had very low/no detectable expression. A summary of expression on naive NK cells and CD8 T cells as detected by the antibodies is shown in Figure 91. Other minor subsets also displayed PVRIG expression and included non-conventional T cells such as NKT cells and γδ T cells. The expression pattern on PBMC subsets was very similar across all donors sourced and analyzed.

[0490] PVRIG is detected on Jurkat cell lines by hybridoma-derived PVRIG antibodies: In addition to screening PBMC for PVRIG protein expression, we wanted to understand whether it was also expressed on cancer cell lines. We chose to screen our antibodies on Jurkat cells given their high expression of PVRIG RNA. We also chose HepG2 as a negative control cell line to further validate the specificity of our antibodies. Most of the hybridoma-derived antibodies did detect PVRIG protein expression on Jurkat cells (Figure 79PVRIG hybridoma antibody binding characteristics to primary human PBMC, cyno over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentrations indicate that at which the gMFIr was calculated. Not tested indicates antibodies that were not tested due to an absence of binding to human HEK hPVRIG, expi cPVRIG cells, or not meeting binding requirements to PBMC subsets. Highlighted antibodies are four antibodies for which humanization was done (See Figure 90).

[0491] Figure 92), but not the HepG2 cells (data not shown). An example of PVRIG detection on Jurkat is shown in Figure 78 with a representative antibody, CHA.7.518.

[0492] Cellular-based biochemical assays: Upon screening our 29 hybridoma antibodies in the cellular biochemical assays, we found that there were 20 clear blockers and 9 non-blockers of the PVRIG-PVRL2 interaction. All of the blocking antibodies were able to inhibit the interaction of PVRIG Fc with HEK cells by at least 50%, with most of these antibodies completely abolishing PVRIG Fc binding. The IC_{50} values associated with those antibodies that did show blocking capacity are reported in Figure 92. The majority of IC_{50} values were between 20-60nM.

Summary and Conclusions

[0493] Using a hybridoma platform, we have been able to successfully generate monoclonal antibodies towards the human PVRIG antigen. Using engineered over-expressing cells as well as a suite of cancer cell lines, we showed that our antibodies are highly specific to the PVRIG antigen, and are able to detect protein expression which correlated with RNA expression. Upon analysis of human PBMC subsets, we showed that the PVRIG protein is most highly expressed on NK and T cells, with low/negative expression on B cells and myeloid cells. We also showed that a proportion of these antibodies are cross-reactive with the cynomolgus monkey (cyno) PVRIG antigen through assessing their binding to over-expressing cells. Furthermore, the expression pattern on cyno PBMC is similar to human PBMC. Lastly, we were able to show through a FACS-based competition assay, that a proportion of our hybridoma antibodies are able to inhibit the interaction of PVRIG with its ligand, PVRL2. The antibodies which showed the best characteristics regarding all the aforementioned data were CHA-7-518, CHA-7-524, CHA-7-530, and CHA-7-538.

EXAMPLE 23. Effect of CHA anti-PVRIG antibodies in the MLR assay

[0494] An assay used to profile the functional effect of anti-human PVRIG antibodies on allo-antigen responses is proliferation of Human CD8+ T Cells in a Mixed Lymphocyte Reaction (MLR) assay. As is known in the art, MLR is an ex vivo cellular immune assay that provides an in vitro correlation of T cell function.

[0495] Anti-PVRIG antibodies are expected to enhance proliferation of human CD4 and CD8 T cells in response to cells from an MHC-mismatched donor. Human T cells are enriched from whole blood of one donor (e.g. donor A) by using Human T cell RosetteSep RTM (StemCell Technologies) as per manufacturer's instructions. After separation, cells are fluorescently labeled with CFSE dye (Molecular Probes). To serve as allogeneic antigen presenting cells (APCs), mononuclear cells are first isolated from whole blood from a MHC-mismatched donor (e.g. donor B) and then depleted of CD3+ T cells. APCs are then irradiated with 2500 rads in a cesium irradiator.

[0496] In general, an MLR assay is done as follows. HumanT cells and allogeneic 150,000 APCs are co-cultured in a 96-well flat-bottom plate with 150,000 CD8+ T cells and APCs for 5 days with anti-PVRIG antibodies at different concentrations. On day 5, cells are harvested, washed and stained with anti-CD8-biotin followed by streptavidin-PerCp. Samples are run by FACS to assess the degree of proliferation as depicted by CFSE
dilution. Functional blocking anti-PVRIG antibodies are expected to enhance T cells proliferation and cytokine secretion in response to cells from a MHC-mismatched donor.

[0497] An MLR assay was used to characterize the biochemical effect of the CHA antibodies of the invention on resting and activated human T cells, and to characterize the capacity of hybridoma-derived antibodies to modulate T cell proliferation in an MLR setting

Protocols

[0498] Mixed Lymphocyte Reaction (MLR): A mixed lymphocyte reaction was established by co-culturing dendritic cells (DCs) and T cells derived from distinct donors in an allogeneic setting. DCs were generated by culturing purified monocytes with 100 ng/ml GM-CSF (R&D systems) and 100ng/ml IL-4 (R&D systems) for 7 days. After 7 days, purified CFSE-labelled CD3 T cells were combined with DCs at a 10:1 ratio and were cultured in X vivo-20 serum free media (Lonza) for 5 days. In some conditions, unconjugated anti-PVRIG antibodies or isotype control antibodies were added to the plates at 10ug/ml. Three MLR assay permutations were set up, where DCs from one donor were co-cultured with CD3 T cells from 3 separate allogeneic donors. All blood products were sourced from Stanford Blood Bank.

[0499] Expression and functional analysis: After the 5 day MLR culture, the level and extent of T cell activation and proliferation was assessed by CFSE dilution and expression of activation markers such as CD25 and PD-1. In-house anti-PVRIG antibodies from both phage and hybridoma campaigns were used to assess the expression of PVRIG. Expression of the PVRIG ligand, PVRL2, was also assessed in a kinetic fashion on DC. All data was acquired using flow cytometry and data analysis was performed using FlowJo (Treestar) and Prism (Graphpad) software.

[0500] FACS-based epitope analysis: As we tested an array of antibodies in the MLR, we were interested in determining whether these antibodies could be epitope 'binned' based on FACS-based binding, and whether this 'binning' would correlate to changes in T cell activation and proliferation in the assay. To do this, T cells harvested from the assay were pre-incubated with unconjugated PVRIG antibodies, and then counter-stained with a conjugated PVRIG antibody of a different clone. The extent to which the conjugated PVRIG antibody gave a signal on T cells indicated the extent to which this antibody had to compete for PVRIG binding on T cells with the unconjugated antibody. A negative or low signal would indicate that there is high competition, indicating the two antibodies are in the same epitope 'bin'. A high signal would indicate low or no competition and thus the antibodies would be considered to be in different 'bins'.

Results

[0501] Expression of PVRL2 on monocyte-derived DC: To determine whether PVRL2 would be expressed on DC for the MLR assay, DC were generated from monocytes, and PVRL2 expression was assessed in a kinetic fashion at daily intervals after addition of GM-CSF and IL-4. As indicated in Figure 72, PVRL2 expression increased from Day 0 until Day 5 where expression peaked. At Day 6, expression decreased slightly compared to Day 5. At Day 7, expression was similar to Day 6 indicating stabilization of PVRL2 expression at these time points. Thus, DC expressed PVRL2 at the appropriate time point for use in the MLR assay.

[0502] Expression of PVRIG on T cells after MLR culture: Many T cell receptors than modulate function in the MLR are expressed on proliferating T cells. Thus, we wanted to determine whether PVRIG is also expressed. We analysed proliferating T cells at Day 5 post MLR co-culture initiation and were characterized by their dilution of CFSE (i.e. CFSE low). As shown in Figure 73 and Figure 74, relative to isotype control (mIgG1), PVRIG was expressed on CFSE low cells as determined by multiple PVRIG antibodies on both CD4 and CD8 T cells across three donors analysed. FACS plots are shown in Figure 73 to indicate PVRIG on CFSE low cells, and bar graphs in Figure 74 indicate the level of expression of PVRIG relative to mIgG1.

[0503] PVRIG antibodies enhance T cell proliferation: Having shown that PVRIG expression is expressed on proliferating T cells in the MLR, we wanted to determine whether treatment with PVRIG antibodies could affect levels of T cell proliferation. As shown in Figure 4, addition of PVRIG antibodies into the MLR assay was able to increase the percentage of CFSE low cells across all the hybridoma antibodies tested compared to control. This was observed across all donors analysed.

[0504] PVRIG antibodies bind to multiple epitopes on PVRIG: To compare the PVRIG antibodies for their ability to bind different epitopes on PVRIG, we performed a competition experiment where T cells from the MLR were cultured with unlabeled anti-PVRIG antibodies derived from our hybridoma campaigns for 5 days. T cells were then harvested at day 5 and counter-stained with a conjugated anti-PVRIG antibody that was derived from our phage campaign (CPA.7.021). As shown in Figure 76, complete or near complete reduction of CPA.7.021 binding was observed in conditions that contained CHA.7.516-M1, CHA.7.518-M1, CHA.7.524-M1, CHA.7.530-M1, and CHA.7.538-M1 when compared to background fluorescence levels, suggesting that these antibodies may overlap in epitope recognition. Partial reduction in CPA.7.021 binding was observed with CHA.7.537-M1, CHA.7.528-M1, and CHA.7.548-M1, suggesting partial overlap in epitope recognition. No reduction in CPA.7.021 binding was observed in cells pre-cultured with CHA.7.543-M1 suggesting an absence of epitope recognition. Collectively, this data indicates that the PVRIG antibodies from our campaigns, when assessed relative to CPA.7.021, could recognize at least 3 different epitopes on PVRIG.

[0505] Conclusions We characterized our PVRIG antibodies for their ability to bind to proliferating and resting T cells, as well as their functional activity in a MLR. Binding of multiple PVRIG antibodies was detected on proliferating T cells and was higher on proliferating T cells as compared to resting, especially the CD8+ subset. This data demonstrates that PVRIG expression is increased upon T cell activation. Furthermore, several PVRIG antibodies increased T cell proliferation as compared to mIgG1 isotype indicating that they can also modulate T cell function. As above, these antibodies all have ability to block PVRIG with its ligand, PVRL2. Based on this, we conclude that by blocking the PVRIG-PVRL2 interaction, these antibodies lead to an increase in T cell activation and proliferation, which is a hallmark indication of a desired effect for an immune checkpoint inhibitor that would be used to treat cancer. Lastly, we performed competition experiments comparing the binding of multiple hybridoma-derived PVRIG antibodies to activated T cells, relative to a phage-derived antibody. From this series of experiments, we provide evidence for epitope diversity of our phage and hybridoma-derived antibodies.

EXAMPLE 24. EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION UPON COMBINATION WITH IMMUNE CHECKPOINT BLOCKADE

[0506] The combination of PVRIG blockade with blocking Abs of a known immune checkpoint (e.g. PD1, PDL-1 or TIGIT), is expected to further enhance the stimulatory effect on T cell activation in the assays depicted above.

EXAMPLE 25. FUNCTIONAL ANALYSIS OF PVRIG ANTIBODIES

[0507] The human PVRIG antibodies of the invention were characterized for the ability to inhibit the interaction of PVRIG with its ligand PVRL2, and their ability to modulate effector lymphocyte function in primary cell-based assays.

Protocols

Cellular-based biochemical assays

[0508] The ability of PVRIG antibodies to inhibit the interaction of PVRIG with its ligand PVRL2 was assessed in a cellular biochemical assay format in two orientations.

[0509] In the first orientation, the ligand PVRL2 is endogenously expressed on un-manipulated HEK cells and soluble biotinylated Fc-tagged PVRIG (manufactured on demand by Genscript) is added. In this case, the ability of PVRIG antibodies to block soluble PVRIG binding to HEK cells were assessed through two permutations. In the first permutation, various concentrations of PVRIG antibodies (range 0.066-66nM) were pre-incubated with 33nM of soluble PVRIG in phosphate buffered saline (PBS, Gibco) for 30 minutes on ice. This complex was subsequently added to 100,000 HEK cells in and incubated for a further 1 hour on ice. After 1 hour, HEK cells were washed twice in PBS and the extent of soluble PVRIG bound to HEK cells was detected by addition of streptavidin conjugated to Alexa 647 (Jackson Laboratories) for 30 minutes on ice. HEK cells were washed twice in PBS, and resuspended in 100ul of PBS for acquisition on the FACS Canto II (BD Biosciences). Data was analysed using FlowJo (Treestar) and Prism (Graphpad) software. In the second permutation, 33nM of soluble PVRIG protein and PVRIG antibodies (0.066-66 nM) were added concomitantly to 100,000 HEK cells and incubated for 1 hour on ice. Subsequent steps to analysis for this permutation are equivalent to the first permutation.

[0510] In the second orientation, HEK cells were engineered to over-express PVRIG and soluble biotinylated Fc-tagged PVRL2 (CD Biosciences) was added. In this case, various concentrations of PVRIG antibodies (range 0-200nM) with 160nM soluble PVRL2 were added concomitantly to 100,000 HEK hPVRIG or parental HEK cells, and incubated in PBS + 1% BSA + 0.1% sodium azide (FACS buffer) for 1hr on ice. Soluble PVRL2 binding was detected by addition of streptavidin Alexa 647 in FACS buffer for 30 minutes on ice. Cells were washed twice in FACS buffer, and re-suspended in 50ul of PBS for acquisition on the Intellicyt HTFC (Intellicyt). Data was analyzed using FlowJo (Treestar), Excel (Microsoft) and Prism (GraphPad).

Primary NK cell assay

[0511] The PBMC subset with the most robust expression profile for PVRIG was on NK cells. As such, we designed an NK cell-based co-culture assay with PVRL2-expressing tumor cells to determine whether our antibodies could modulate NK cell-mediated cytotoxicity towards these targets. The targets we chose were the acute B cell lymphocytic leukemia cell line, Reh (ATCC cell bank), and the acute myeloid leukemia cell line, MOLM-13 (DSMZ cell bank). Reh and MOLM-13 cells were grown in RPMI media (Gibco) + 20% fetal calf serum (Gibco), glutamax (Gibco), penicillin/streptomycin (Gibco), non-essential amino acids (Gibco), sodium pyruvate (Gibco), HEPES (Gibco), and beta-mercaptoethanol (Gibco).

[0512] Two days prior to the co-culture assay, primary NK cells were isolated using the human NK cell isolation kit (Miltenyi Biotec) and cultured in RPMI media + 20% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, HEPES, beta-mercaptoethanol, and 250U/ml IL-2 (R&D systems). On the day of the assay NK cells were harvested, enumerated and pre-incubated with PVRIG antibodies for 15-30 minutes at room temperature. During this incubation, target cells were harvested from culture, labelled with Calcein AM (Life Technologies) for 30 minutes at 37°c, washed in media, and enumerated for the assay. NK cell-mediated cytotoxicity assays were set up where a constant number of target cells (50,000) were co-cultured with increasing concentrations of NK cells pre-incubated with 5 ug/ml of PVRIG antibodies (thus altering the NK cell to target ratio). Alternatively, a fixed NK cell to target ratio was used in the assay, but NK cells were pre-incubated with altering concentrations of PVRIG antibody (range 3.9 ng/ml - 5 ug/ml) in a dose titration. Upon addition of the NK cells and targets, plates were pulse spun at 1,400 rpm for 1 minute and placed at 37°c in a 5% CO_2 atmosphere for 4 hours. After 4 hours, plates were spun at 1,400 rpm for 4 minutes, and 80ul of supernatant was harvested to quantitate the release of Calcein AM from the target cells. The quantity of Calcein AM released from targets was assessed by a Spectramax Gemini XS fluorometer (Molecular Devices). As controls for Calcein AM release, total and spontaneous release was assessed by exposing target cells to 70% ethanol or media only for the duration of the assay. Levels of killing (as a percentage) by NK cells were calculated using the following formula:

(Sample release - spontaneous release) / (total release – spontaneous release)*100

[0513] In addition to PVRIG antibodies, in some cases, other antibodies towards NK cell receptors such as TIGIT (Genentech, clone 10A7, Patent number: WO2009126688 A2) and DNAM-1 (Biolegend, clone 11A8) were also added as comparators.

Results

[0514] Cellular-based biochemical assays: Upon screening a panel of our PVRIG antibodies in the cellular biochemical assays, we found that there was variable levels of inhibition across the antibodies tested, and the level of inhibition was dependent on the permutation and orientation of the assay (Figure 98). Four antibodies are specifically shown in Figure 93 to illustrate these points. The orientation and permutation of the assay which gave the most robust inhibitory effect relative to control, was when soluble PVRIG pre-incubated with PVRIG antibodies was added to HEK cells (Figure 93a). In this permutation, CPA.7.021 showed the best absolute blocking capacity compared to the other three antibodies (CPA.7.002, CPA.7.005, and CPA.7.050). Despite the differences in level of blocking, all antibodies in this permutation showed similar IC50 values which were in the low nanomolar range, and the blocking capacity plateaued at higher concentrations.

[0515] When the absolute level of inhibition invoked by the four PVRIG antibodies was then measured when soluble PVRIG and PVRIG antibodies were concomitantly added to HEK cells, more variability of blocking in the assay was observed (Figure 93b). CPA.7.021 remained the best blocking antibody. However, CPA.7.002 and CPA.7.005 showed markedly less ability to inhibit soluble PVRIG binding to HEK cells relative to the control antibody. CPA.7.050 showed an intermediate level of blocking as compared to CPA.7.021, CPA.7.002, and CPA.7.005. This difference in absolute level of inhibition also corresponded to differences in the IC50 values of each antibody. CPA.7.021 and CPA.7.050 again showed low nanomolar IC50 values, although they were both higher than in the first permutation of the assay. In contrast, the IC50 values of CPA.7.002 and CPA.7.005 increased substantially, CPA.7.002 by approximately 20-fold, and CPA.7.005 by approximately 30-fold. This data indicates that how the antibody has to compete for PVRIG binding with its cognate ligand, will indicate the potency with which the antibody can block this interaction.

[0516] When the orientation of the biochemical assay was reversed (i.e. PVRL2Fc was assessed to bind to HEK hPVRIG cells), the ability of the four PVRIG antibodies to block PVRL2 Fc interaction was variable

(Figure 93c). Consistent with the biochemical assays which used HEK cells as targets (Figure 93a-b), CPA.7.021 and CPA.7.050 inhibited PVRL2 Fc binding to HEK hPVRIG cells, and their ability to block the binding was similar. Surprisingly however, we saw enhancement of PVRL2 Fc binding in the presence of CPA.7.002 and CPA.7.005 antibodies which we did not observe when HEK cells were used as targets.

[0517] NK cell cytotoxicity assay with Reh cells: The first target we investigated in the NK cell cytotoxicity assay was the Reh line. Reh was initially selected as it showed robust levels of PVRL2 by flow cytometry, but a low frequency of other activating ligands such as NKG2D ligands, and low expression of PVR (Figure 94). Traditional NK cell targets were not used, such as K562, due to their expression of a high frequency of NKG2D ligands, and high expression of PVR, which may mask a functional effect of the PVRIG antibodies. Importantly, Reh cells did not express any NK cell receptors known to interact with PVRL2 and PVR such as TIGIT, DNAM-1, and PVRIG.

[0518] Upon screening our panel of PVRIG antibodies in this assay, we found four antibodies that were able to modulate NK cell-mediated cytotoxicity (Figure 99). These four antibodies were those that were discussed in the biochemical assay results section-CPA.7.002, CPA.7.005, CPA.7.021, and CPA.7.050. In all cases, addition of these antibodies enhanced NK cell-mediated cytotoxicity against Reh cells (Figure 95a-c). Addition of CPA.7.002 and CPA.7.005 enhanced cytotoxicity most robustly (Figure 95a-b), followed by CPA.7.021 and CPA.7.050 which showed similar levels of enhancement (Figure 95c). Figure 95d shows a concentration-dependent analysis of enhancement of NK cell-mediated cytotoxicity by CPA.7.002 and CPA.7.021. Blocking antibodies towards receptors that have been reported to also bind PVRL2 such as TIGIT and DNAM-1 were added to the assay with Reh cells as comparators. As shown in Figure 95e-f, the addition of TIGIT and DNAM-1 antibodies did not show functional effects in this assay.

[0519] NK cell assay with MOLM-13 cells: To assess whether PVRIG antibodies were able to modulate NK cell-mediated cytotoxicity against a second target, MOLM-13 cells were utilized. MOLM-13 also express PVRL2 analogous to Reh cells, but also have robust expression of PVR (Figure 94). Like the Reh cells, MOLM-13 did not express any NK cell receptors. Utilization of this cell line, in addition to Reh cells, would indicate whether PVRIG antibodies can modulate NK cell-mediated cytotoxicity in the context of different receptor-ligand interactions, particularly when PVR is expressed.

[0520] Upon screening our PVRIG antibodies in this assay, we found that the functional effect of CPA.7.021 was diminished and did not show significant enhancement of NK cell-mediated cytotoxicity above control levels (Figure 97a). In contrast, CPA.7.002 and CPA.7.005 were able to enhance NK cell-mediated cytotoxicity in this assay (Figure 97a). Using a comparator antibody, blockade of TIGIT did not show functional effects in this assay when compared to control (Figure 97b).

Summary and Conclusions

[0521] Using our antibody phage platform, we generated a panel of antibodies against the human PVRIG antigen that showed an ability to block the interaction of PVRIG with its ligand PVRL2, and enhance NK cell-mediated cytotoxicity against two hematological cell lines. The ability of the PVRIG antibodies to inhibit PVRIG and PVRL2 interaction was influenced by the orientation of the assay as well as pre-incubation steps, representative of potential antibody dynamics with PVRIG in physiological settings such as cancer. Four antibodies showed an ability to enhance NK cell-mediated cytotoxicity against the Reh cell line, but only two antibodies showed an ability to enhance cytotoxicity against MOLM-13 cells. This difference may be attributed to the alternate receptor-ligand interactions involved in NK cell-mediated recognition of each cell line, and/or differential properties of the antibodies and their potency in modulating the function of

PVRIG.

EXAMPLE 26. EFFECT OF ANTI-PVRIG ANTIBODIES ON GD T CELL ACTIVATION USING PVRL2 ECTOPIC OR NATURALLY EXPRESSING CELLS

[0522] A cell based assay is used to test the effect of anti-PVRIG antibodies on gamma delta T cell activation, proliferation and cytokine secretion. Purified human gamma delta T cells are activated with HMBPP or IPP and co-cultured with target cells (e.g. REH, MOLM-13)_that naturally express PVRL2 or with target cells ectopically expressing PVRL2 or empty vector (e.g. CHO, Raji, 721.221). Gamma delta T cell function is assessed by examining cytokine production (e.g. IFN-γ, IL-17)_in cultured supernatants or cytotoxic activity on the target cells. PVLR2 expression is expected to have a basal stimulatory effect on gamma delta T cell activation, mediated through endogenous DNAM1 - a known costimulatory counterpart receptor of PVRL2 on gamma delta T cells. In the presence of antagonistic anti-PVRIG Abs, cytokine production or cytotoxic activity is expected to be further enhanced, due to their blocking of the inhibitory function of endogenous PVRIG on gamma delta T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of gamma delta T cell activation.

Example 27: Effect of Proteins On Human T Cells Activated Using Anti-CD3 and Anti-CD28 in the Presence of Autologous PBMCs

MATERIALS AND METHODS

[0523] In these experiments the effects of PVRIG on human T cells which were activated using anti-CD3 and anti-CD28 in the presence of autologous PBMCS is evaluated. Conversely, this assay can also be used to assay the effects of anti-PVRIG antibodies on T cell activation.

[0524] PVRIG hECD-hIg fusion protein (Figure 92BA), composed of the ECD of human PVRIG fused to the Fc of human IgG1 bearing C220, C226 and C229 to S mutations at the hinge, was produced at GenScript (China) by transient transfection in CHO-3E7 cells which were cultured for 6 days, followed by protein A purification of cell harvest. The final product was formulated in PBS pH 7.2. Expression vector used was Mammalian Expression Vector pTT5, in which PVRIG gene is driven by CMV promoter.

[0525] CD4+ Human T cell Isolation Kit II is purchased from Miltenyi (Cat. #130-094-131). hIgG1 control (Synagis®) is obtained from Medimmune Inc. Anti-human CD3 Ab (OKT3, Cat# 16-0037) and anti-human CD28 Ab (clone CD28. 2; Cat# 16-0289) are purchased from eBioscience. Dynabeads M-450 Epoxy (Cat. # 140. 11) are purchased from Invitrogen. Buffy coats of human blood are obtained from LifeSource. Ficoll-Paque Plus (Cat. #17-1440-02), is purchased from GE HealthCare.

[0526] Isolation of PBMCs from buffy coats using Ficoll separation: Total PBMCs are suspended in Ex-Vivo 20 medium, and irradiated at 3000rad. Naive CD4+ T cells are isolated from buffy coats of three healthy human donors' blood using CD4+ Human T cell Isolation Kit II (Miltenyi) according to manufacturer's instructions and co-cultured with irradiated autologous PBMCs at a ratio of 1:1 (1. 5×10^5 T cells with 1. 5×10^5 irradiated PBMCs per well). The cultures are activated with anti-CD3 (0. 5ug/ml) and anti-CD28 (0. 5 ug/ml) antibodies. Either an anti-PVRIG antibody or a PVRIG ECD protein are added to the culture at the indicated concentrations. After 24 hr in culture, cells are pulsed with H3-thymidine. Cells are harvested after 72 hours in culture.

[0527] For the ECD experiment, the results are expected to cause a dose dependent inhibition of T cell proliferation and/or activation, supporting the therapeutic potential of immunoinhibitory PVRIG based therapeutic agents (e.g. PVRIG polypeptides or PVRIG fusion proteins according to at least some embodiments of the invention) for treating T cell-driven autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease, as well as for treating other immune related diseases and/or for reducing the undesirable immune activation that follows gene or cell therapy. Essentially, immunoinhibitory PVRIG proteins that agonize PVRIG should prevent or reduce the activation of T cells and the production of proinflammatory cytokines involved in the disease pathology of such conditions.

[0528] In addition, these results are also expected to support a therapeutic potential of immunostimulatory anti-PVRIG antibodies that reduce the inhibitory activity of PVRIG for treating conditions which will benefit from enhanced immune responses such as immunotherapy of cancer, infectious diseases, particularly chronic infections and sepsis. Essentially, immunostimulatory anti=PVRIG antibodies will promote the activation of T cells and elicit the production of proinflammatory cytokines thereby promoting the depletion of cancerous or infected cells or infectious agents.

Example 28: Inhibition of T cell Activation Assay.

[0529] In these experiments the effects of PVRIG ECDs or anti-PVRIG antibodies on T cell activation in a bead assay.

MATERIALS & METHODS

[0530] Isolation of human T Cells: Buffy coats are obtained from Stanford Blood Bank from healthy human donors. CD3+ T cells are isolated from buffy coats using RosetteSep kit (StemCell Technologies) following manufacturer's instructions. Cells are analyzed with anti-CD45 and anti-CD3 by flow cytometry to evaluate the % of CD3+ cells obtained. Viability is evaluated after thawing prior to the assay.

[0531] Bead Coating and QC: Tosyl activated beads (Invitrogen, Cat# 14013) at 500×10^6/ml are coated with anti-CD3 mAb and either PVRIG ECD proteins or anti-PVRIG antibodies in a two-step protocol: with 50ug/ml human anti-CD3 clone UTCH1 (R&D systems, Cat# mab 100) in sodium phosphate buffer at 37 ° C. overnight, followed with 0-320ug/ml of either PVRIG ECD proteins or anti-PVRIG antibodies for another overnight incubation at 37° C.

[0532] The amount of PVRIG protein (either ECD or antibody) bound to the beads is analyzed.

[0533] Bead assay setup: 100k human CD3+ T cells are cultured with 100k or 200k beads coated with various concentrations of the PVRIG protein for 5 days in complete IMDM (Gibco, Cat #12440-053) supplemented with 2% AB human serum (Gibco, Cat# 34005-100), Glutmax (Gibco, Cat #35050-061), sodium pyruvate (Gibco, Cat #11360-070), MEM Non-Essential Amino Acids Solution (Gibco, Cat #11140-050), and 2-mercaptoethanol (Gibco, Cat #21985). At the end of 5 day culture, cells are stained with anti-CD25, anti-CD4, anti-CD8, and fixable live dead dye to determine CD25 expression levels on each subset of cells. Supernatants are collected and assayed for IFNγ secretion by ELISA (Human INFγ duoset, R&D systems, DY285).

[0534] In these experiments human CD3 T cells co-cultured with beads coated with various concentration of PVRIG-protein are analyzed for their level of expression of CD25. Both CD4+ and CD8+ cells are anticipated to show dose dependent inhibition by the PVRIG-ECD- fusion protein, or, conversely, both CD4+ and CD8+ cells are anticipated to show dose dependent activation by the PVRIG-antibody.

Example 29: Epitope mapping of anti-human PVRIG antibodies based on cynomolgus cross-reactivity

Rationale and Objectives

[0535] The objective of this study is to identify the epitopes on the PVRIG protein that determine cross-reactivity of anti-human PVRIG antibodies against the cynomolgus monkey (cyno) orthologue. Many of the lead antibodies against human PVRIG target show varied degrees of cyno cross-reactivity despite the fact that many of these antibodies belong to the same epitope bin. To shed light on the molecular basis of human/cyno cross-reactivity (or lack thereof), several cyno-to-human mutations of the PVRIG recombinant proteins were designed, expressed and purified, and tested for binding to a panel of anti-human PVRIG antibodies in ELISA.

Methods

[0536] Design of cyno-to-human PVRIG variants: Sequence alignment of human and PVRIG extracellular domains (ECDs) shows 90% sequence identity and 93% sequence homology between human and cyno orthologs (Figure 100). Based on the nature of the mutations (conserved vs non-conserved) and the secondary structure prediction (coil vs extended) of the mutation region, three site-directed mutants of the cyno PVRIG were designed to probe the cyno-cross reactivity focused epitope mapping. These mutants include H61R, P67S, and L95R/T97I cyno PVRIG. Wild type cyno and human PVRIG were also generated.

[0537] Expression and purification of cyno, human, and hybrid PVRIG variants: All the PVRIG variants were expressed as ECD fusions with a C-terminal 6XHis tag in mammalian cells. The proteins were purified by affinity purification, ion-exchange chromatography, and size-exclusion chromatography. The purified proteins were buffer-exchanged into PBS buffer (pH 7.4) and stored at 4°C.

[0538] ELISA to determine PVRIG-antibody interaction: The functional ELISA was performed as follows: cyno, human, and cyno/human hybrid PVRIG (His-tagged) recombinant proteins were adsorbed on an IA plate overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound PVRIG variants were incubated with anti-human PVRIG mAbs (human IgG1 isotype) in solution (linear range of 0.1 µg/mL to 8 µg/mL in a 50 µL/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well of a HRP-conjugated secondary antibody was added (Human IgG Fc domain specific, Jackson ImmunoResearch). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H_2SO_4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer. The data were exported to Excel (Microsoft) and plotted in GraphPad Prism

(GraphPad Software, Inc.).

Results

[0539] S67, R95, and 197 residues as determinants of cyno cross-reactivity: The binding data shown in Figure 101 clearly shows that the S67, R95, and 197 residues affect the cyno cross-reactivity of various antibodies. While the P67S cyno-to-human mutation negatively impacts the binding of CPA.7.002 and CPA.7.041, the L95R/T97I cyno-to-human mutation significantly improves the binding of CPA.7.002, CPA.7.021, CPA.7.028, and CPA.7.041. On the other hand, H61R cyno-to-human mutation does not affect the binding of any of the antibodies tested.

[0540] Relative binding to cyno-to-human variants suggests three epitope groups: The relative binding of the antibodies to cyno, human and hybrid PVRIG variants suggests 3 distinct epitope groups: Group 1 binds to R95/I97 residues (CPA.7.021 and CPA.7.028). Group 2 binds to S67 and R95/I97 residues (CPA.7.002 and CPA.7.041). Group 3 does not bind to S67 or R95/I97 residues (CPA.7.024 and CPA.7.050). The epitope groups show strong correlation to the degree of cyno cross-reactivity of these antibodies (

Figure 102).

Summary and Conclusions

[0541] The restricted epitope mapping based on cyno-to-human variations in the PVRIG ECD identified S67, R95, and 197 residues as determinants of cyno cross-reactivity of anti-human PVRIG antibodies. The complete restoration of binding to L95R/T97I cyno PVRIG for CPA.7.021 and CPA.7.028 antibodies and improved binding of CPA.7.002 to this mutant strongly suggests that R95 and 197 residues are critical human PVRIG epitopes for these antibodies. These findings also suggest a possible way to predict cross-reactivity to non-human primate PVRIG orthologs based on their primary amino acid sequence.

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Patentkrav

1. Anti-PVRIG-antistof til anvendelse i behandlingen af kræft,
hvor antistoffet aktiverer T-celler og/eller NK-celler,
idet antistoffet specifikt binder til human PVRIG og konkurrerer med et antistof, som omfatter vhCDR1 som fremsat i SEQ ID NO:885, vhCDR2 som fremsat i SEQ ID NO:886, vhCDR3 som fremsat i SEQ ID NO:887, vlCDR1 som fremsat i SEQ ID NO:889, vlCOR2 som fremsat i SEQ ID NO:890 og vlCDR3 som fremsat i SEQ ID NO:891 til specifikt at binde til PVRIG-molekylet.

2. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 1, hvor anti-PVRIG-antistoffet anvendes i kombination med antagonistiske antistoffer, som er målrettet mod yderligere immune kontrolpunkter.

3. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 2, hvor de antagonistiske antistoffer, som er målrettet mod yderligere immune kontrolpunkter, inkluderer anti-CTLA-4-antistoffer, anti-PD-1-antistoffer, anti-PD-L1-antistoffer, anti-LAG-3-antistoffer, anti-TIM-3-antistoffer, anti-BTLA-antistoffer, anti-B7-H4-antistoffer, anti-B7-H3-antistoffer og anti-VISTA-antistoffer.

4. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 3, hvor anti-CTLA4-antistoffer inkluderer ipilimumab og tremelimumab.

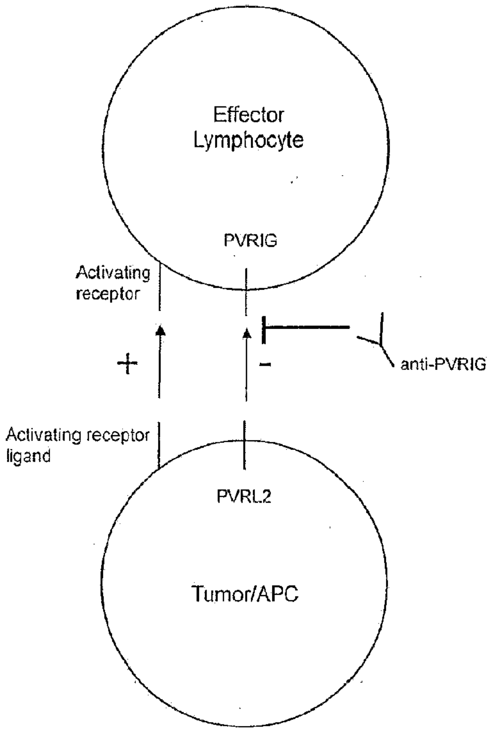
5. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 3, hvor anti-PD-1-antistoffer inkluderer nivolumab, pidilizumab og pembrolizumab.

6. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 3, hvor anti-PDL-1-antistoffer inkluderer atezolizumab og durvalumab.

7. Anti-PVRIG-antistoffet til anvendelse i behandlingen af kræft ifølge krav 1, hvor anti-PVRIG-antistoffet anvendes i kombination med et anti-TIGIT-antistof.

DRAWINGS

Figure 1



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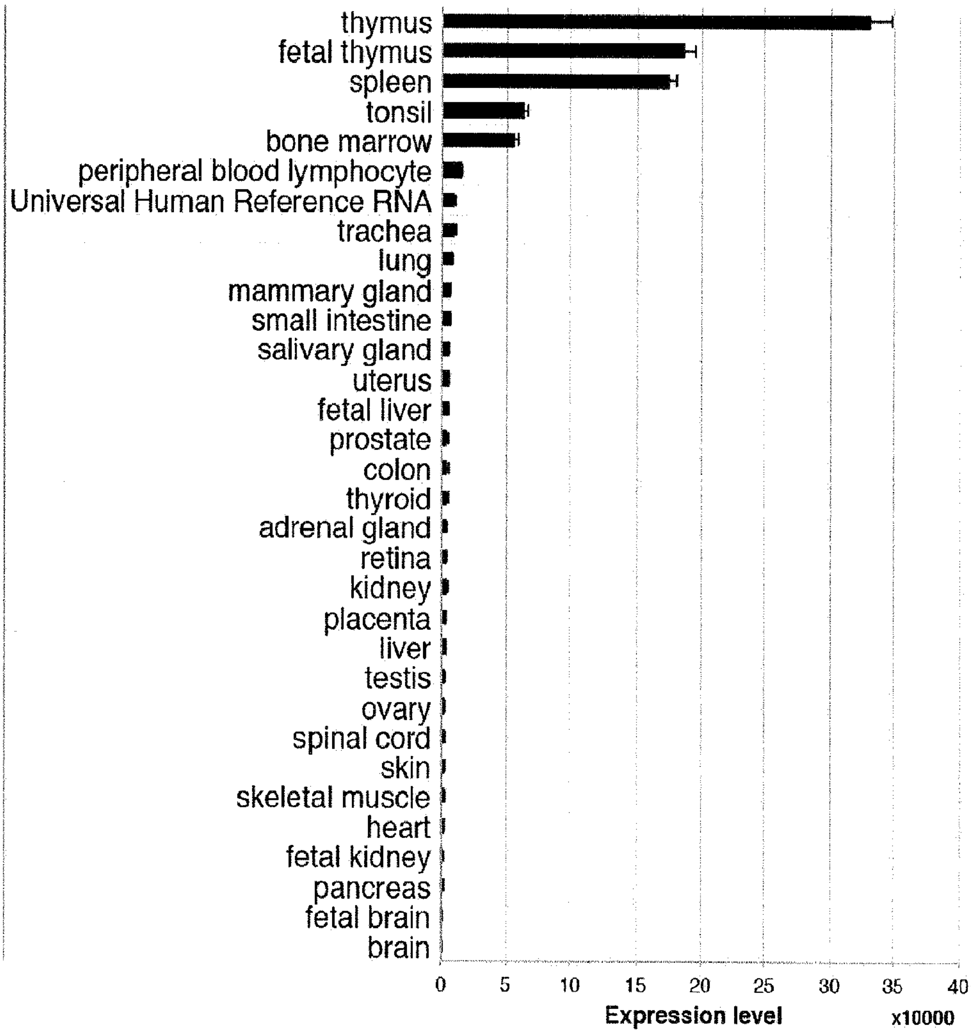
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spleen
tonsil
bone marrow
peripheral blood lymphocyte
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trachea
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mammary gland
small intestine
salivary gland
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fetal liver
prostate
colon
thyroid
adrenal gland
retina
kidney
placenta
liver
testis
ovary
spinal cord
skin
skeletal muscle
heart
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Expression level
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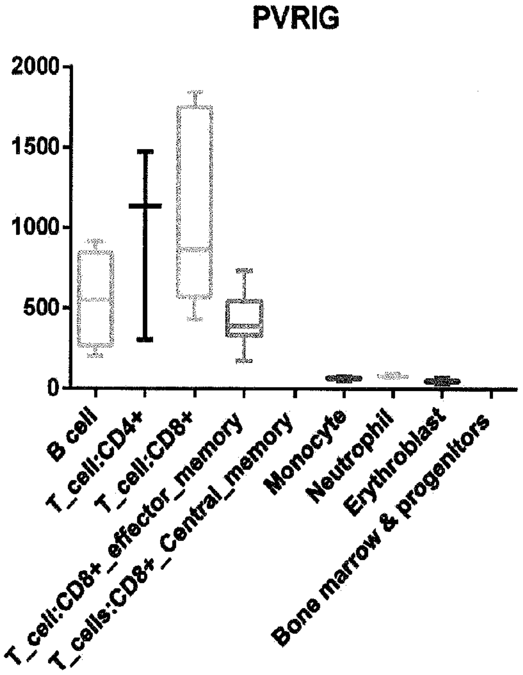
Figure 3: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1000
500
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T_cell:CD8+
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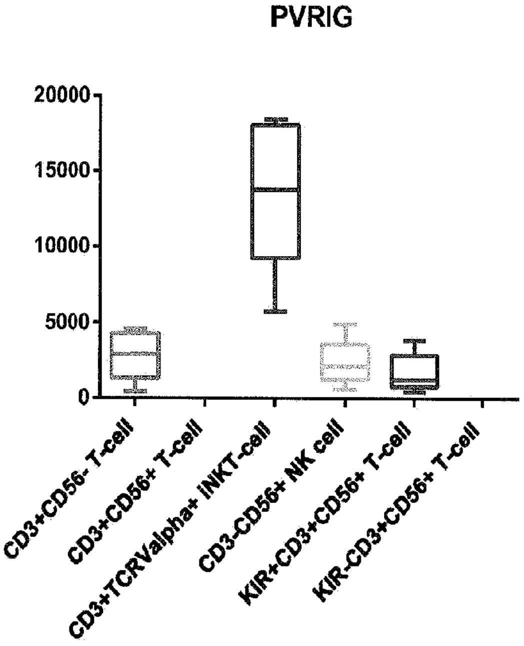
Monocyte
Neutrophil
Erythroblast
Bone marrow & progenitors

Figure 4


PVRIG
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10000
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CD3+CD56+ T-cell
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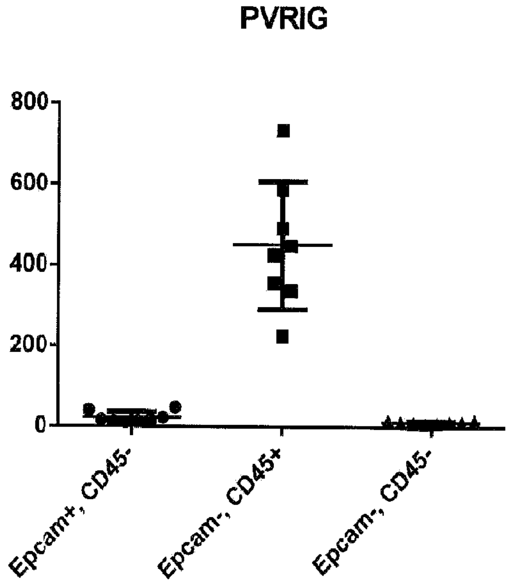

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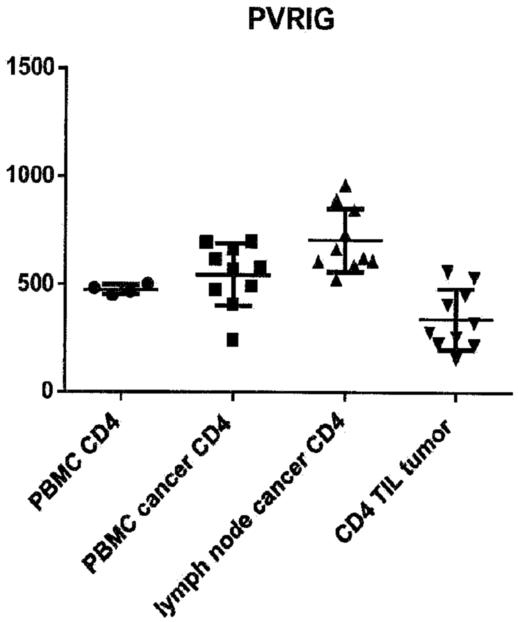
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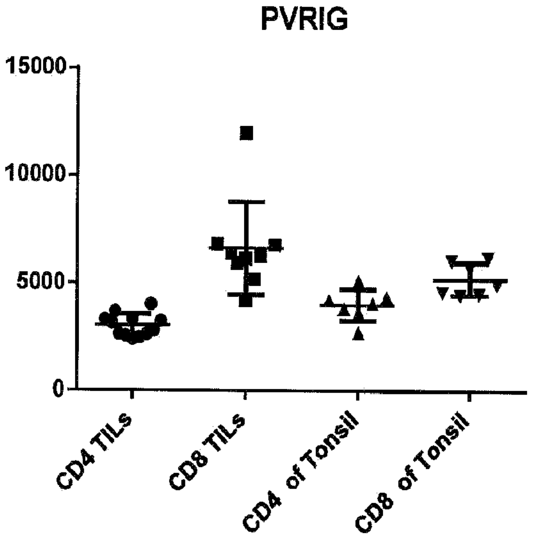
PBMC CD4
PBMC cancer CD4
lymph node cancer CD4
CD4 TIL tumor

Figure 5c: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5000
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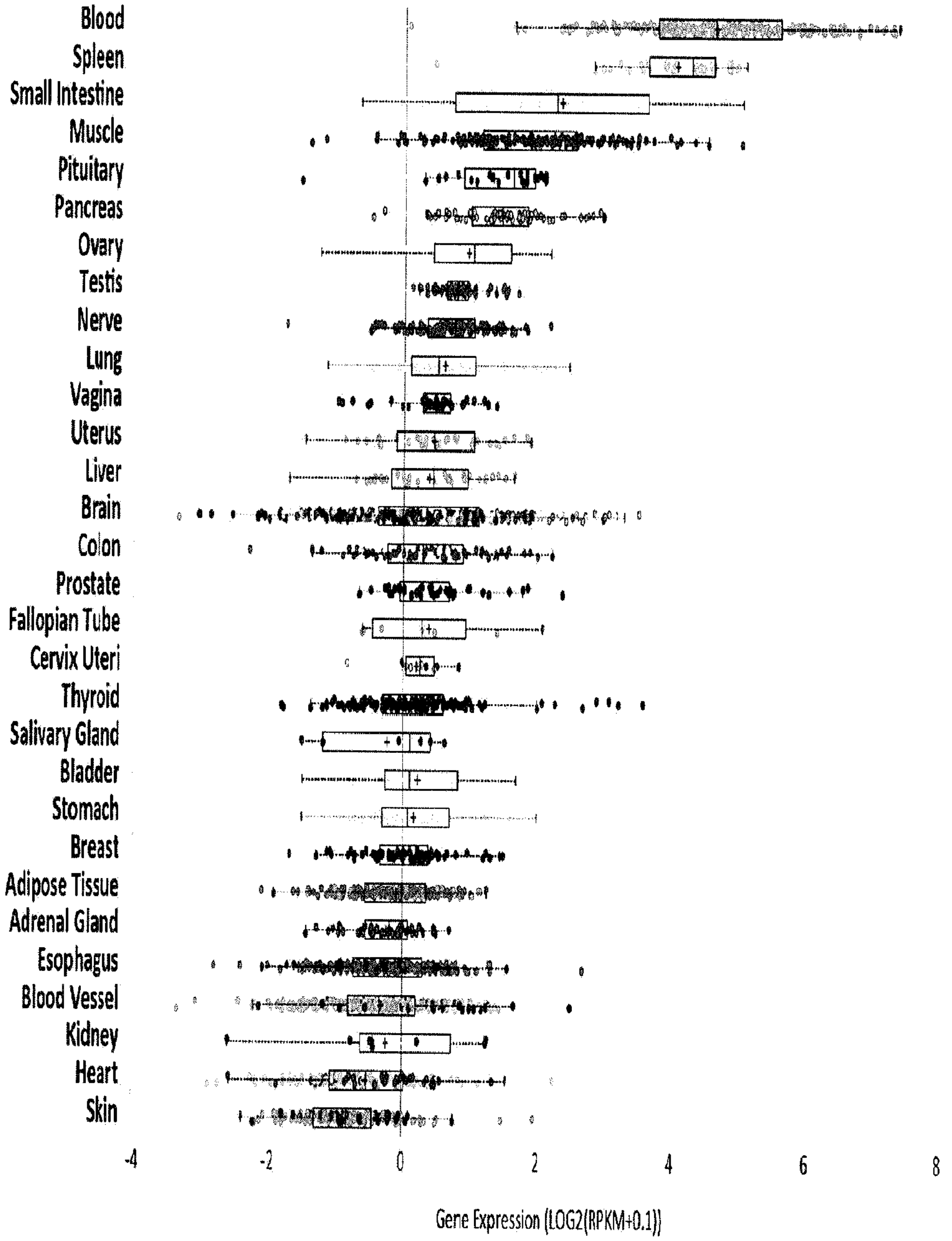
CD8 TILs
CD4 of Tonsil
CD8 of Tonsil

Figure 6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Pancreas
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Testis
Nerve
Lung
Vagina
Uterus
Liver
Brain
Colon
Prostate
Fallopian Tube
Cervix Uteri
Thyroid
Salivary Gland
Bladder
Stomach
Breast
Adipose Tissue
Adrenal Gland
Esophagus
Blood Vessel
Kidney
Heart
Skin
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-2
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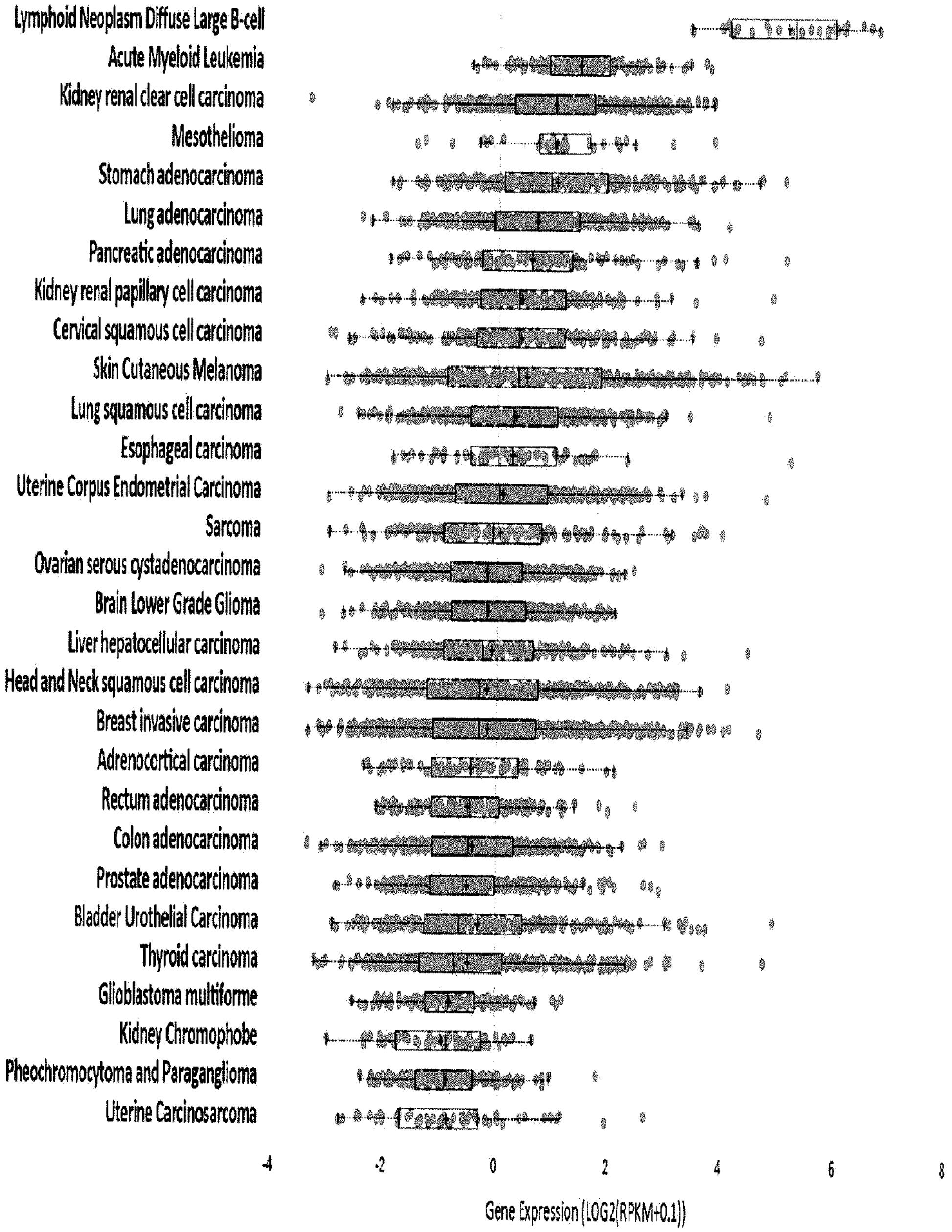
2
4
6
8
Gene Expression (LOG2(RPKM+0.1))

Figure 7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Acute Myeloid Leukemia
Kidney renal clear cell carcinoma
Mesothelioma
Stomach adenocarcinoma
Lung adenocarcinoma
Pancreatic adenocarcinoma
Kidney renal papillary cell carcinoma
Cervical squamous cell carcinoma
Skin Cutaneous Melanoma
Lung squamous cell carcinoma
Esophageal carcinoma
Uterine Corpus Endometrial Carcinoma
Sarcoma
Ovarian serous cystadenocarcinoma
Brain Lower Grade Glioma
Liver hepatocellular carcinoma
Head and Neck squamous cell carcinoma
Breast invasive carcinoma
Adrenocortical carcinoma
Rectum adenocarcinoma
Colon adenocarcinoma
Prostate adenocarcinoma
Bladder Urothelial Carcinoma
Thyroid carcinoma
Glioblastoma multiforme
Kidney Chromophobe
Pheochromocytoma and Paraganglioma
Uterine Carcinosarcoma
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-2
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2
4
6
8
Gene Expression (LOG2(RPKM+0.1))

Figure 8

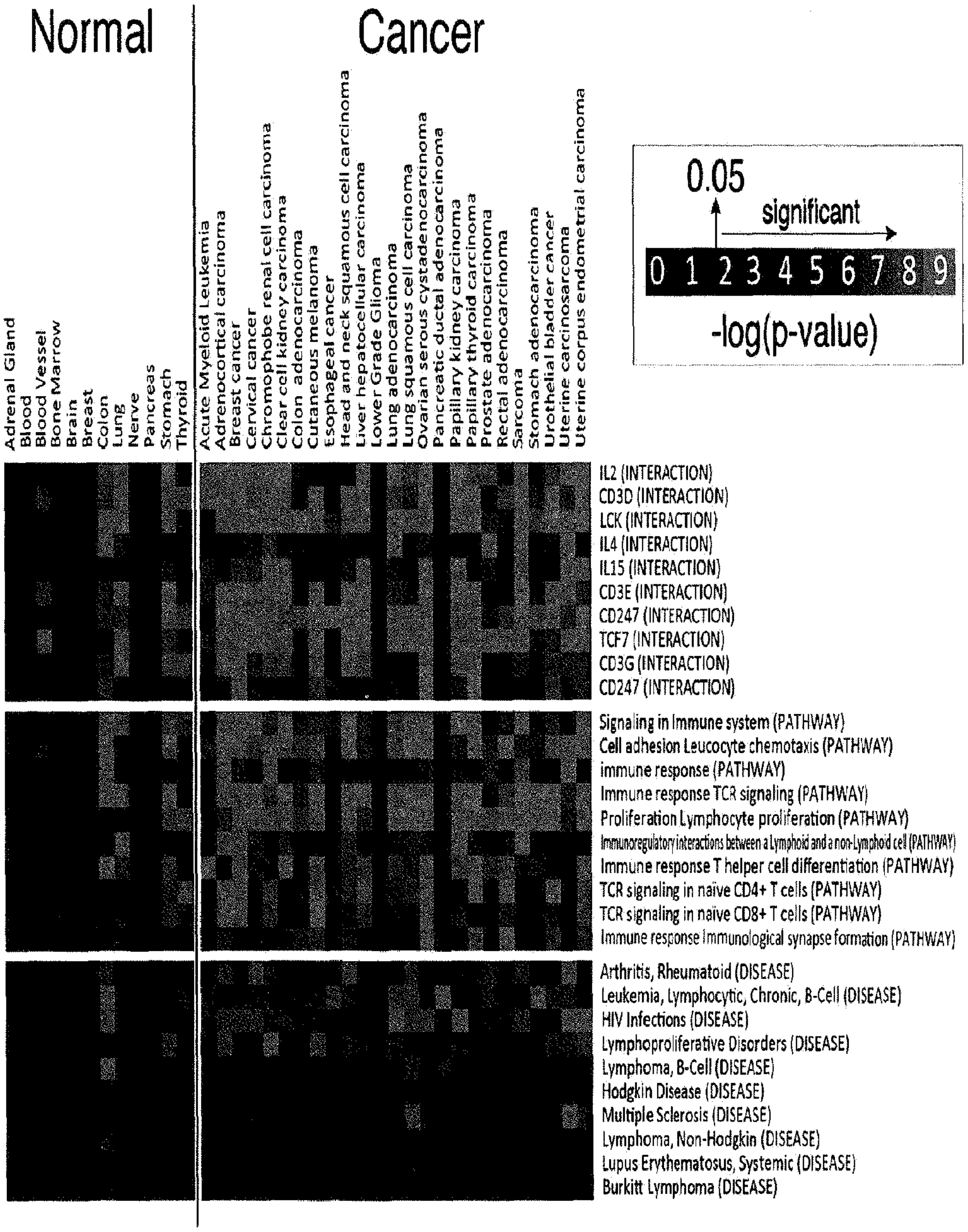

Normal
Cancer
Adrenal Gland
Blood
Blood Vessel
Bone Marrow
Brain
Breast
Colon
Lung
Nerve
Pancreas
Stomach
Thyroid
Acute Myeloid Leukemia
Adrenocortical carcinoma
Breast cancer
Cervical cancer
Chromophobe renal cell carcinoma
Clear cell kidney carcinoma
Colon adenocarcinoma
Cutaneous melanoma
Esophageal cancer
Head and neck squamous cell carcinoma
Liver hepatocellular carcinoma
Lower Grade Glioma
Lung adenocarcinoma
Lung squamous cell carcinoma
Ovarian serous cystadenocarcinoma
Pancreatic ductal adenocarcinoma
Papillary kidney carcinoma
Papillary thyroid carcinoma
Prostate adenocarcinoma
Rectal adenocarcinoma
Sarcoma
Stomach adenocarcinoma
Urothelial bladder cancer
Uterine carcinosarcoma
Uterine corpus endometrial carcinoma
0.05
significant
0 1 2 3 4 5 6 7 8 9
-log(p-value)
IL2 (INTERACTION)
CD3D (INTERACTION)
LCK (INTERACTION)
IL4 (INTERACTION)
IL15 (INTERACTION)
CD3E (INTERACTION)
CD247 (INTERACTION)
TCF7 (INTERACTION)
CD3G (INTERACTION)
CD247 (INTERACTION)
Signaling in Immune system (PATHWAY)
Cell adhesion Leucocyte chemotaxis (PATHWAY)
immune response (PATHWAY)
Immune response TCR signaling (PATHWAY)
Proliferation Lymphocyte proliferation (PATHWAY)
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell (PATHWAY)
Immune response T helper cell differentiation (PATHWAY)
TCR signaling in naive CD4+ T cells (PATHWAY)
TCR signaling in naive CD8+ T cells (PATHWAY)
Immune response Immunological synapse formation (PATHWAY)
Arthritis, Rheumatoid (DISEASE)
Leukemia, Lymphocytic, Chronic, B-Cell (DISEASE)
HIV Infections (DISEASE)
Lymphoproliferative Disorders (DISEASE)
Lymphoma, B-Cell (DISEASE)
Hodgkin Disease (DISEASE)
Multiple Sclerosis (DISEASE)
Lymphoma, Non-Hodgkin (DISEASE)
Lupus Erythematosus, Systemic (DISEASE)
Burkitt Lymphoma (DISEASE)

Figure 9

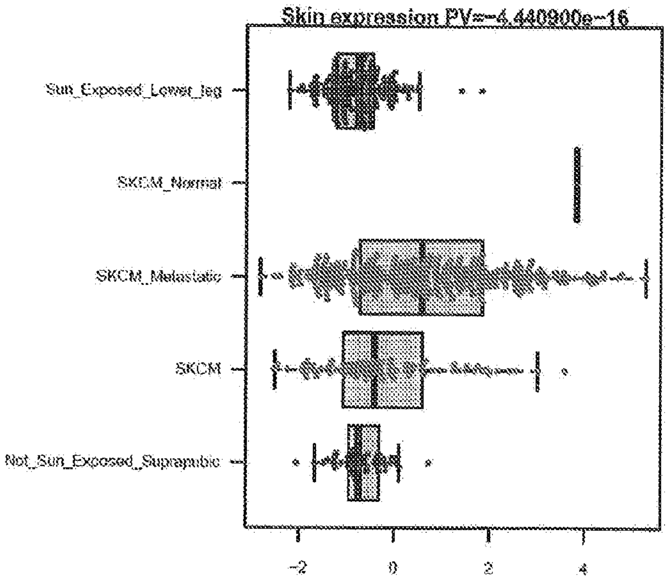


Figure 10

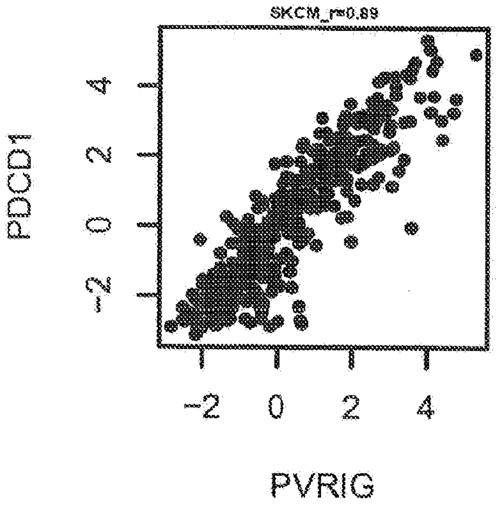


Figure 11

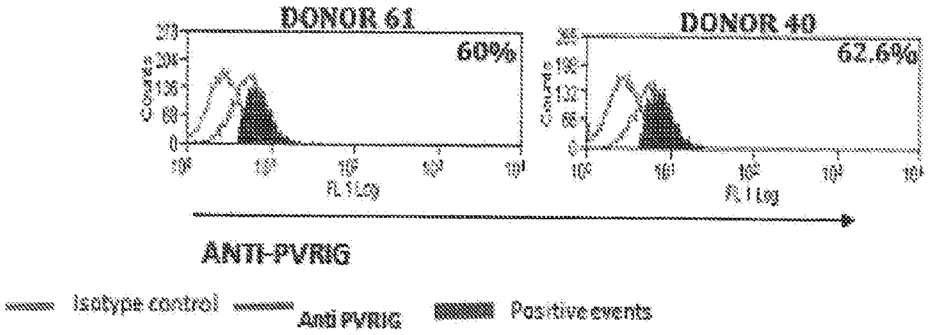

DONOR 61
60%
Counts
FL1 Log
DONOR 40
62.6%
Counts
FL1 Log
ANTI-PVRIG
Isotype control
Anti PVRIG
Positive events

Figure 12

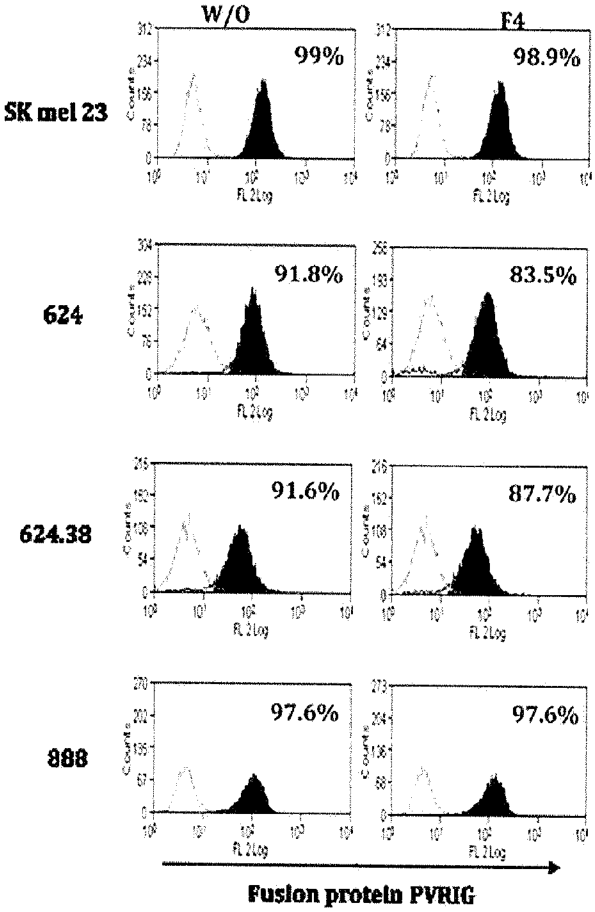

W/O
F4
SK mel 23
99%
98.9%
624
91.8%
83.5%
624.38
91.6%
87.7%
888
97.6%
97.6%
Counts
FL 2 Log
Fusion protein PVRIG

Figure 13

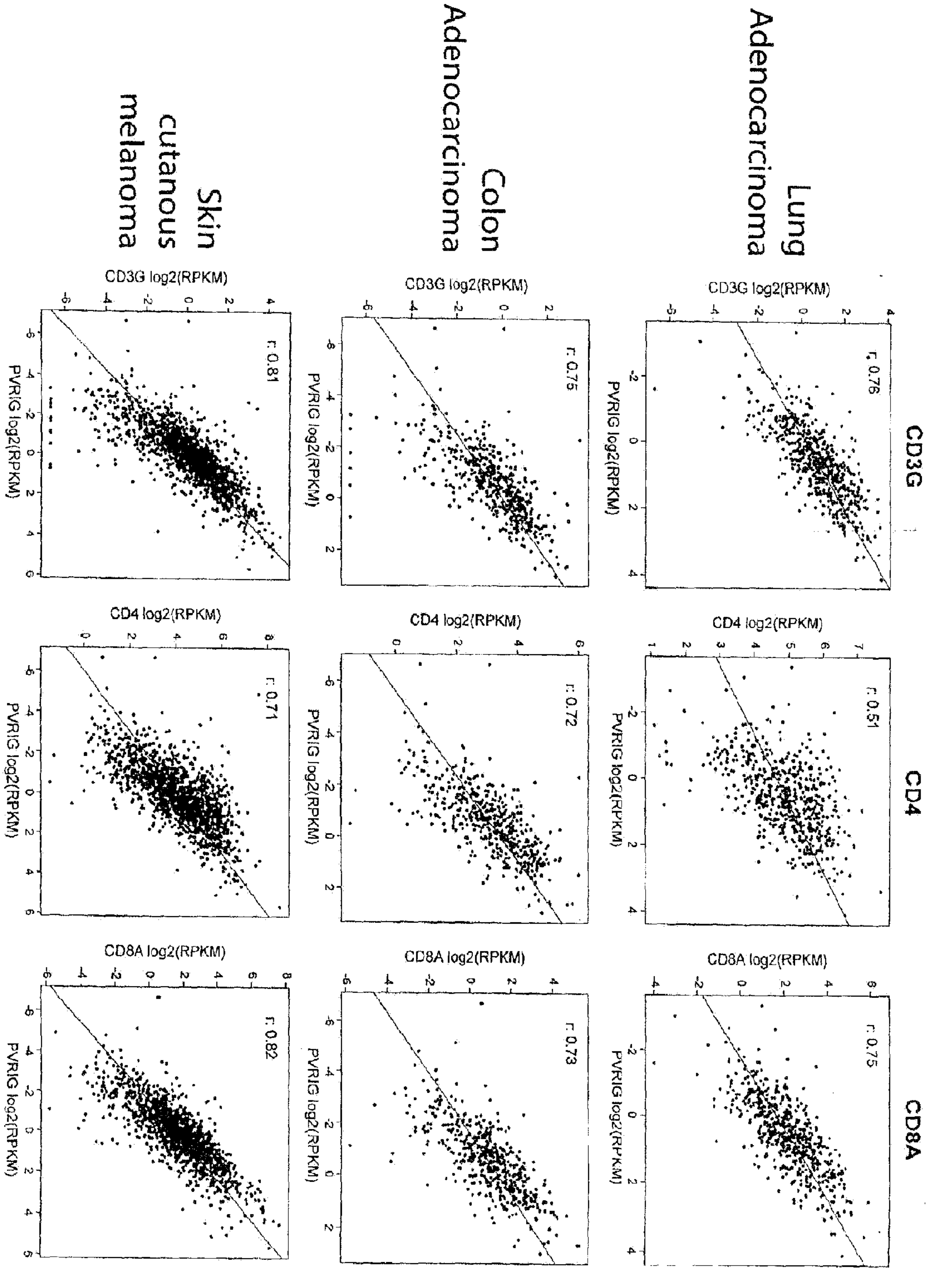
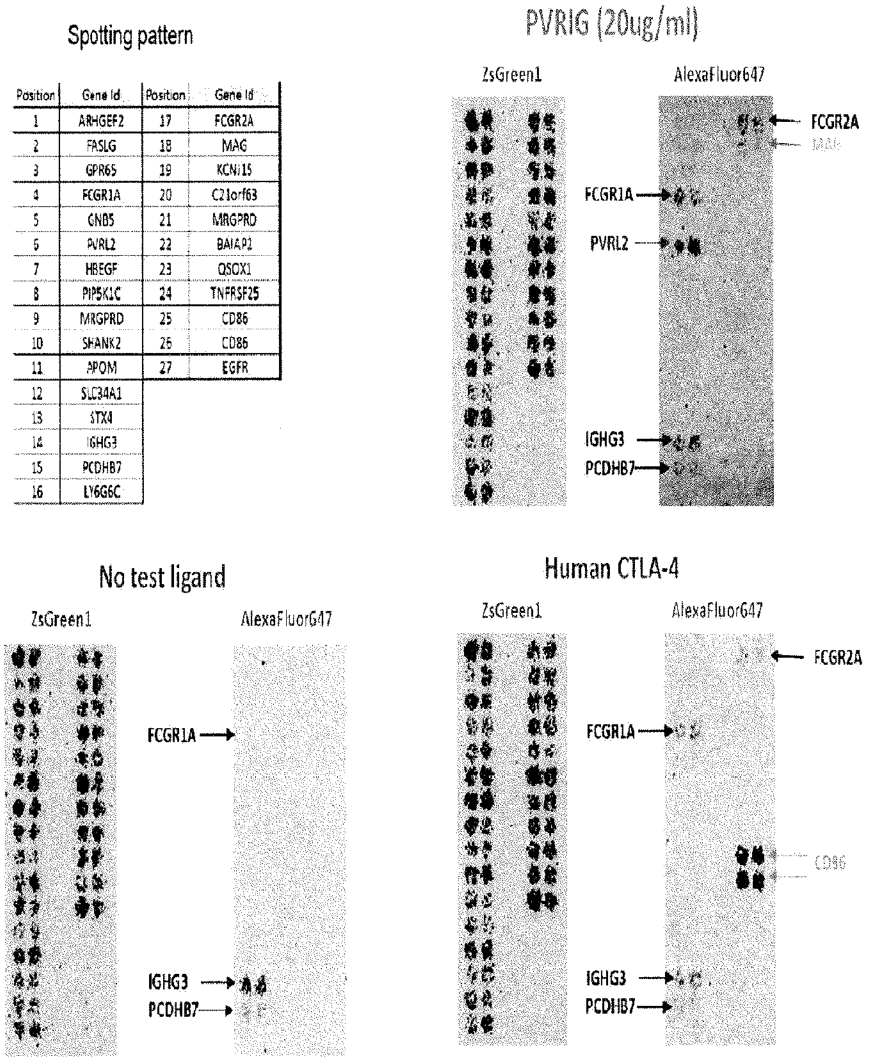

Lung Adenocarcinoma
Colon Adenocarcinoma
Skin cutanous melanoma
CD3G
CD4
CD8A
CD3G log2(RPKM)
CD4 log2(RPKM)
CD8A log2(RPKM)
PVRIG log2(RPKM)
r: 0.76
r: 0.51
r: 0.75
r: 0.75
r: 0.72
r: 0.73
r: 0.81
r: 0.71
r: 0.82

Figure 14:



Spotting pattern

Position	Gene Id	Position	Gene Id
1	ARHGEF2	17	FCGR2A
2	FASLG	18	MAG
3	GPR65	19	KCNJ15
4	FCGR1A	20	C21orf63
5	GNB5	21	MRGPRD
6	PVRL2	22	BAIAP2
7	HBEGF	23	QSOX1
8	PIP5K1C	24	TNFRSF25
9	MRGPRD	25	CD86
10	SHANK2	26	CD86
11	APOM	27	EGFR
12	SLC34A1		
13	STX4		
14	IGHG3		
15	PCDHB7		
16	LY6G6C		

PVRIG (20ug/ml)

No test ligand

Human CTLA-4

FIG. 15A

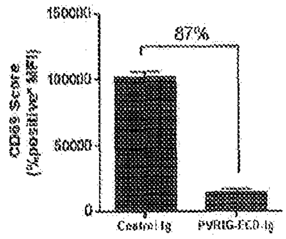


FIG. 15B

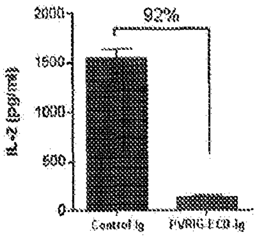


FIG. 15C

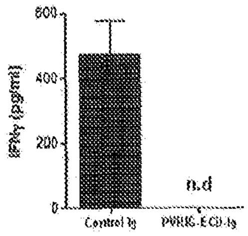


FIG. 15D

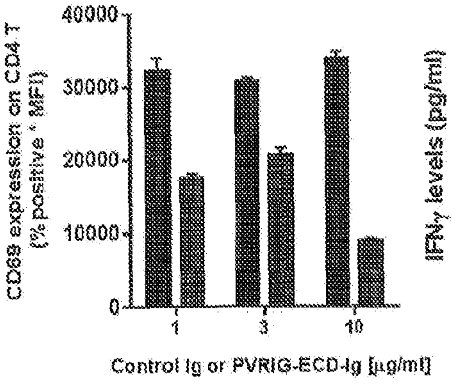


FIG. 15E

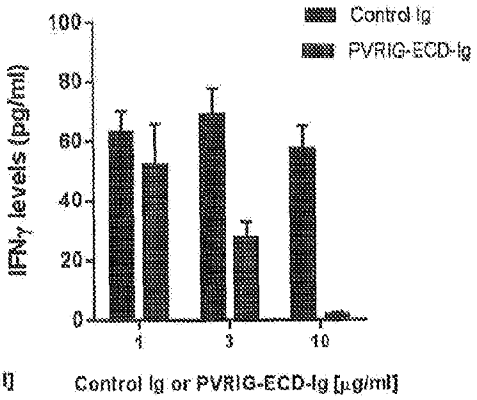


Figure 16

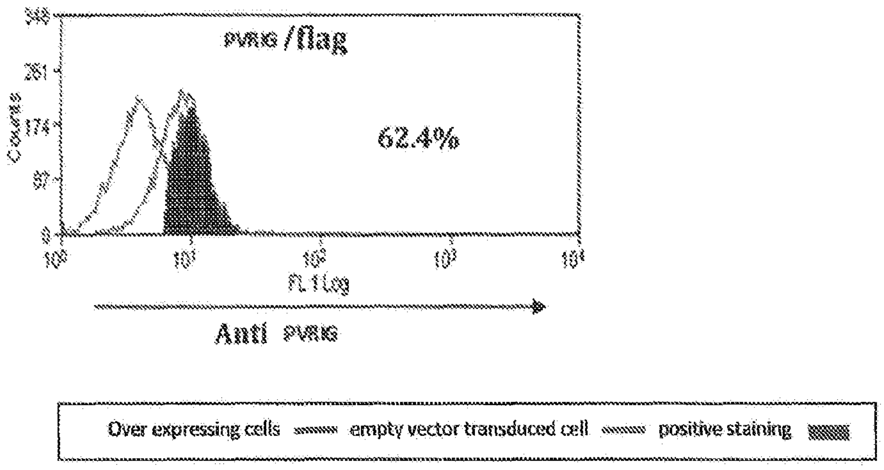


Figure 17

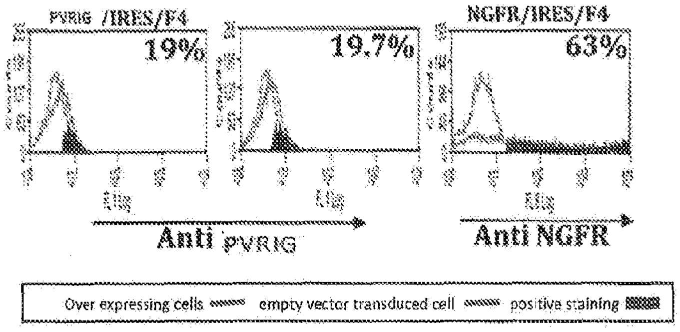


Figure 18A

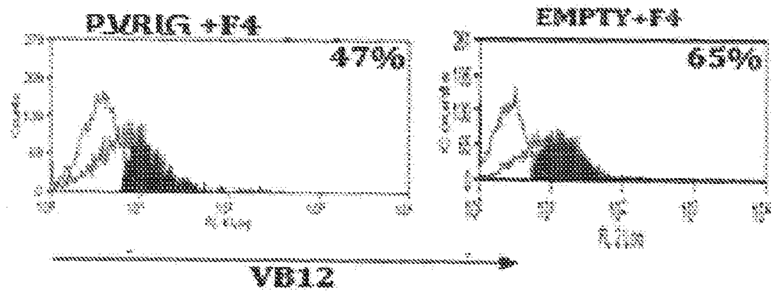


Figure 18B

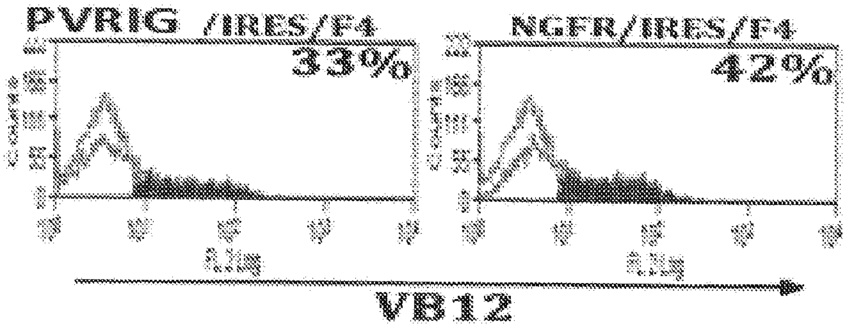


Figure 19

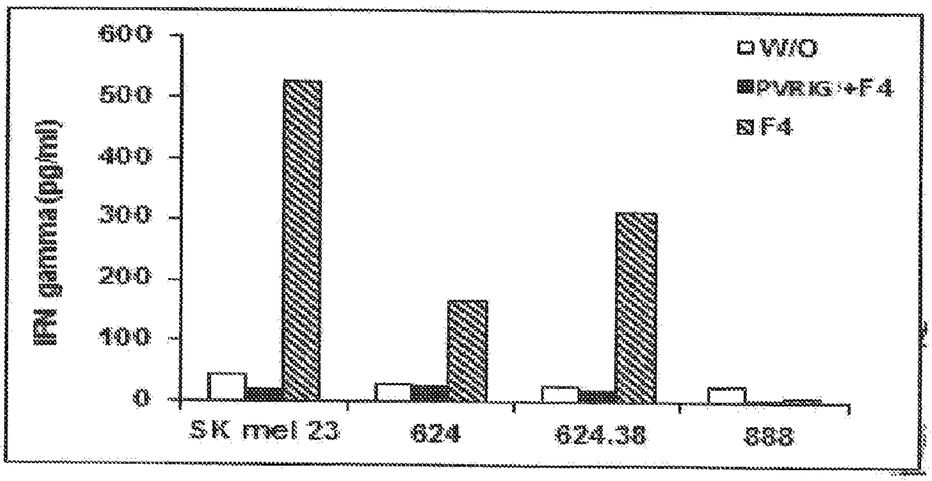


Figure 20A

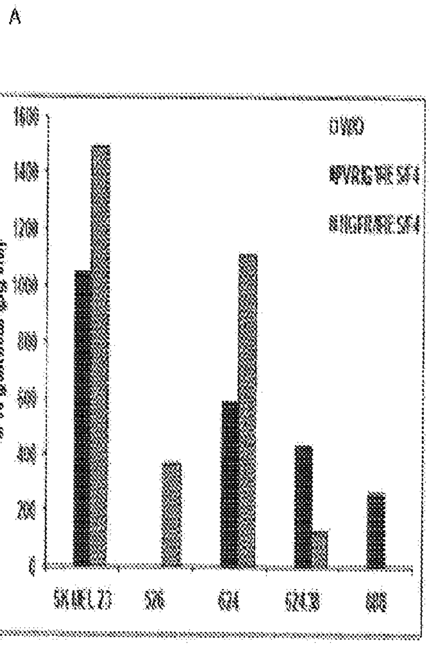


Figure 20B

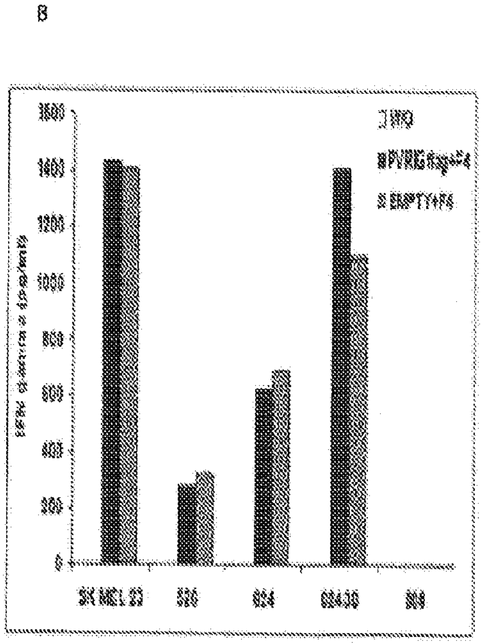


Figure 21

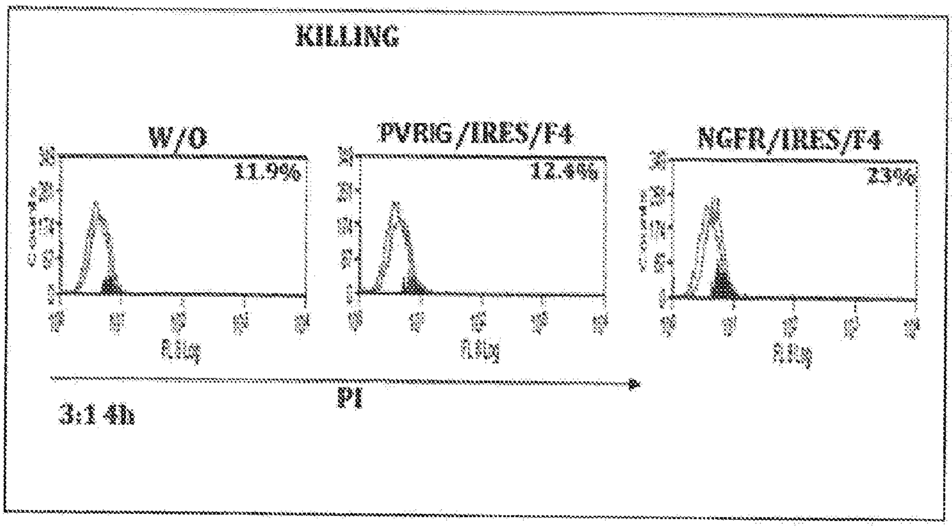
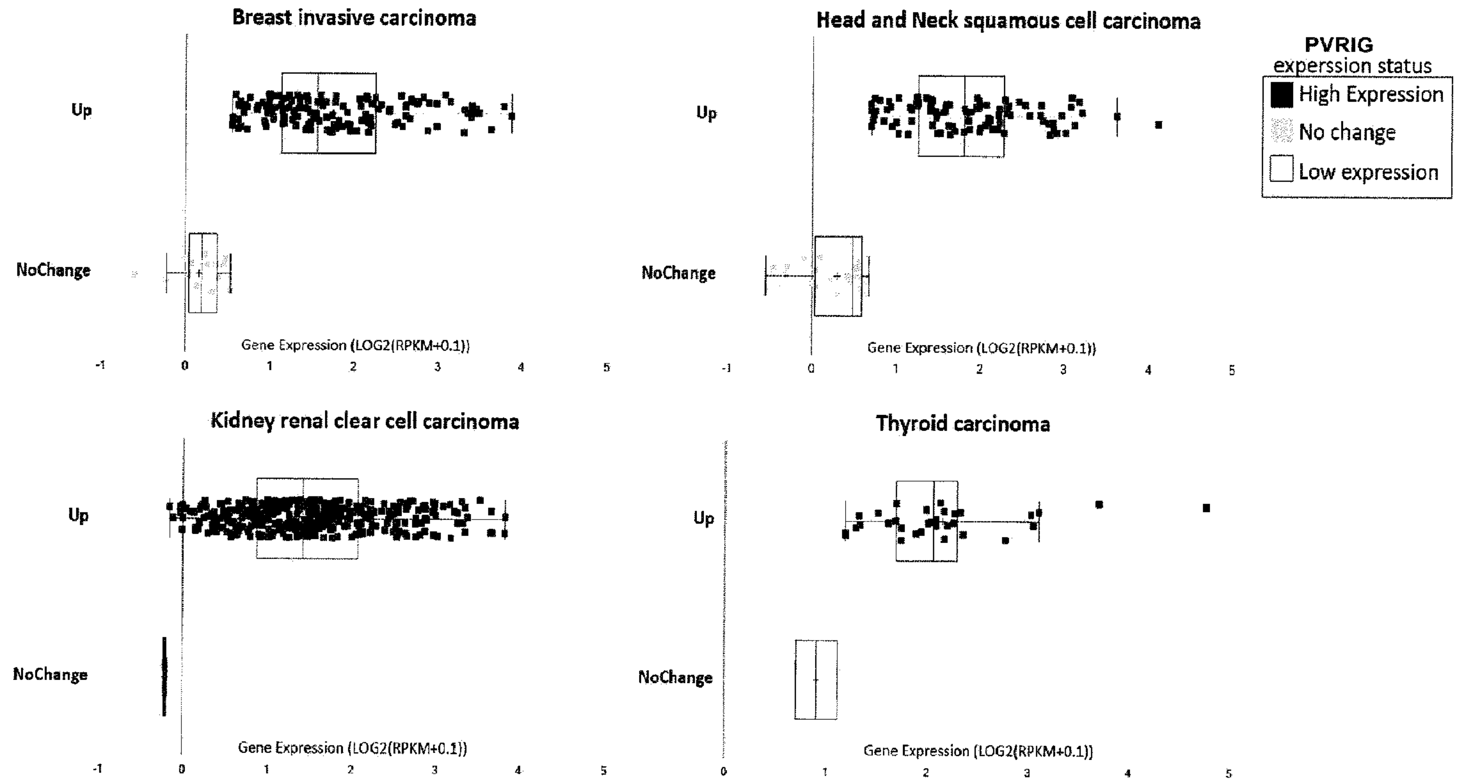


Figure 22


Breast invasive carcinoma
Head and Neck squamous cell carcinoma
Kidney renal clear cell carcinoma
Thyroid carcinoma
Up
NoChange
Gene Expression (LOG2(RPKM+0.1))
-1
0
1
2
3
4
5
PVRIG
experssion status
■ High Expression
▨ No change
☐ Low expression

Fiure 23A

Figure 23B

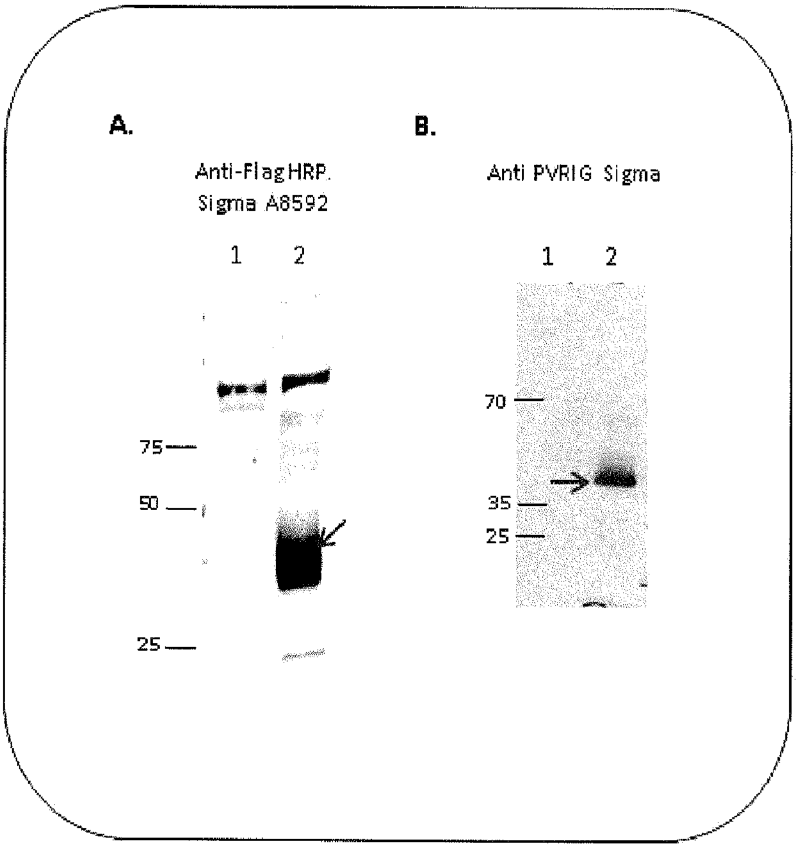


Figure 24

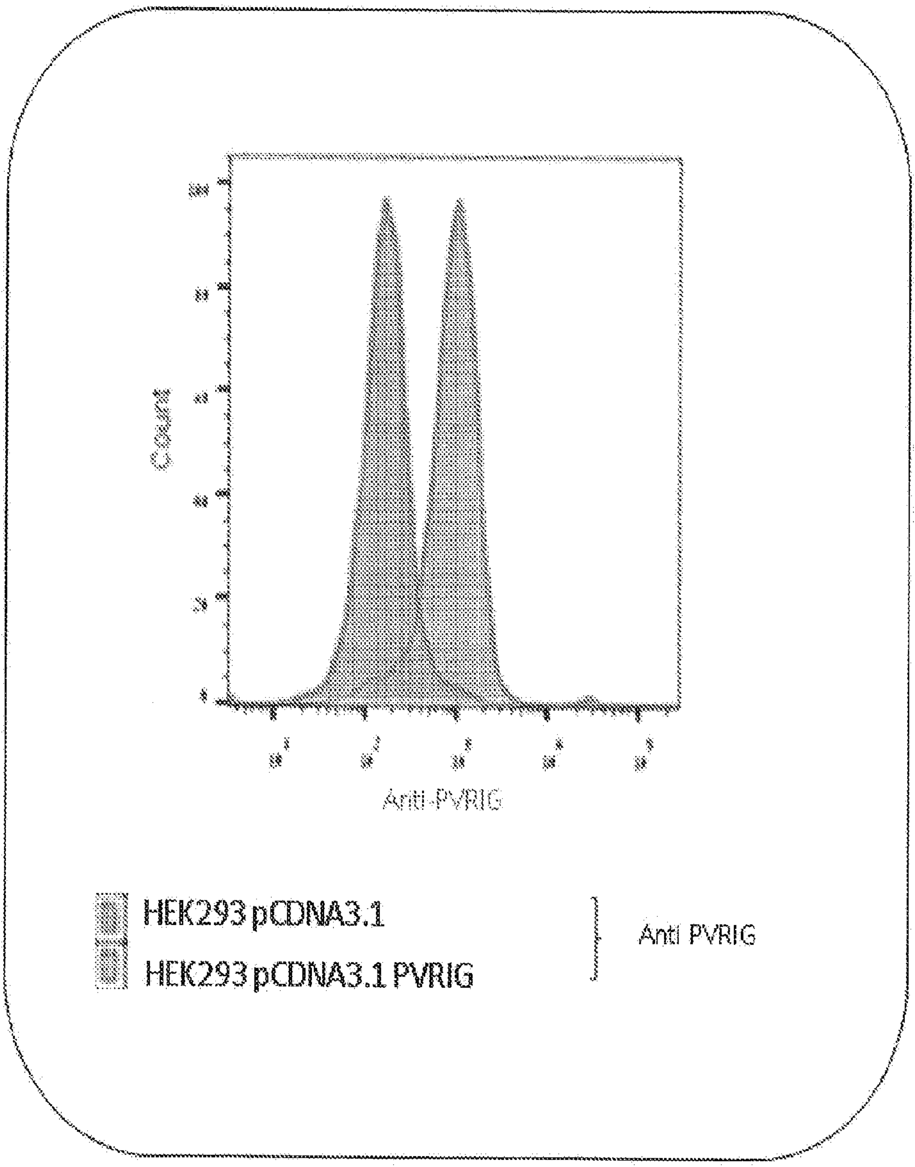

Count
Anti-PVRIG
HEK293 pCDNA3.1
HEK293 pCDNA3.1 PVRIG
Anti PVRIG

Figure 25

Human PVRIG WT Full length

Human PVRIG sequence starting from position 21 - alternative methionine

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR (SEQ ID NO: 8)

Human PVRIG sequence starting from position 1 methionine

MRTEAQVPALQPPEPGLEGAMGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR (SEQ ID NO: 9)

Alternative signal P with three C to S Mutations at the Fc domain
CGEN-PVRIGHH-2 Alternative SP (MGWSCIILFLVATATGVHS (SEQ ID NO: 10)) + CGEN-PVRIG (41-171 of PVRIG_HUMAN) + Human IgG1 Fc mutated at C220S, C226S, C229S of hinge

MGWSCIILFLVATATGVHSTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 11)

Figure 26

Human PVLR2 alpha isoform

>gi|5360210|ref|NP_002847.1| nectin-2 isoform alpha precursor [Homo sapiens]

MARAAALLPSRSPPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVELPCHLLPPVPGLYISLVTW

QRPDAPANHQNVAAFHPKMGPSFPSPKPGSERLSFVSAKQSTGQDTEAELQDATLALHGLTVEDEGNYTC

EFATFPKGSVRGMTWLRVIAKPKNQAEAQKVTFSQDPTTVALCISKEGRPPARISWLSSLDWEAKETQVS

GTLAGTVTVTSRFTLVPSGRADGVTVTCKVEHESFEEPALIPVTLSVRYPPEVSISGYDDNWYLGRTDAT

LSCDVRSNPEPTGYDWSTTSGTFPTSAVAQGSQLVIHAVDSLFNTTFVCTVTNAVGMGRAEQVIFVRETP

RASPRDVGPLVWGAVGGTLLVLLLLAGGSLAFILLRVRRRRKSPGGAGGGASGDGGFYDPKAQVLGNGDP

VFWTPVVPGPMEPDGKDEEEEEEEEKAEKGLMLPPPPALEDDMESQLDGSLISRRAVYV (SEQ ID NO: 12)

Human PVLR2 delta isoform
>gi|112789532|ref|NP_001036189.1| nectin-2 isoform delta precursor [Homo sapiens]
MARAAALLPSRSPPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVELPCHLLPPVPGLYISLVTW
QRPDAPANHQNVAAFHPKMGPSFPSPKPGSERLSFVSAKQSTGQDTEAELQDATLALHGLTVEDEGNYTC
EFATFPKGSVRGMTWLRVIAKPKNQAEAQKVTFSQDPTTVALCISKEGRPPARISWLSSLDWEAKETQVS
GTLAGTVTVTSRFTLVPSGRADGVTVTCKVEHESFEEPALIPVTLSVRYPPEVSISGYDDNWYLGRTDAT
LSCDVRSNPEPTGYDWSTTSGTFPTSAVAQGSQLVIHAVDSLFNTTFVCTVTNAVGMGRAEQVIFVRETP
NTAGAGATGGIIGGIIAAIIATAVAATGILICRQQRKEQTLQGAEEDEDLEGPPSYKPPTPKAKLEAQEM
PSQLFTLGASEHSPLKTPYFDAGASCTEQEMPRYHELPTLEERSGPLHPGATSLGSPIPVPPGPPAVEDV
SLDLEDEEGEEEEEYLDKINPIYDALSY (SEQ ID NO: 13)

Figure 27

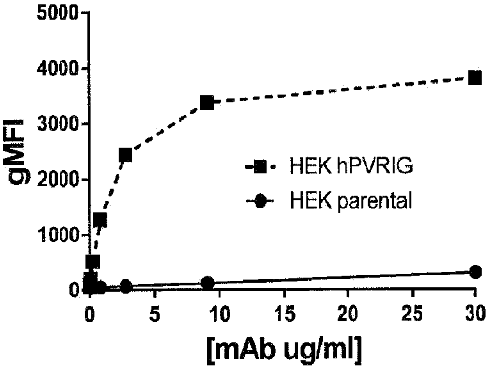


Figure 28

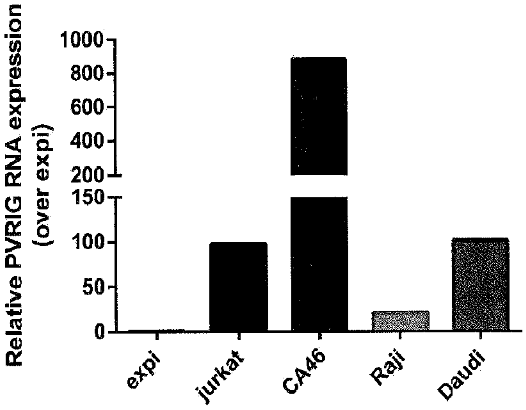


Figure 29

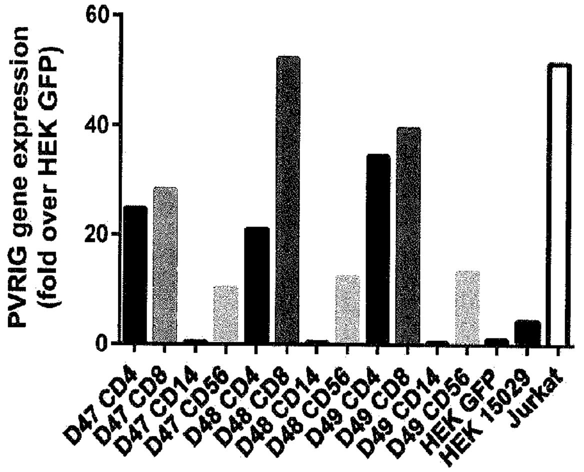

PVRIG gene expression
(fold over HEK GFP)
60
40
20
0
D47 CD4
D47 CD8
D47 CD14
D47 CD56
D48 CD4
D48 CD8
D48 CD14
D48 CD56
D49 CD4
D49 CD8
D49 CD14
D49 CD56
HEK GFP
HEK 15029
Jurkat

Figure 30A

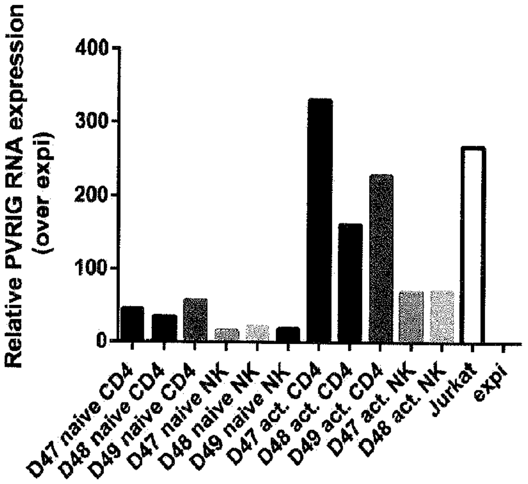


Figure 30B

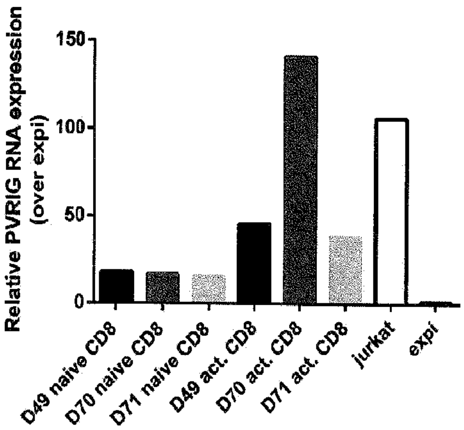


Figure 31A

Antibody (hIgG1)	EC_{50} (HEK OE, nM)	HEK OE/par (5ug/ml, gMFIr)	Jurkat (5ug/ml, gMFIr)	CA46 (5ug/ml, gMFIr)
CPA.7.002	53.47	11.97	1.25	1.16
CPA.7.003	7.12	1.28	3.85	6.71
CPA.7.004	43.74	25.18	1.03	1.16
CPA.7.005	125.50	15.77	0.93	1.02
CPA.7.007	0.99	2.90	4.42	9.82
CPA.7.008	Not tested	Not tested	Not tested	Not tested
CPA.7.009	67.30	8.35	0.93	0.99
CPA.7.010	30.00	18.76	1.08	1.01
CPA.7.011	128.80	10.86	0.93	0.96
CPA.7.012	0.40	2.01	4.89	8.23
CPA.7.014	19.66	5.90	1.14	0.92
CPA.7.015	1.74	6.64	4.3	7.33
CPA.7.016	28.38	2.20	1.11	1.04
CPA.7.017	2.62	4.56	0.99	1.49
CPA.7.018	20.38	2.06	1.05	0.96
CPA.7.019	2.11	1.94	4.66	9.23
CPA.7.020	108.30	1.37	1.64	1.45
CPA.7.021	12.41	30.59	4.47	7.96
CPA.7.022	7.73	4.60	1.01	1.24
CPA.7.023	10.90	20.31	0.9	1.01
CPA.7.024	22.91	9.92	1.14	1.25
CPA.7.025	No binding	No binding	Not tested	Not tested
CPA.7.026	8.169	1.16	1.00	0.99
CPA.7.027	No binding	No binding	Not tested	Not tested
CPA.7.028	21.5	2.34	9.57	3.16
CPA.7.029	3.87	1.24	1.17	0.85
CPA.7.031	No binding	No binding	Not tested	Not tested
CPA.7.032	49.62	4.50	1.81	1.64
CPA.7.033	221	1.8221999	1.94	1.17
CPA.7.034	55.69	4.12	1.25	1.01
CPA.7.035	No binding	No binding	Not tested	Not tested
CPA.7.036	14.19	5.68	1.69	1.46
CPA.7.037	Not reliable fit	4.56	1.78	1.28

Figure 31B

Antibody (hIgG1)	EC_{50} (HEK OE, nM)	HEK OE/par (5ug/ml, gMFIr)	Jurkat (5ug/ml, gMFIr)	CA46 (5ug/ml, gMFIr)
CPA.7.038	2436	4.36	Not tested	Not tested
CPA.7.039	2.99	6.52	1.55	1.40
CPA.7.040	0.84	7.91	1.78	1.59
CPA.7.041	14.35	3.25	Not tested	Not tested
CPA.7.042	5.42	4.20	1.57	1.28
CPA.7.043	7.63	5.47	1.20	1.08
CPA.7.044	43.64	3.24	1.03	0.97
CPA.7.045	5.17	4.04	1.68	1.05
CPA.7.046	Not reliable fit	Not tested	Not tested	Not tested
CPA.7.047	4.61	5.89	0.81	0.92
CPA.7.049	1.94	2.56	Not tested	Not tested
CPA.7.050	121.5	4.29	2.72	2.02

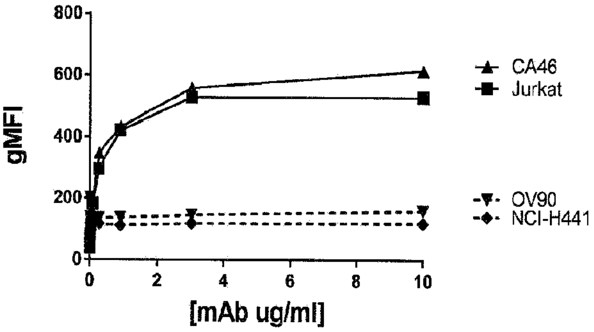
Figure 32A

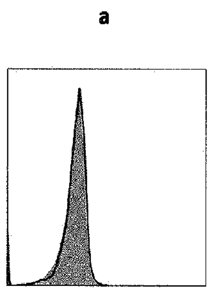
Antibody (hIgG1)	Human CD56 int. NK (gMFIr, 5ug/ml)	Human CD8+ T cells (gMFIr, 5ug/ml)	Expi cyno OE/par (gMFIr, 5ug/ml)	Cyno NK cells (gMFIr, 5ug/ml)	Cyno CD3+ T cells (gMFIr, 5ug/ml)
CPA.7.002	3.21	1.01	1.16	2.24	1.01
CPA.7.003	16.21	1.41	1.36	Not tested	Not tested
CPA.7.004	1.30	0.98	1.1	Not tested	Not tested
CPA.7.005	2.35	1.07	1.21	1.12	0.99
CPA.7.007	5.31	1.32	1.11	Not tested	Not tested
CPA.7.008	Not tested	Not tested	Not tested	Not tested	Not tested
CPA.7.009	1.34	0.98	1.08	Not tested	Not tested
CPA.7.010	2.09	1.06	1.41	Not tested	Not tested
CPA.7.011	1.19	1.02	1.06	Not tested	Not tested
CPA.7.012	1.62	1.18	1.98	Not tested	Not tested
CPA.7.014	1.57	0.98	1.06	Not tested	Not tested
CPA.7.015	2.82	1.14	1.11	Not tested	Not tested
CPA.7.016	2.39	1.05	1.43	Not tested	Not tested
CPA.7.017	1.73	0.86	1.30	Not tested	Not tested
CPA.7.018	1.62	1.04	1.06	Not tested	Not tested
CPA.7.019	4.23	1.36	1.23	Not tested	Not tested
CPA.7.020	3.12	1.07	3.10	Not tested	Not tested
CPA.7.021	3.74	1.23	1.08	1.91	1.01
CPA.7.022	1.27	0.87	Not tested	Not tested	Not tested
CPA.7.023	1.11	0.99	1.08	Not tested	Not tested
CPA.7.024	1.20	1.02	5.67	0.30	1.06
CPA.7.025	Not tested	Not tested	1.41	Not tested	Not tested
CPA.7.026	1.72	0.92	1.31	Not tested	Not tested
CPA.7.027	Not tested	Not tested	1.45	Not tested	Not tested
CPA.7.028	21.42	1.40	1.34	Not tested	Not tested
CPA.7.029	1.24	0.90	1.29	Not tested	Not tested
CPA.7.031	Not tested	Not tested	1.47	Not tested	Not tested
CPA.7.032	10.43	0.93	1.46	Not tested	Not tested
CPA.7.033	2.46	0.93	1.39	Not tested	Not tested
CPA.7.034	1.05	0.89	1.33	Not tested	Not tested
CPA.7.035	Not tested	Not tested	1.24	Not tested	Not tested
CPA.7.036	4.17	0.87	1.24	Not tested	Not tested
CPA.7.037	1.64	1.01	1.40	Not tested	Not tested
CPA.7.038	18.08	1.03	7.88	3.35	1.01
CPA.7.039	1.43	0.92	7.53	0.31	1.01

Figure 32B

Antibody (hIgG1)	Human CD56 int. NK (gMFIr, 5ug/ml)	Human CD8+ T cells (gMFIr, 5ug/ml)	Expi cyno OE/par (gMFIr, 5ug/ml)	Cyno NK cells (gMFIr, 5ug/ml)	Cyno CD3+ T cells (gMFIr, 5ug/ml)
CPA.7.040	1.73	0.88	1.32	Not tested	Not tested
CPA.7.041	6.15	1.01	3.31	1.26	0.98
CPA.7.042	6.10	0.92	3.71	1.38	0.99
CPA.7.043	1.10	0.83	1.50	Not tested	Not tested
CPA.7.044	8.79	0.88	1.31	Not tested	Not tested
CPA.7.045	1.28	0.84	1.43	Not tested	Not tested
CPA.7.046	Not tested	Not tested	4.42	Not tested	Not tested
CPA.7.047	0.99	0.90	1.29	Not tested	Not tested
CPA.7.049	Not tested	Not tested	1.37	Not tested	Not tested
CPA.7.050	4.98	1.23	1.47	0.64	1.03

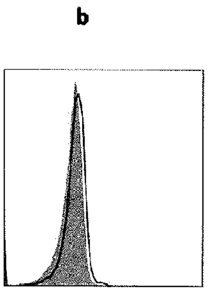
Figure 33


800
600
400
200
0
gMFI
0
2
4
6
8
10
[mAb ug/ml]
CA46
Jurkat
OV90
NCI-H441



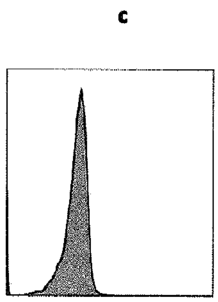
expi parental

Figure 34A



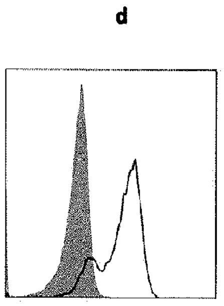
cPVRIG transient

Figure 34B



expi parental

Figure 34C



cPVRIG transient

Figure 34D

Figure 35

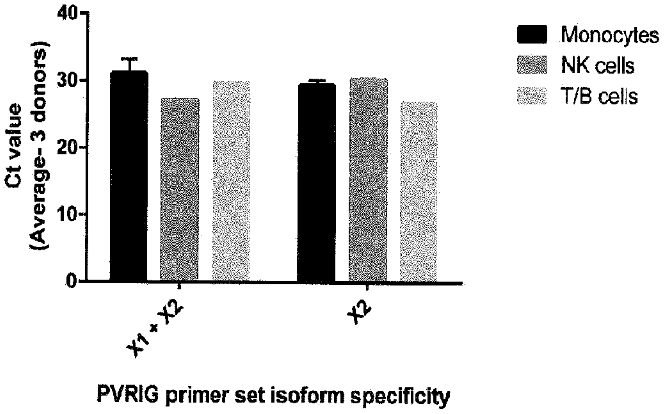

40
30
20
10
0
Ct value
(Average- 3 donors)
X1 + X2
X2
PVRIG primer set isoform specificity
Monocytes
NK cells
T/B cells

Figure 36A

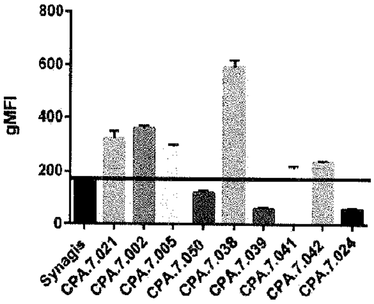


Figure 36B

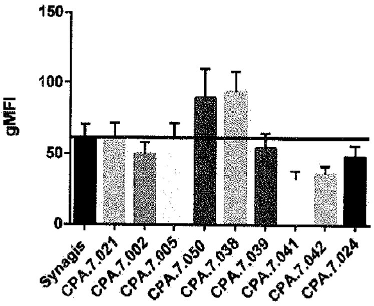


Figure 36C

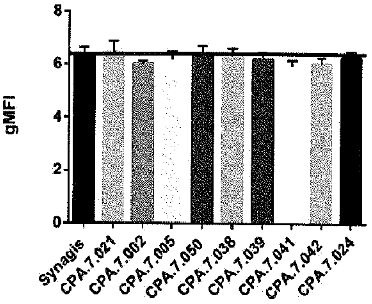


Figure 37A

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.001	GGTFSSYA	14	IIPIFGTA	15	AREEVSSPYGMDV	16	TGAVTSGHY	17	DTG	18	LLSYSGASWV	19
CPA.7.003	GFSLSHFS	20	FDPEEGGT	21	ATGIWYSSGWPVDY	22	QSLLDSSGYNY	23	LGS	24	MQALQTPIT	25
CPA.7.004	GYTLTELS	26	FDPEDGET	27	ATVSRVRGVINYYYYMDV	28	QSLLYRNGNNY	29	LGS	30	MQALQTPPT	31
CPA.7.006	GGTFGTYA	32	ITPISATI	33	ARGFEYSDGLLDD	34	QSLFYSDDGNTY	35	RLS	36	MQHMEFPLT	37
CPA.7.008	SGSISSTNW	38	IYHSGST	39	ARVGPAAIYY	40	SNNVGYEG	41	RNN	42	SAWDSSLNAVV	43
CPA.7.009	GYTLTELS	44	FDPEDGET	45	ATAKPGIAVAGQNYYYYYMDV	46	QSLLYRNGNNY	47	LGS	48	MQALQTPPT	49
CPA.7.010	GFTFSSYA	50	ISYDGSNK	51	ASSPIGYSYGYWGGMDV	52	SGIDVRTNK	53	FQSDSDK	54	LIWHTSGWV	55
CPA.7.011	GYTLTELS	56	FDPEDGET	57	ATGPAAAGVGYYYYMDV	58	QSLLYRNGYNY	59	LGS	60	MQALQTPPT	61
CPA.7.012	GFTFSSYA	62	ISYDGSNK	63	ARDVMVYCSSTSCYFYGMDV	64	QDIRDY	65	DAS	66	QQFENLPIT	67
CPA.7.013	GYTLTELS	68	FDPEDGET	69	ATGGYSSGFNYYYYYMDV	70	QSLLYRNGNNY	71	LGS	72	MQALQTPPT	73
CPA.7.014	GYTLTELS	74	FDPEDGET	75	ATGVTTYYYYGMDV	76	QSLLYSNGNNF	77	LGS	78	MQALQTPPT	79
CPA.7.015	GFTFSSYG	80	IRYDGSNK	81	ARDLFDFWWDGMDV	82	QSVSSMY	83	GAS	84	QQYVSSPMYT	85
CPA.7.017	GGTFNNYG	86	IIPLFGTT	87	ARDRMAADGMAVFDY	88	SSNIGRHF	89	KND	90	SSWDAALNGVV	91
CPA.7.018	GYTLTELS	92	FDPEDGET	93	ATEVPMVRGARRYYYYMDV	94	QTLLYINENNY	95	LGS	96	MQGLQTPPT	97

Figure 37B

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.019	GGSISNSNYY	98	IYYSGST	99	ARGAWELSLGDWFDP	100	SSNIGAGYD	101	GNN	102	QSYDSSLSVYVV	103
CPA.7.021	GFTFGTSS	104	ISFDGTEI	105	AKGSGNIYFYSGMDV	106	QSISGW	107	ETS	108	QQYYSYPLT	109
CPA.7.022	GYTLTELS	110	FDPEDGET	111	ATGVPAAIGVYYYYYYMDV	112	QSLLYSNGYNY	113	LGS	114	MQALQSPVT	115
CPA.7.023	GYTLTELS	116	FDPEDGET	117	ATDSRDGPAARGGYYYYMDV	118	QSLLYINGYNY	119	LGS	120	MQALQTPPT	121
CPA.7.024	GGTFSSYA	122	IIPIFGTA	123	ARDAYYYDSSGYYNPDAFDI	124	QSLLHSNGYNY	125	LGS	126	MQGLQTPRT	127
CPA.7.033	GGTFSSSA	128	IIPIYGIT	129	ARDDTARRVRGVPYYYYYAMDV	130	QDIDDD	131	EAS	132	LQHDNLPLT	133
CPA.7.034	GYTLTELS	134	FDPEDGET	135	ATEDPGPVAGPYYYYGMDV	136	QSLLYINGYHY	137	LGS	138	MQALQTPPT	139
CPA.7.036	GGTFSSSA	140	IIPIYGIT	141	ARDDTARRVRGVPYYYYYAMDV	142	QSLLDSDDGNTY	143	TLS	144	MQRLQFPLT	145
CPA.7.040	GYTLTELS	146	FDPEDGET	147	ATGVPAAIGVYYYYYYMDV	148	QSLLYRNGYNY	149	WGS	150	MQAVQNPPT	151
CPA.7.046	GGTFSSSA	152	IIPIYGIT	153	ARDDTARRVRGVPYYYYYAMDV	154	QTMNNY	155	DAS	156	QQYGDWLPIT	157
CPA.7.047	GYTLTELS	158	FDPEDGET	159	ATAFPEATISYYYYMDV	160	QSLLYRNGYNY	161	WGS	162	MQAVQNPPT	163
CPA.7.049	GGTFSSSA	164	IIPIYGIT	165	ARDDTARRVRGVPYYYYYAMDV	166	RSLLDSDDGNTH	167	SLS	168	MQRKEFPLT	169
CPA.7.050	GGTFSSYA	170	IIPIFGTA	171	ARGPWYYDSSGYSSYAYYMDV	172	QSLLHSDGYNY	173	LGS	174	MQALHTPGVT	175

Figure 38A

CPA.7.001

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREEVSSPYGMDVWGQGTTVTVSS	176
vhCDR1	GGTFSSYA	177
vhCDR2	IIPIFGTA	178
vhCDR3	AREEVSSPYGMDV	179
Full length HC	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREEVSSPYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	180
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGTSTGAVTSGHYPYWFQQKPGQAPKTLIYDTGNKHSWTPARFSGSLLGGKAALTLSGAQPEDEADYYCLLSYSGASWVFGGGTKLTVLG	181
vlCDR1	TGAVTSGHY	182
vlCDR2	DTG	183
vlCDR3	LLSYSGASWV	184
Full length light chain	QAVVTQEPSLTVSPGGTVTLTCGTSTGAVTSGHYPYWFQQKPGQAPKTLIYDTGNKHSWTPARFSGSLLGGKAALTLSGAQPEDEADYYCLLSYSGASWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	185

Figure 38B
CPA.7.003

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVSCKVSGFSLSHFSMHWVRQVPGKGLEWLGGFDPEEGGTIPAQKFQGRLTMTEDTSTETAYMELSSLRSEDTAVYYCATGIWYSSGWPVDYWGPGTLVTVSS	186
vhCDR1	GFSLSHFS	187
vhCDR2	FDPEEGGT	188
vhCDR3	ATGIWYSSGWPVDY	189
Full length HC	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGPWYYDSSGYSSYAYYMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	190
Variable light (vl) domain	DVVMTQSPLSLPVTPGGPASISCRSSQSLLDSSGYNYVDWYLQKPGQSPQLLISLGSDRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTPITFGQGTRLEIKR	191
vlCDR1	QSLLDSSGYNY	192
vlCDR2	LGS	193
vlCDR3	MQALQTPIT	194
Full length light chain	DVVMTQSPLSLPVTPGGPASISCRSSQSLLDSSGYNYVDWYLQKPGQSPQLLISLGSDRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	195

Figure 38C
CPA.7.004

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATVSRVRGVINYYYYMDV WGKGTTVTVSS	196
vhCDR1	GYTLTELS	197
vhCDR2	FDPEDGET	198
vhCDR3	ATVSRVRGVINYYYYMDV	199
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATVSRVRGVINYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	200
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKR	201
vlCDR1	QSLLYRNGNNY	202
vlCDR2	LGS	203
vlCDR3	MQALQTPPT	204
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	205

Figure 38D
CPA.7.006

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVRRPGSSVRVSCKPSGGTFGTYAFTWVRQAPGQGLEWMGGITPISATINRAQNLQDRLTITADESTTTVHMDLTSLRSEDTAVYYCARGFEYSDGLLDDWGQGTLVTVSS	206
vhCDR1	GGTFGTYA	207
vhCDR2	ITPISATI	208
vhCDR3	ARGFEYSDGLLDD	209
Full length HC	QVQLVQSGAEVRRPGSSVRVSCKPSGGTFGTYAFTWVRQAPGQGLEWMGGITPISATINRAQNLQDRLTITADESTTTVHMDLTSLRSEDTAVYYCARGFEYSDGLLDDWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	210
Variable light (vl) domain	DIVMTQTPLSLPVIPGEPASISCRSSQSLFYSDDGNTYLDWYLQKPGQSPQLLIYRLSHRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHMEFPLTFGGGTKVEIKR	211
vlCDR1	QSLFYSDDGNTY	212
vlCDR2	RLS	213
vlCDR3	MQHMEFPLT	214
Full length light chain	DIVMTQTPLSLPVIPGEPASISCRSSQSLFYSDDGNTYLDWYLQKPGQSPQLLIYRLSHRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHMEFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	215

Figure 38E
CPA.7.008

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGPGLVKPSGTLSLTCVVSSGSISSTNWWTWVRQPPGKGLEWIGEIYHSGSTSYNSSLKSRVTISEDKSKNQISLRLSSVTAADTAVYYCARVGPAAIYYWGQGTLVTVSS	216
vhCDR1	SGSISSTNW	217
vhCDR2	IYHSGST	218
vhCDR3	ARVGPAAIYY	219
Full length HC	QVQLQESGPGLVKPSGTLSLTCVVSSGSISSTNWWTWVRQPPGKGLEWIGEIYHSGSTSYNSSLKSRVTISEDKSKNQISLRLSSVTAADTAVYYCARVGPAAIYYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	220
Variable light (vl) domain	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGYEGAAWLQQHQGHAPKLLLYRNNNRPSGISKRFSASRSGNTASLTITGLQPEDEADYYCSAWDSSLNAVVFGGGTQLTVLG	221
vlCDR1	SNNVGYEG	222
vlCDR2	RNN	223
vlCDR3	SAWDSSLNAVV	224
Full length light chain	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGYEGAAWLQQHQGHAPKLLLYRNNNRPSGISKRFSASRSGNTASLTITGLQPEDEADYYCSAWDSSLNAVVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	225

Figure 38F
CPA.7.009

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATAKPGIAVAGQNYYYYYMDVWGKGTTVTVSS	226
vhCDR1	GYTLTELS	227
vhCDR2	FDPEDGET	228
vhCDR3	ATAKPGIAVAGQNYYYYYMDV	229
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATAKPGIAVAGQNYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	230
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKR	231
vlCDR1	QSLLYRNGNNY	232
vlCDR2	LGS	233
vlCDR3	MQALQTPPT	234
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	235

Figure 38G
CPA.7.010

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCASSPIGYSYGYWGGMDVWGQGTTVTVSS	236
vhCDR1	GFTFSSYA	237
vhCDR2	ISYDGSNK	238
vhCDR3	ASSPIGYSYGYWGGMDV	239
Full length HC	EVQLVESGGGLVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCASSPIGYSYGYWGGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	240
Variable light (vl) domain	QAVLTQPASLSASPGASASLTCTLRSGIDVRTNKIFWYQVKPGSPPQHLLTFQSDSDKQQGSGVPSRFSGSKDASANAGILIISGLQSEDEADYYCLIWHTSGWVFGGGTQLTVLG	241
vlCDR1	SGIDVRTNK	242
vlCDR2	FQSDSDK	243
vlCDR3	LIWHTSGWV	244
Full length light chain	QAVLTQPASLSASPGASASLTCTLRSGIDVRTNKIFWYQVKPGSPPQHLLTFQSDSDKQQGSGVPSRFSGSKDASANAGILIISGLQSEDEADYYCLIWHTSGWVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	245

Figure 38H
CPA.7.011

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGPAAAGVGYYYYMDV WGKGTTVTVSS	246
vhCDR1	GYTLTELS	247
vhCDR2	FDPEDGET	248
vhCDR3	ATGPAAAGVGYYYYMDV	249
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGPAAAGVGYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	250
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKR	251
vlCDR1	QSLLYRNGYNY	252
vlCDR2	LGS	253
vlCDR3	MQALQTPPT	254
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	255

Figure 38I
CPA7.012

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVETGGGLIQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDVMVYCSSTSCYFYGMDVWGQGTTVTVSS	256
vhCDR1	GFTFSSYA	257
vhCDR2	ISYDGSNK	258
vhCDR3	ARDVMVYCSSTSCYFYGMDV	259
Full length HC	EVQLVETGGGLIQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDVMVYCSSTSCYFYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	260
Variable light (vl) domain	DIQMTQSPSSLSASVGDRVTVTCQASQDIRDYLNWYQKKPGKAPKLLIYDASNLEAGVPSRFSGSGSGTDFTFTISGLQPEDVATYYCQQFENLPITFGQGTRLEIKR	261
vlCDR1	QDIRDY	262
vlCDR2	DAS	263
vlCDR3	QQFENLPIT	264
Full length light chain	DIQMTQSPSSLSASVGDRVTVTCQASQDIRDYLNWYQKKPGKAPKLLIYDASNLEAGVPSRFSGSGSGTDFTFTISGLQPEDVATYYCQQFENLPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	265

Figure 38J
CPA.7.013

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGGYSSGFNYYYYYMDV WGKGTTVTVSS	266
vhCDR1	GYTLTELS	267
vhCDR2	FDPEDGET	268
vhCDR3	ATGGYSSGFNYYYYYMDV	269
Full length HC	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGGYSSGFNYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	270
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKR	271
vlCDR1	QSLLYRNGNNY	272
vlCDR2	LGS	273
vlCDR3	MQALQTPPT	274
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	275

Figure 38K
CPA.7.014

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVTTYYYYGMDVWGQGTTVTVSS	276
vhCDR1	GYTLTELS	277
vhCDR2	FDPEDGET	278
vhCDR3	ATGVTTYYYYGMDV	279
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVTTYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	280
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGNNFLDWYLQKPGQSPRLLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTPPTFGQGTKVEIKR	281
vlCDR1	QSLLYSNGNNF	282
vlCDR2	LGS	283
vlCDR3	MQALQTPPT	284
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGNNFLDWYLQKPGQSPRLLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	285

Figure 38L
CPA.7.015

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QITLKESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIRYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLFDFWWDGMDVWGQGTTVTVSS	286
vhCDR1	GFTFSSYG	287
vhCDR2	IRYDGSNK	288
vhCDR3	ARDLFDFWWDGMDV	289
Full length HC	QITLKESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIRYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLFDFWWDGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	290
Variable light (vl) domain	EIVLTQSPGTLSLSPGEKATLSCRVSQSVSSMYLAWYQQKPGQAPRLLIYGASYRATGIPDRFSGSGSGTDFNLTISRLEPEDFAVYYCQQYVSSPMYTFGLGTKLEIKR	291
vlCDR1	QSVSSMY	292
vlCDR2	GAS	293
vlCDR3	QQYVSSPMYT	294
Full length light chain	EIVLTQSPGTLSLSPGEKATLSCRVSQSVSSMYLAWYQQKPGQAPRLLIYGASYRATGIPDRFSGSGSGTDFNLTISRLEPEDFAVYYCQQYVSSPMYTFGLGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	295

Figure 38M
CPA.7.017

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFNNYGISWVRQAPGKGLEWMGQIIPLFGTTKYAQKFQGRVTIAADEPTSTAYMELGSLRSEDTAIYYCARDRMAADGMAVFDYWGQGTLVTVSS	296
vhCDR1	GGTFNNYG	297
vhCDR2	IIPLFGTT	298
vhCDR3	ARDRMAADGMAVFDY	299
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFNNYGISWVRQAPGKGLEWMGQIIPLFGTTKYAQKFQGRVTIAADEPTSTAYMELGSLRSEDTAIYYCARDRMAADGMAVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	300
Variable light (vl) domain	QSVLTQPPSVSGTPGQKVIISCSGSSSNIGRHFVFWYQQLPGTAPKLLIYKNDERPSGVPDRFSGSKSGTSASLAVSGLRSEDEADYYCSSWDAALNGVVFGGGTKLTVLG	301
vlCDR1	SSNIGRHF	302
vlCDR2	KND	303
vlCDR3	SSWDAALNGVV	304
Full length light chain	QSVLTQPPSVSGTPGQKVIISCSGSSSNIGRHFVFWYQQLPGTAPKLLIYKNDERPSGVPDRFSGSKSGTSASLAVSGLRSEDEADYYCSSWDAALNGVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	305

Figure 38N
CPA.7.018

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEVPMVRGARRYYYYMDVWGKGTTVTVSS	306
vhCDR1	GYTLTELS	307
vhCDR2	FDPEDGET	308
vhCDR3	ATEVPMVRGARRYYYYMDV	309
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEVPMVRGARRYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	310
Variable light (vl) domain	DVVMTQSPLFLAVTPGEPASISCRSSQTLLYINENNYLDWYVQKPGQSPQLLIYLGSTRASGVPDRFSGGGSGTDFTLTISRVEAEDVGLYYCMQGLQTPPTFGQGTRLEIKR	311
vlCDR1	QTLLYINENNY	312
vlCDR2	LGS	313
vlCDR3	MQGLQTPPT	314
Full length light chain	DVVMTQSPLFLAVTPGEPASISCRSSQTLLYINENNYLDWYVQKPGQSPQLLIYLGSTRASGVPDRFSGGGSGTDFTLTISRVEAEDVGLYYCMQGLQTPPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	315

Figure 38O
CPA.7.019

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGPGLVKSSETLSLTCSVSGGSISNSNYYWGWIRQPPGKGLEWIGGIYYSGSTYYNPSLESRVTISEDTSKNQISLKLSSVTAADTAVYYCARGAWELSLGDWFDPWGPGTLVTVSS	316
vhCDR1	GGSISNSNYY	317
vhCDR2	IYYSGST	318
vhCDR3	ARGAWELSLGDWFDP	319
Full length HC	QVQLQESGPGLVKSSETLSLTCSVSGGSISNSNYYWGWIRQPPGKGLEWIGGIYYSGSTYYNPSLESRVTISEDTSKNQISLKLSSVTAADTAVYYCARGAWELSLGDWFDPWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	320
Variable light (vl) domain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIQWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSRSGTSASLAITGLQAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLG	321
vlCDR1	SSNIGAGYD	322
vlCDR2	GNN	323
vlCDR3	QSYDSSLSVYVV	324
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIQWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSRSGTSASLAITGLQAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	325

Figure 38P
CPA.7.021

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGVVKPGGSLRLSCAASGFTFGTSSMNWVRQAPGKGLEWVAVISFDGTEIHYADSVKGRFTISRDNSKSTVFLQMNSLRPDDTALYYCAKGSGNIYFYSGMDVWGQGTTVTVSS	326
vhCDR1	GFTFGTSS	327
vhCDR2	ISFDGTEI	328
vhCDR3	AKGSGNIYFYSGMDV	329
Full length HC	EVQLVESGGGVVKPGGSLRLSCAASGFTFGTSSMNWVRQAPGKGLEWVAVISFDGTEIHYADSVKGRFTISRDNSKSTVFLQMNSLRPDDTALYYCAKGSGNIYFYSGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	330
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRAGQSISGWLAWFQQKPGKAPNLLIYETSTLESGVPSRFSGSGSGTEYTLTISSLQPDDFATYYCQQYYSYPLTFGQGTKVEIKR	331
vlCDR1	QSISGW	332
vlCDR2	ETS	333
vlCDR3	QQYYSYPLT	334
Full length light chain	DIQMTQSPSTLSASVGDRVTITCRAGQSISGWLAWFQQKPGKAPNLLIYETSTLESGVPSRFSGSGSGTEYTLTISSLQPDDFATYYCQQYYSYPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	335

Figure 38Q
CAP.7.022

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYYYMDVWGKGTTVTVSS	336
vhCDR1	GYTLTELS	337
vhCDR2	FDPEDGET	338
vhCDR3	ATGVPAAIGVYYYYYYMDV	339
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	340
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQLLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQSPVTFGGGTKVEIKR	341
vlCDR1	QSLLYSNGYNY	342
vlCDR2	LGS	343
vlCDR3	MQALQSPVT	344
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQLLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQSPVTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	345

Figure 38R
CPA.7.023

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDSRDGPAARGGYYYYMDVWGQGTTVTVSS	346
vhCDR1	GYTLTELS	347
vhCDR2	FDPEDGET	348
vhCDR3	ATDSRDGPAARGGYYYYMDV	349
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDSRDGPAARGGYYYYMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	350
Variable light (vl) domain	DVVMTQSPLSLPVTLGQPASISCRSSQSLLYINGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKR	351
vlCDR1	QSLLYINGYNY	352
vlCDR2	LGS	353
vlCDR3	MQALQTPPT	354
Full length light chain	DVVMTQSPLSLPVTLGQPASISCRSSQSLLYINGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	355

Figure 38S
CPA.7.024

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAYYYDSSGYYNPDAFDIWGQGTMVTVSS	356
vhCDR1	GGTFSSYA	357
vhCDR2	IIPIFGTA	358
vhCDR3	ARDAYYYDSSGYYNPDAFDI	359
Full length HC	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAYYYDSSGYYNPDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	360
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSVSGTDFTLKISRVEAEDVGVYYCMQGLQTPRTFGRGTKLEIKR	361
vlCDR1	QSLLHSNGYNY	362
vlCDR2	LGS	363
vlCDR3	MQGLQTPRT	364
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSVSGTDFTLKISRVEAEDVGVYYCMQGLQTPRTFGRGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	365

Figure 38T

CPA.7.033

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	366
vhCDR1	GGTFSSSA	367
vhCDR2	IIPIYGIT	368
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	369
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	370
Variable light (vl) domain	ETTLTQSPAFMSATPGDEVNISCKASQDIDDDVSWYQQKPGGAPIFLIQEASTLVPGIPPRFSGSGFGTDFTLTIKNMESEDAAYYFCLQHDNLPLTFGGGTKVDIKR	371
vlCDR1	QDIDDD	372
vlCDR2	EAS	373
vlCDR3	LQHDNLPLT	374
Full length light chain	ETTLTQSPAFMSATPGDEVNISCKASQDIDDDVSWYQQKPGGAPIFLIQEASTLVPGIPPRFSGSGFGTDFTLTIKNMESEDAAYYFCLQHDNLPLTFGGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	375

Figure 38U

CPA.7.034

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEDPGPVAGPYYYYGMDVWGQGTTVTVSS	376
vhCDR1	GYTLTELS	377
vhCDR2	FDPEDGET	378
vhCDR3	ATEDPGPVAGPYYYYGMDV	379
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEDPGPVAGPYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	380
Variable light (vl) domain	DVVMTQSPLVLPVTPGEPASISCRSSQSLLYINGYHYLDWYVQRPGQSPQLLIFLGSTRASGVPDRFSGSGSGTDFTLEISKVEAEDVGIYFCMQALQTPPTFGGGTKVEIKR	381
vlCDR1	QSLLYINGYHY	382
vlCDR2	LGS	383
vlCDR3	MQALQTPPT	384
Full length light chain	DVVMTQSPLVLPVTPGEPASISCRSSQSLLYINGYHYLDWYVQRPGQSPQLLIFLGSTRASGVPDRFSGSGSGTDFTLEISKVEAEDVGIYFCMQALQTPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	385

Figure 38V

CPA.7.036

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	386
vhCDR1	GGTFSSSA	387
vhCDR2	IIPIYGIT	388
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	389
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	390
Variable light (vl) domain	DIVMTQTPLSLPVTPGEPASISCRPSQSLLDSDDGNTYLDWYLQKPGQSPQLLIHTLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRLQFPLTFGGGTKVEIKR	391
vlCDR1	QSLLDSDDGNTY	392
vlCDR2	TLS	393
vlCDR3	MQRLQFPLT	394
Full length light chain	DIVMTQTPLSLPVTPGEPASISCRPSQSLLDSDDGNTYLDWYLQKPGQSPQLLIHTLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRLQFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	395

Figure 38W

CPA.7.040

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYYYMDVWGKGTTVTVSS	396
vhCDR1	GYTLTELS	397
vhCDR2	FDPEDGET	398
vhCDR3	ATGVPAAIGVYYYYYYMDV	399
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	400
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAVQNPPTFGQGTKVDIKR	401
vlCDR1	QSLLYRNGYNY	402
vlCDR2	WGS	403
vlCDR3	MQAVQNPPT	404
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAVQNPPTFGQGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	405

Figure 38X

CPA.7.046

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	406
vhCDR1	GGTFSSSA	407
vhCDR2	IIPIYGIT	408
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	409
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	410
Variable light (vl) domain	DVVMTQSPAFLSVTPGERVTLSCKASQTMNNYLAWYQQKPGQAPRLLIYDASTRATDTPPRFSGSGSGTEFTLTISSVQSEDFALYYCQQYGDWLPITFGQGTRLEIKR	411
vlCDR1	QTMNNY	412
vlCDR2	DAS	413
vlCDR3	QQYGDWLPIT	414
Full length light chain	DVVMTQSPAFLSVTPGERVTLSCKASQTMNNYLAWYQQKPGQAPRLLIYDASTRATDTPPRFSGSGSGTEFTLTISSVQSEDFALYYCQQYGDWLPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	415

Figure 38Y

CPA.7.047

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATAFPEATISYYYYMDVWGKGTTVTVSS	416
vhCDR1	GYTLTELS	417
vhCDR2	FDPEDGET	418
vhCDR3	ATAFPEATISYYYYMDV	419
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATAFPEATISYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	420
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAVQNPPTFGQGTKVEIKR	421
vlCDR1	QSLLYRNGYNY	422
vlCDR2	WGS	423
vlCDR3	MQAVQNPPT	424
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAVQNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	425

Figure 38Z

CPA.7.049

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	426
vhCDR1	GGTFSSSA	427
vhCDR2	IIPIYGIT	428
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	429
Full length HC	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	430
Variable light (vl) domain	DIVMTQTPLSLPVTPGEPASMSCRSSRSLLDSDDGNTHLDWYLQKPGQSPQLLIQSLSYRASGVPDRFSGSGSGTDFTLEISRVEAEDVGIYYCMQRKEFPLTFGGGTKVEIKR	431
vlCDR1	RSLLDSDDGNTH	432
vlCDR2	SLS	433
vlCDR3	MQRKEFPLT	434
Full length light chain	DIVMTQTPLSLPVTPGEPASMSCRSSRSLLDSDDGNTHLDWYLQKPGQSPQLLIQSLSYRASGVPDRFSGSGSGTDFTLEISRVEAEDVGIYYCMQRKEFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	435

Figure 38AA

CPA.7.050

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGPWYYDSSGYSSYAYYMDVWGQGTTVTVSS	436
vhCDR1	GGTFSSYA	437
vhCDR2	IIPIFGTA	438
vhCDR3	ARGPWYYDSSGYSSYAYYMDV	439
Full length HC	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGPWYYDSSGYSSYAYYMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	440
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALHTPGVTFGGGTKVEIKR	441
vlCDR1	QSLLHSDGYNY	442
vlCDR2	LGS	443
vlCDR3	MQALHTPGVT	444
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSDGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALHTPGVTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	445

Figure 39A
CPA.7.028

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QLQLQESGPGLVKPSETLSLTCTVTGGSISSSSYYWAWIRQPPGKGLEWIGGIYYSGSTYYNVSLESRVTISQDTSKNQFSLKLTSVTAADTAVYYCARGAWELRLGDWFDPWGQGTLVTVSS	446
vhCDR1	GGSISSSSYY	447
vhCDR2	IYYSGST	448
vhCDR3	ARGAWELRLGDWFDP	449
Full length HC	QLQLQESGPGLVKPSETLSLTCTVTGGSISSSSYYWAWIRQPPGKGLEWIGGIYYSGSTYYNVSLESRVTISQDTSKNQFSLKLTSVTAADTAVYYCARGAWELRLGDWFDPWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	450
Variable light (vl) domain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLGQPKAA	451
vlCDR1	SSNIGAGYD	452
vlCDR2	GYS	453
vlCDR3	QSYDSSLSVYVV	454
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	455

Figure 39B
CPA.7.030

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGGGVVQPGGSLRLSCAASRFTFEDYAMHWVRQPPGKGLEWVSGISWKSGGINYADSVKGRFTISRDNAQNSLYLQMNSLRAEDTALYYCVKDPTLVATDRAFNIWGQGTMVTVSS	456
vhCDR1	RFTFEDYA	457
vhCDR2	ISWKSGGI	458
vhCDR3	VKDPTLVATDRAFNI	459
Full length HC	QVQLQESGGGVVQPGGSLRLSCAASRFTFEDYAMHWVRQPPGKGLEWVSGISWKSGGINYADSVKGRFTISRDNAQNSLYLQMNSLRAEDTALYYCVKDPTLVATDRAFNIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	460
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQYLQTPDTFGQGTKLEIKRAAPS	461
vlCDR1	QSLLHSNGYNY	462
vlCDR2	LGS	463
vlCDR3	MQYLQTPDT	464
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQYLQTPDTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	465

Figure 39C
CPA.7.041

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	466
vhCDR1	GGTFSSSA	467
vhCDR2	IIPIYGIT	468
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	469
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	470
Variable light (vl) domain	EIVLTQSPDFQSVTPKEKVTITCRASQNIDSSLHWYQQKPGQSPKLLINYASQSFSGVPSRFSGSGSGTDFTLTIDSLEPEDAATYFCHQSSSLPLTFGGGTKVEIRRTVAAPS	471
vlCDR1	QNIDSS	472
vlCDR2	YAS	473
vlCDR3	HQSSSLPLT	474
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	475

Figure 39D
CPA.7.016

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	476
vhCDR1	GGTFSSSA	477
vhCDR2	IIPIYGIT	478
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	479
Full length HC	EVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	480
Variable light (vl) domain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYDDWPQTFGQGTRLEIKRTVAAPS	481
vlCDR1	QSVSSY	482
vlCDR2	DAS	483
vlCDR3	QQYDDWPQT	484
Full length light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYDDWPQTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	485

Figure 39E
CPA.7.020

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	486
vhCDR1	GGTFSSSA	487
vhCDR2	IIPIYGIT	488
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	489
Full length HC	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	490
Variable light (vl) domain	EIVMTQSPATLSLSTGERATLFCRTSQNVYGEVAWYQQKPGQAPRLLIYDTFERAAGIPAKFSGSGSGTDFTLTISRVEPEDFAVYYCQQRRDWPITFGQGTRLEIKRTVAAPS	491
vlCDR1	QNVYGE	492
vlCDR2	DTF	493
vlCDR3	QQRRDWPIT	494
Full length light chain	EIVMTQSPATLSLSTGERATLFCRTSQNVYGEVAWYQQKPGQAPRLLIYDTFERAAGIPAKFSGSGSGTDFTLTISRVEPEDFAVYYCQQRRDWPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	495

Figure 39F
CPA.7.038

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	496
vhCDR1	GGTFSSSA	497
vhCDR2	IIPIYGIT	498
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	499
Full length HC	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	500
Variable light (vl) domain	DIQMTQSPSSLSASVGDRVTITCQASRDISDSLSWYQQKPGKAPKLLIFDASNLKTGVSSRFSGSGSGTDFTFTISSLQPEDIATYYCHQYDNLPLTFGGGTKVEIKRTVAAPS	501
vlCDR1	RDISDS	502
vlCDR2	DAS	503
vlCDR3	HQYDNLPLT	504
Full length light chain	DIQMTQSPSSLSASVGDRVTITCQASRDISDSLSWYQQKPGKAPKLLIFDASNLKTGVSSRFSGSGSGTDFTFTISSLQPEDIATYYCHQYDNLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	505

Figure 39G
CPA.7.044

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSS	506
vhCDR1	GGTFSSSA	507
vhCDR2	IIPIYGIT	508
vhCDR3	ARDDTARRVRGVPYYYYYAMDV	509
Full length HC	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPIYGITDYAQKFQGRVTITTDESTSTAYMELSSLTSEDTAVYYCARDDTARRVRGVPYYYYYAMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	510
Variable light (vl) domain	EIVMTQSPATLSLSPGERATLSCRASESVTTFLAWYQQKPGQAPRLLITDASNRATGIPGRFSGSGSGTDFTLTISSLEPEDFAVYYCHQHTNWPLTFGGGTKLEIKRTVAAPS	511
vlCDR1	ESVTTF	512
vlCDR2	DAS	513
vlCDR3	HQHTNWPLT	514
Full length light chain	EIVMTQSPATLSLSPGERATLSCRASESVTTFLAWYQQKPGQAPRLLITDASNRATGIPGRFSGSGSGTDFTLTISSLEPEDFAVYYCHQHTNWPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	515

Figure 39H
CPA.7.045

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEVGYCSGGSCYISYYYGMDVWGQGTTVTVSS	516
vhCDR1	GYTLTELS	517
vhCDR2	FDPEDGET	518
vhCDR3	ATEVGYCSGGSCYISYYYGMDV	519
Full length HC	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATEVGYCSGGSCYISYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	520
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGHNFLDWYVQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKLEIKRTVAAPS	521
vlCDR1	QSLLYRNGHNF	522
vlCDR2	LGS	523
vlCDR3	MQALQTPPT	524
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYRNGHNFLDWYVQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	525

Figure 40A

CDRs

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.001	GGTFSSYA	526	IIPIFGTA	527	AREEVSSPYGMDV	528	TGAVTSGHY	529	DTG	530	LLSYSGASWV	531
CPA.7.002	GGTFSSSA	532	IIPIYGIT	533	ARDDTARRVRGVPYYYYYAMDV	534	QGIGNY	535	SAS	536	QQLKDYPIT	537
CPA.7.003	GFSLSHFS	538	FDPEEGGT	539	ATGIWYSSGWPVDY	540	QSLLDSSGYNY	541	LGS	542	MQALQTPIT	543
CPA.7.004	GYTLTELS	544	FDPEDGET	545	ATVSRVRGVINYYYYMDV	546	QSLLYRNGNNY	547	LGS	548	MQALQTPPT	549
CPA.7.005	GGTFSSSA	550	IIPIYGIT	551	ARDDTARRVRGVPYYYYYAMDV	552	QSVDSS	553	DAS	554	QQYKDWPFT	555
CPA.7.006	GGTFGTYA	556	ITPISATI	557	ARGFEYSDGLLDD	558	QSLFYSDDGNTY	559	RLS	560	MQHMEFPLT	561
CPA.7.007	GGSISSSSYY	562	IYYSGST	563	ARGAWELRLGDWFDP	564	SSNIGAGYD	565	GNN	566	QSYDSSLSIYVV	567
CPA.7.008	SGSISSTNW	568	IYHSGST	569	ARVGPAAIYY	570	SNNVGYEG	571	RNN	572	SAWDSSLNAVV	573
CPA.7.009	GYTLTELS	574	FDPEDGET	575	ATAKPGIAVAGQNYYYYYMDV	576	QSLLYRNGNNY	577	LGS	578	MQALQTPPT	579
CPA.7.010	GFTFSSYA	580	ISYDGSNK	581	ASSPIGYSYGYWGGMDV	582	SGIDVRTNK	583	FQSDSDK	584	LIWHTSGWV	585
CPA.7.011	GYTLTELS	586	FDPEDGET	587	ATGPAAAGVGYYYYMDV	588	QSLLYRNGYNY	589	LGS	590	MQALQTPPT	591
CPA.7.012	GFTFSSYA	592	ISYDGSNK	593	ARDVMVYCSSTSCYFYGMDV	594	QDIRDY	595	DAS	596	QQFENLPIT	597
CPA.7.013	GYTLTELS	598	FDPEDGET	599	ATGGYSSGFNYYYYYMDV	600	QSLLYRNGNNY	601	LGS	602	MQALQTPPT	603

Figure 40B

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.014	GYTLTELS	604	FDPEDGET	605	ATGVTTYYYYGMDV	606	QSLLYSNGNNF	607	LGS	608	MQALQTPPT	609
CPA.7.015	GFTFSSYG	610	IRYDGSNK	611	ARDLFDFWWDGMDV	612	QSVSSMY	613	GAS	614	QQYVSSPMYT	615
CPA.7.016	GGTFSSSA	616	IIPIYGIT	617	ARDDTARRVRGVPYYYYYAMDV	618	QSVSSY	619	DAS	620	QQYDDWPQT	621
CPA.7.017	GGTFNNYG	622	IIPLFGTT	623	ARDRMAADGMAVFDY	624	SSNIGRHF	625	KND	626	SSWDAALNGVV	627
CPA.7.018	GYTLTELS	628	FDPEDGET	629	ATEVPMVRGARRYYYYMDV	630	QTLLYINENNY	631	LGS	632	MQGLQTPPT	633
CPA.7.019	GGSISNSNYY	634	IYYSGST	635	ARGAWELSLGDWFDP	636	SSNIGAGYD	637	GNN	638	QSYDSSLSVYVV	639
CPA.7.020	GGTFSSSA	640	IIPIYGIT	641	ARDDTARRVRGVPYYYYYAMDV	642	QNVYGE	643	DTF	644	QQRRDWPIT	645
CPA.7.021	GFTFGTSS	646	ISFDGTEI	647	AKGSGNIYFYSGMDV	648	QSISGW	649	ETS	650	QQYYSYPLT	651
CPA.7.022	GYTLTELS	652	FDPEDGET	653	ATGVPAAIGVYYYYYYMDV	654	QSLLYSNGYNY	655	LGS	656	MQALQSPVT	657
CPA.7.023	GYTLTELS	658	FDPEDGET	659	ATDSRDGPAARGGYYYYMDV	660	QSLLYINGYNY	661	LGS	662	MQALQTPPT	663
CPA.7.024	GGTFSSYA	664	IIPIFGTA	665	ARDAYYYDSSGYYNPDAFDI	666	QSLLHSNGYNY	667	LGS	668	MQGLQTPRT	669
CPA.7.025	GFSLTSGGMS	670	IDWNDDK	671	ARIRGMTWGFDS	672	QSVSSSY	673	GAS	674	QQYGTTPFA	675
CPA.7.026	GYTFTAYY	676	INPNSGGT	677	ARDGAFYYGSENYYNAGWFDP	678	QSLLSGNGYNY	679	LGS	680	MQALKSPLT	681
CPA.7.027	GYTFTNYY	682	INPSGGIT	683	ARAGLGYNWNYAPSGMDV	684	SSDVGGYNY	685	EVS	686	SSYAGSNNLV	687
CPA.7.028	GGSISSSSYY	688	IYYSGST	689	ARGAWELRLGDWFDP	690	SSNIGAGYD	691	GYS	692	QSYDSSLSVYVV	693

Figure 40C

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.029	GFTFDDYA	694	ISWNSGSI	695	AKDVNRILVAGMVDY	696	QSLLHSNGYNY	697	LGS	698	MQGLQTPIT	699
CPA.7.030	RFTFEDYA	700	ISWKSGGI	701	VKDPTLVATDRAFNI	702	QSLLHSNGYNY	703	LGS	704	MQYLQTPDT	705
CPA.7.031	GGTFSSSA	706	IIPIYGIT	707	ARDDTARRVRGVPYYYYYAMDV	708	QSLLDSDDGNIH	709	TLS	710	MQRKEFPLT	711
CPA.7.032	GFTFSSYG	712	ISYDGSNK	713	AKEDRLRFLEWLFYGMDV	714	HDIYTY	715	DAS	716	QQYDNHPPEVT	717
CPA.7.033	GGTFSSSA	718	IIPIYGIT	719	ARDDTARRVRGVPYYYYYAMDV	720	QDIDDD	721	EAS	722	LQHDNLPLT	723
CPA.7.034	GYTLTELS	724	FDPEDGET	725	ATEDPGPVAGPYYYYGMDV	726	QSLLYINGYHY	727	LGS	728	MQALQTPPT	729
CPA.7.035	GYMFTNYP	730	INAGTGNT	731	AREGMFYYGLESYYKGGWFDP	732	QSLLHRNGYNY	733	LAS	734	MQALQTPLT	735
CPA.7.036	GGTFSSSA	736	IIPIYGIT	737	ARDDTARRVRGVPYYYYYAMDV	738	QSLLDSDDGNTY	739	TLS	740	MQRLQFPLT	741
CPA.7.037	TYTFTTYY	742	IYPSGGNT	743	VRDQNYYYSAMDV	744	QDIRNY	745	DAS	746	QQFENLPIT	747
CPA.7.038	GGTFSSSA	748	IIPIYGIT	749	ARDDTARRVRGVPYYYYYAMDV	750	RDISDS	751	DAS	752	HQYDNLPLT	753
CPA.7.039	GFNFRGYA	754	ISGSGGTT	755	AQSYAQIGYGGHIDH	756	SGIDVATYM	757	YKSDSDK	758	LIWHGSHYV	759
CPA.7.040	GYTLTELS	760	FDPEDGET	761	ATGVPAAIGVYYYYYYMDV	762	QSLLYRNGYNY	763	WGS	764	MQAVQNPPT	765
CPA.7.041	GGTFSSSA	766	IIPIYGIT	767	ARDDTARRVRGVPYYYYYAMDV	768	QNIDSS	769	YAS	770	HQSSSLPLT	771
CPA.7.042	GGTFSSSA	772	IIPIYGIT	773	ARDDTARRVRGVPYYYYYAMDV	774	QSVYNNY	775	DAS	776	QQYNSWPPYT	777
CPA.7.043	GGTFSSYA	778	IIPIFGTA	779	ARDAYYYDSSGYYNPDAFDI	780	QSLLYSNGYNY	781	LGS	782	MQARQTPYT	783

Figure 40D

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.044	GGTFSSSA	784	IIPIYGIT	785	ARDDTARRVRGVPYYYYYAMDV	786	ESVTTF	787	DAS	788	HQHTNWPLT	789
CPA.7.045	GYTLTELS	790	FDPEDGET	791	ATEVGYCSGGSCYISYYYGMDV	792	QSLLYRNGHNF	793	LGS	794	MQALQTPPT	795
CPA.7.046	GGTFSSSA	796	IIPIYGIT	797	ARDDTARRVRGVPYYYYYAMDV	798	QTMNNY	799	DAS	800	QQYGDWLPIT	801
CPA.7.047	GYTLTELS	802	FDPEDGET	803	ATAFPEATISYYYYMDV	804	QSLLYRNGYNY	805	WGS	806	MQAVQNPPT	807
CPA.7.049	GGTFSSSA	808	IIPIYGIT	809	ARDDTARRVRGVPYYYYYAMDV	810	RSLLDSDDGNTH	811	SLS	812	MQRKEFPLT	813
CPA.7.050	GGTFSSYA	814	IIPIFGTA	815	ARGPWYYDSSGYSSYAYYMDV	816	QSLLHSDGYNY	817	LGS	818	MQALHTPGVT	819

Figure 41A

CHA.7.502

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLVESGGDLVQPGGSRKLSCTASGFTFSNFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPENTLFLQMTSLRSEDTAMYYCTRLDYYTNSYSMDHWGQGTSVTVSS	820
vhCDR1	GFTFSNFG	821
vhCDR2	ISSGSSTI	822
vhCDR3	TRLDYYTNSYSMDH	823
Variable light (vl) domain	QIVLTQSPALMSASPGEKVTLTCSASSSLPYIYWYQQKPGSSPKPWIYLTSNLASGVPARFSGSRSGTSYSLTISSVEAEDAATYYCQQWSSNPFTFGSGTKLEIK	824
vlCDR1	SSLPY	825
vlCDR2	LTS	826
vlCDR3	QQWSSNPFT	827

Figure 41B

CHA.7.503

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELAKPGASVKMSCKASGYTFTSNWMHWVKQRPGQGLEWIGYINPSNGYTECNQKFRDKATLSADKSSSTAYMQLNSLTSADSAVYYCALMISAWLPYWGQGTLVTVSA	828
vhCDR1	GYTFTSNW	829
vhCDR2	INPSNGYT	830
vhCDR3	ALMISAWLPY	831
Variable light (vl) domain	DIVLTQSPASLAISLGQRATISCRASQSVSASSYSYVHWYQQKPGQPPKLLIKYASSLESGVPARFSGSGSGTDFTLNIHPVEEEDTATYYCLHTWEIPYTFGGGTKLEIK	832
vlCDR1	QSVSASSYSY	833
vlCDR2	YAS	834
vlCDR3	LHTWEIPYT	835

Figure 41C

CHA.7.506

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELTRPGASVNLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPGDGDTRFNQKFKGKATLTADESSSTAYMQLSSLASEDSAVYYCATYYRYDDYWGQGTTLTVSS	836
vhCDR1	GYTFTSYW	837
vhCDR2	IYPGDGDT	838
vhCDR3	ATYYRYDDY	839
Variable light (vl) domain	QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPTRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPYTFGGGTKLEIK	840
vlCDR1	SSVSY	841
vlCDR2	DTS	842
vlCDR3	QQWSSNPYT	843

Figure 41D

CHA.7.508

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPDLVKPGASMKISCKASGYTFIDYNIHWVKQSQGKSLDWIGYIYPYNGGTGYNQKFKNKATLTVDSSSSTAYMEVRSLTFEDSAVYFCAREADYYGNRGQFDYWGQGTLVTVSA	844
vhCDR1	GYTFIDYN	845
vhCDR2	IYPYNGGT	846
vhCDR3	AREADYYGNRGQFDY	847
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTITCRASENIFSNLAWYQQKQGKSPQLLVYGEANLADGVPSRFSGSGSGTQYSLKINSLQSEDFGNYYCQHFWGTPYTFGGGTTLEIK	848
vlCDR1	ENIFSN	849
vlCDR2	GEA	850
vlCDR3	QHFWGTPYT	851

Figure 41E

CHA.7.510

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVTLKESGPGILQPSQTLSLTCSFSGFSLNTSGTGVGWIRQPSGKGLEWLTHIWWNDNKFYNTFLKSRLTISKETSNNQVFLKIASVDTADAATYYCARMAYGNLWFVNWGQGTLVAVST	852
vhCDR1	GFSLNTSGTG	853
vhCDR2	IWWNDNK	854
vhCDR3	ARMAYGNLWFVN	855
Variable light (vl) domain	DIVLTQSPASLAVSLGQRASISCRASRSVTISGYSYMYWYQQKPGQPPRLLFYLASNLASGVPARFSGSGSGTDFTLNIHPVEEEDAAIYYCQHSRELPYTFGGGTKLEIK	856
vlCDR1	RSVTISGYSY	857
vlCDR2	LAS	858
vlCDR3	QHSRELPYT	859

Figure 41F

CHA.7.512

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPELKKPRETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMGWINTETGEPTYADDFKGRFAFSLEASASSAYLQINILKDEDTATYFCARSRGGYYEDYYALDYWGQGTSVTVSS	860
vhCDR1	GYTFTDYS	861
vhCDR2	INTETGEP	862
vhCDR3	ARSRGGYYEDYYALDY	863
Variable light (vl) domain	DIQMTQSPASLSASVGESVTITCRASGNIHYYLAWYQQKQGKSPQLLVYNAKNLADGVPSRFSGSGSGTQFSLKINSLQPEDFGSYYCQHFWISPPTFGGGTKLEIK	864
vlCDR1	GNIHYY	865
vlCDR2	NAK	866
vlCDR3	QHFWISPPT	867

Figure 41G

CHA.7.514

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLRQSGADLVKPGASVKLSCTASGFNIKDTYIDWVKQRPEQGLDWIGRIDPANGNTKYDPKFQGKATIITDTSSNTAYLQLSNLTSEDTAVYYCARYGSYPYFDYWGRGTTLAVSS	868
vhCDR1	GFNIKDTY	869
vhCDR2	IDPANGNT	870
vhCDR3	ARYGSYPYFDY	871
Variable light (vl) domain	SIVMTQTPKFLLISAGDRVTITCKASQSVRNDVAWYQQKPGQSPKLLMYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSPPTFGGGTKLEIK	872
vlCDR1	QSVRND	873
vlCDR2	YAS	874
vlCDR3	QQDYSSPPT	875

Figure 41H

CHA.7.516

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGPELVRPGVSVKISCKVSGYTFTDYVMHWVKQSHAKSLEWIGIISPYSGNTNYNQNFKGKATMTVDKSSSTAYMALARLTSEDSAIYYCAREGDLPMFAYWGQGTLVTVSA	876
vhCDR1	GYTFTDYV	877
vhCDR2	ISPYSGNT	878
vhCDR3	AREGDLPMFAY	879
Variable light (vl) domain	QIVLTQSPTIMSASPGEKVTMTCSASSSVSYIYWYQQNPGSSPRLLIYDTSILASGVPFRFSGSGSGTSYSLTISRMEAEDAATYYCQQWTSYPLTFGSGTKLELK	880
vlCDR1	SSVSY	881
vlCDR2	DTS	882
vlCDR3	QQWTSYPLT	883

Figure 41I

CHA.7.518

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKASGYTFTDYNINWVKQSHGKSLEWIGYIYPYIGGS GYNQKFKSKATLSADNPSSTAYMELRSLTSEDSAVYYCAREDKTARNAMDYWGQG TPVTVSS	884
vhCDR1	GYTFTDYN	885
vhCDR2	IYPYIGGS	886
vhCDR3	AREDKTARNAMDY	887
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTIICRVSENIYSNLAWYQQKQGKSPQLLVYEATNLAEG VPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGTPYTFGGGTKLEIK	888
vlCDR1	ENIYSN	889
vlCDR2	EAT	890
vlCDR3	QHFWGTPYT	891

Figure 41J

CHA.7.520_1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIRQFPGNKLEWMGYISYGSTSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARHYRYPPYAMDYWGQGTSVTVSS	892
vhCDR1	GYSITSDYA	893
vhCDR2	ISYSGST	894
vhCDR3	ARHYRYPPYAMDY	895
Variable light (vl) domain	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSSNQKNYLAWYQQKPGQSPKLLVYFASTRESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQHYSTPFTFGSGTKLEIK	896
vlCDR1	QSLLNSSNQKNY	897
vlCDR2	FAS	898
vlCDR3	QQHYSTPFT	899

Figure 41K

CHA.7.520_2

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSFGVHWVRQSPGKGLEWLGVIWSGGTTVYDAAFISRLSISKDNSKSQVFFKMNSLQTNDTAIYYCARKRGNFYVMDYWGQGTSVTVSS	900
vhCDR1	GFSLTSFG	901
vhCDR2	IWSGGTT	902
vhCDR3	ARKRGNFYVMDY	903
Variable light (vl) domain	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSSNQKNYLAWYQQKPGQSPKLLVYFASTRESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQHYSTPFTFGSGTKLEIK	904
vlCDR1	QSLLNSSNQKNY	905
vlCDR2	FAS	906
vlCDR3	QQHYSTPFT	907

Figure 41L

CHA.7.522

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQPGSELVRPGTSVKLSCKASGYTFTSYWVHWVRQRHGQGLEWIGNVYPGSGSTNYDEKFKSKGTLTVDTSSSTAYMHLSSLTSEDSAVYYCTRGVLRFPLDYWGQGTTLTVSS	908
vhCDR1	GYTFTSYW	909
vhCDR2	VYPGSGST	910
vhCDR3	TRGVLRFPLDY	911
Variable light (vl) domain	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPHLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK	912
vlCDR1	KSLLHSNGNTY	913
vlCDR2	RMS	914
vlCDR3	MQHLEYPLT	915

Figure 41M

CHA.7.524

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGPEVVRPGVSVKISCKGSGYKFPDYVMHWVKQSHAKSLEWIGIISIYSGNTNYNQKFKGKATMTVDKSSSTAYMELARLTSEDSAIYYCAREGDLPMFAYWGQGTLVTVSA	916
vhCDR1	GYKFPDYV	917
vhCDR2	ISIYSGNT	918
vhCDR3	AREGDLPMFAY	919
Variable light (vl) domain	QIVLTQSPAIMSASPGEKVTMTCNASSSVSYMYWYQQKPISSPRLLIYDTSNLASGVPVRFSGSGSGTSYSLTIGRMEAEDAATYYCQQWSSYPLTFGAGTKVEVK	920
vlCDR1	SSVSY	921
vlCDR2	DTS	922
vlCDR3	QQWSSYPLT	923

Figure 41N

CHA.7.526

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSLSITCTVSGFSLTAYNINWVRQPPGKGLEWLGMIWGDG NTDYNSPLKSRLTISKDNSKSQVFLKMDSLQTDDTARYYCARDLKVRRDSPYTMDY WGQGTSVTVSS	924
vhCDR1	GFSLTAYN	925
vhCDR2	IWGDGNT	926
vhCDR3	ARDLKVRRDSPYTMDY	927
Variable light (vl) domain	NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIY WASNRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK	928
vlCDR1	QSVLYSSNQKNY	929
vlCDR2	WAS	930
vlCDR3	HQYLSSYT	931

Figure 410

CHA.7.527

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLQESGPGLVKPSQSLSLTCTVTGYSLTSDYAWNWIRQFPGNKLEWMGYITYSGGTTYNPSLKSRISITRDTSKNQFFLQLTSVTTEDTATYYCARRGSGTTVVGDWYFDVWGAGTTVTVSS	932
vhCDR1	GYSLTSDYA	933
vhCDR2	ITYSGGT	934
vhCDR3	ARRGSGTTVVGDWYFDV	935
Variable light (vl) domain	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSFNQKYYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTPTISSVTAEDLAVYYCQQFYTYPYTFGGGTKLEMK	936
vlCDR1	QSLLYSFNQKYY	937
vlCDR2	WAS	938
vlCDR3	QQFYTYPYT	939

Figure 41P

CHA.7.528

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQVQQSGPELVKPGASVKISCKASGYTFTKSNMHWVKQSHGKSLEWIGYIYPYNGGTGYNQNFKSKATLTVDISSSTAYMELRSLTLEDSAVYLCAREADYYGNRGQFDYWGQGTLVTVSA	940
vhCDR1	GYTFTKSN	941
vhCDR2	IYPYNGGT	942
vhCDR3	AREADYYGNRGQFDY	943
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTITCRASDNIFSNLAWYHQKQGKSPHLLVYGATNLADGVPSRFSGSGSGTQYSLKINSLQSEDFGDYYCQHFWGTPYTFGGGTKLEIK	944
vlCDR1	DNIFSN	945
vlCDR2	GAT	946
vlCDR3	QHFWGTPYT	947

Figure 41Q

CHA.7.530

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELMRPGTSVKVSCKASGYAFTNHLIEWIKQRPGQGLEWIGVINPGSDSTDYNEKFKDKATLTADKSSSTAYMQLSSLTSDDSAVYFCARSLYYNSWFVYWGQGTLVTVSA	948
vhCDR1	GYAFTNHL	949
vhCDR2	INPGSDST	950
vhCDR3	ARSLYYNSWFVY	951
Variable light (vl) domain	DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKRGKSPQLLVYNAKTLVEGVPSRFSGSGSGTQFSLKINSLQPEDFGSYYCQHHYGTPYTFGGGTKLEIK	952
vlCDR1	ENIYSY	953
vlCDR2	NAK	954
vlCDR3	QHHYGTPYT	955

Figure 41R

CHA.7.534

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGAELVKPGASVRLSCTASGFSIRDTYIHWVKQRPEQGLDWIGKIDPANGKSEYDPKFQGRATMTTDTSSNTAYLQLSSLTSEDTAVYYCTRYGYYPYFDVWGAGTTVTVFS	956
vhCDR1	GFSIRDTY	957
vhCDR2	IDPANGKS	958
vhCDR3	TRYGYYPYFDV	959
Variable light (vl) domain	SIVMTQTPKFLLVSAGDRVAITCKASQSVRHDVVWYQQKPGQSPKLLIYYASSRYTGVPDRFTGSGYGTDFTFTISTVQAEDLALYFCLQDFSSPWTFGGGTKLEIK	960
vlCDR1	QSVRHD	961
vlCDR2	YAS	962
vlCDR3	LQDFSSPWT	963

Figure 41S

CHA.7.535

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKTSGYTFTKNTMHWVRQSHGKSLEWIGGINPNSGGASFNQKFMGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARDGYDGDWFFDVWGAGTTVTVSS	964
vhCDR1	GYTFTKNT	965
vhCDR2	INPNSGGA	966
vhCDR3	ARDGYDGDWFFDV	967
Variable light (vl) domain	DIQMNQSPFSLSASLGDTVTITCHASQNIYVWLSWYQQKPGNIPKLLIYKASDLHTGVPSRFSGSGSGTDFTLNISSLQPEDIATYYCQQGQSYPRTFGGGTKLEIK	968
vlCDR1	QNIYVW	969
vlCDR2	KAS	970
vlCDR3	QQGQSYPRT	971

Figure 41T

CHA.7.537

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSLSITCTVSGFSLIGHGVNWIRQPPGKGLEWLGVIWGDGNTDYNSALKSRLSISKDNSKSQVFLKMNSLQTDDTARYFCAVNSAMDYWGQGTAVTVSS	972
vhCDR1	GFSLIGHG	973
vhCDR2	IWGDGNT	974
vhCDR3	AVNSAMDY	975
Variable light (vl) domain	NIVMTQSPKSMSMSVGERVTLNCTASENVASFVSWYQQKPEQSPKLLIYGTSNRYTGVPDRFTGSGSATDFTLTISSVQAEDLGDYHCGQSYNYPFTFGSGTKLEIE	976
vlCDR1	ENVASF	977
vlCDR2	GTS	978
vlCDR3	GQSYNYPFT	979

Figure 41U

CHA.7.538_1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELVRPGASVKVSCKTSGYAFTNYLIEWVKQRPGQGLEWIGVINPGSGGIYYNDKFKVKTTLTADKSSSTAYMQLSSLTSDDSAVYFCARSETHDTWFAYWGQGTLVTVSA	980
vhCDR1	GYAFTNYL	981
vhCDR2	INPGSGGI	982
vhCDR3	ARSETHDTWFAY	983
Variable light (vl) domain	DIVMTQSQKFISTSVGDRVSITCKASQSVRIAVAWFQQKPGQSPKALIYLASTRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPYTFGGGTKLEIKR	984
vlCDR1	QSVRIA	985
vlCDR2	LAS	986
vlCDR3	LQHWNYPYT	987

Figure 41V

CHA.7.538_2

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELVRPGTSVKMSCKAAGYTFTNYWIGWVKQRPGHGLEWIGDIYPGGGYTNYNEKFKGKATLTADTSSSTAYMQLSSLTSEDSAIYYCASPYYGSSYGFAFWGQGTLVTVSA	988
vhCDR1	GYTFTNYW	989
vhCDR2	IYPGGGYT	990
vhCDR3	ASPYYGSSYGFAF	991
Variable light (vl) domain	DIVMTQSQKFISTSVGDRVSITCKASQSVRIAVAWFQQKPGQSPKALIYLASTRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPYTFGGGTKLEIKR	992
vlCDR1	QSVRIA	993
vlCDR2	LAS	994
vlCDR3	LQHWNYPYT	995

Figure 41W

CHA.7.543

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSLSITCTVSGFSLSGYGIKWVRQPPGKGLEWLGTIWGDGSTDYNSALKSRLSISKDNSKSQVFLKMTSLQTDDTARYYCASDSLGITFGYWGQGTLVTVSA	996
vhCDR1	GFSLSGYG	997
vhCDR2	IWGDGST	998
vhCDR3	ASDSLGITFGY	999
Variable light (vl) domain	DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPLTFGAGTKLELK	1000
vlCDR1	QDISNY	1001
vlCDR2	YTS	1002
vlCDR3	QQGNTLPLT	1003

Figure 41X

CHA.7.544

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSLSITCTVSGFSLTSYAITWVRQPPGKGLEWLGVIWPGGGTNYNSALKSRLSISKDNSKSHLFLKMNSLQTDDTARYYCVRSYDGYLDWYFDVWGTGTTVTVSS	1004
vhCDR1	GFSLTSYA	1005
vhCDR2	IWPGGGT	1006
vhCDR3	VRSYDGYLDWYFDV	1007
Variable light (vl) domain	NIVMTQSPKSMSMSVGERVTLSCKASENVGTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLIISSVQAEDLSDYHCGQSYSYPYTFGGGTKLEII	1008
vlCDR1	ENVGTY	1009
vlCDR2	GAS	1010
vlCDR3	GQSYSYPYT	1011

Figure 41Y

CHA.7.545

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPDLKKPGETVKISCKASGYTFTTYGMSWVKQAPGRGLKWMGWINTYSGVSTFPDDFKGRFAFSLETSASTAYLQINNLKNEDSATYFCARLGMGSTTGAGYFDVWGTGTTVTVSS	1012
vhCDR1	GYTFTTYG	1013
vhCDR2	INTYSGVS	1014
vhCDR3	ARLGMGSTTGAGYFDV	1015
Variable light (vl) domain	DIVLTQSPAIMSASPGEKVTMTCSASSSVSSWYLHWYQQKSGASPKLWIYGTSNLASGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYRSDPYTFGSGTKLEIK	1016
vlCDR1	SSVSSWY	1017
vlCDR2	GTS	1018
vlCDR3	QQYRSDPYT	1019

Figure 41Z

CHA.7.546

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPELKKPGETVKISCKASGYTFTTYGMSWVKQAPGKGLKWMGWINTYSGVSTYADDFEGRFAFSLETSVSTAYLQINNLKNEDTATYFCARLGRGSTTGAGYLDVWGTGTTVTVSS	1020
vhCDR1	GYTFTTYG	1021
vhCDR2	INTYSGVS	1022
vhCDR3	ARLGRGSTTGAGYLDV	1023
Variable light (vl) domain	DIVLTQSPAIMSASPGEKVSMTCSASSSVSSWYLHWYQQKSGASPKLWIYGTSNLASGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYHSDPYTFGSGTKLEIK	1024
vlCDR1	SSVSSWY	1025
vlCDR2	GTS	1026
vlCDR3	QQYHSDPYT	1027

Figure 41AA

CHA.7.547

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPDLKKPGETVKISCKASGYTFTTYGMSWVKQAPGRGLKWMGWINTYSGVSTFPDDFKGRFAFSLETSASTAYLQINNLKNEDSATYFCARLGMGSTTGAGYFDVWGTGTTVTVSS	1028
vhCDR1	GYTFTTYG	1029
vhCDR2	INTYSGVS	1030
vhCDR3	ARLGMGSTTGAGYFDV	1031
Variable light (vl) domain	ENVLTQSPAIMSASLGEKVTLSCRASSSVNYMYWYQQKSDASPKLWIYYTSNLAPGVPARFSGSGSGNSYSLTISSVEGEDAATYYCQQFTSSPWTFGGGTKLEIK	1032
vlCDR1	SSVNY	1033
vlCDR2	YTS	1034
vlCDR3	QQFTSSPWT	1035

Figure 41BB

CHA.7.548

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKASGYTFTDYSMNWVKQSHGKSLEWIGDITPNNGSPNYNQKFKDKATLTVDKSSSTVYMELRSLTSEDSAVYYCASLFFDYWGHGTTLTVSS	1036
vhCDR1	GYTFTDYS	1037
vhCDR2	ITPNNGSP	1038
vhCDR3	ASLFFDY	1039
Variable light (vl) domain	DIVMTQSPSSLSVSAGEKVTMSCKSSQSLLNSGNQKNYLAWYQQKPGQPPKLLIYGASTRDSGVPDRFTGSGSGTDFTLTITSVQAEDLAVYYCQNDHTYPYTFGGGTKLEIK	1040
vlCDR1	QSLLNSGNQKNY	1041
vlCDR2	GAS	1042
vlCDR3	QNDHTYPYT	1043

Figure 41CC

CHA.7.549

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQPGTELVKPGASVKLSCKASGFTFTTHWMHWVRQRPGQGLEWIGNIYPSNGGSNYNEKFKTKATLTVDRSSSTAYMHLSSLTSEDSAVYYCARRVNWDGYYFDYWGQGTTLTVSS	1044
vhCDR1	GFTFTTHW	1045
vhCDR2	IYPSNGGS	1046
vhCDR3	ARRVNWDGYYFDY	1047
Variable light (vl) domain	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKLLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYNSYPLTFGGGTKLEIK	1048
vlCDR1	QNVGTN	1049
vlCDR2	SAS	1050
vlCDR3	QQYNSYPLT	1051

Figure 41DD

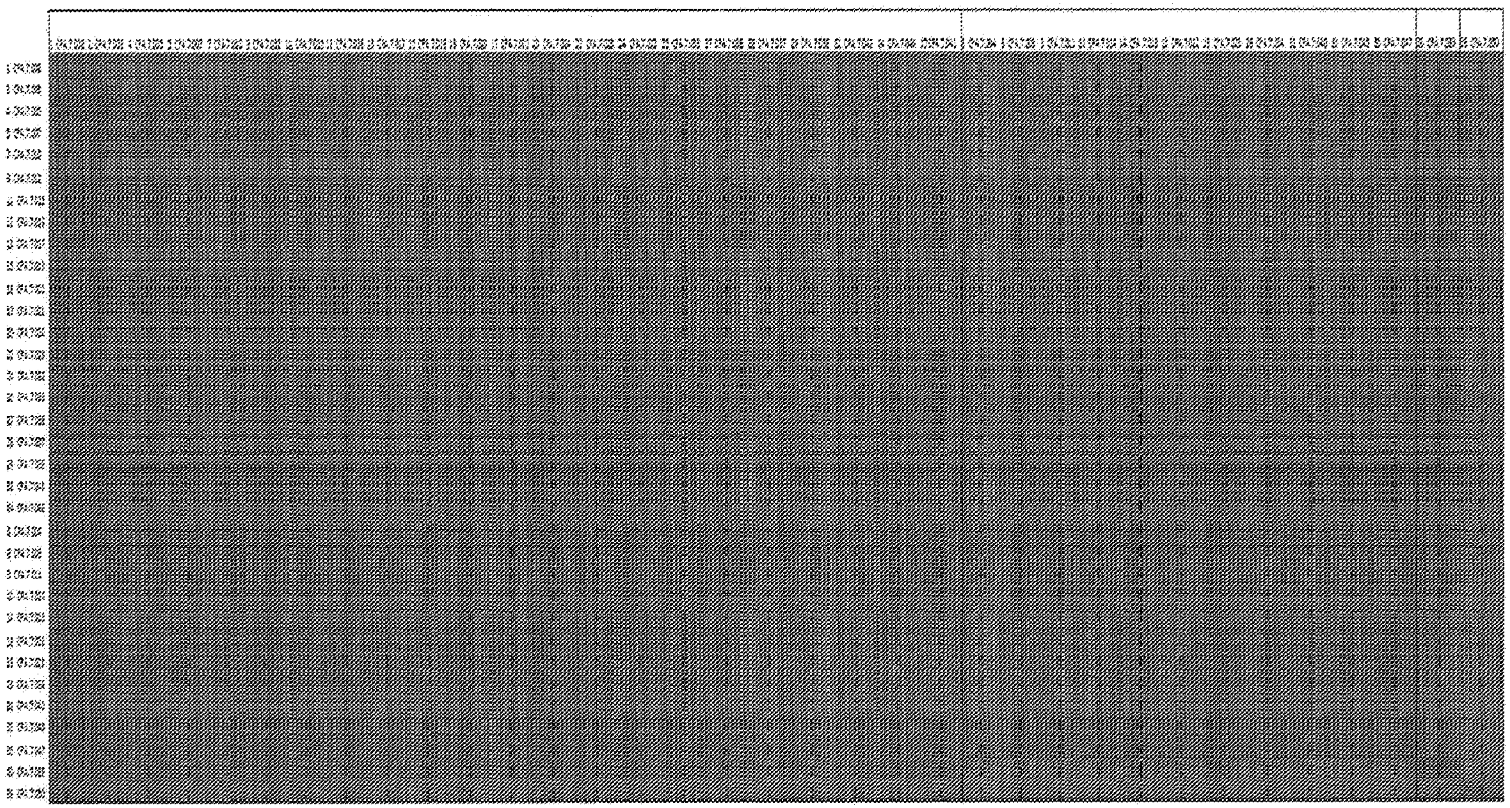
CHA.7.550

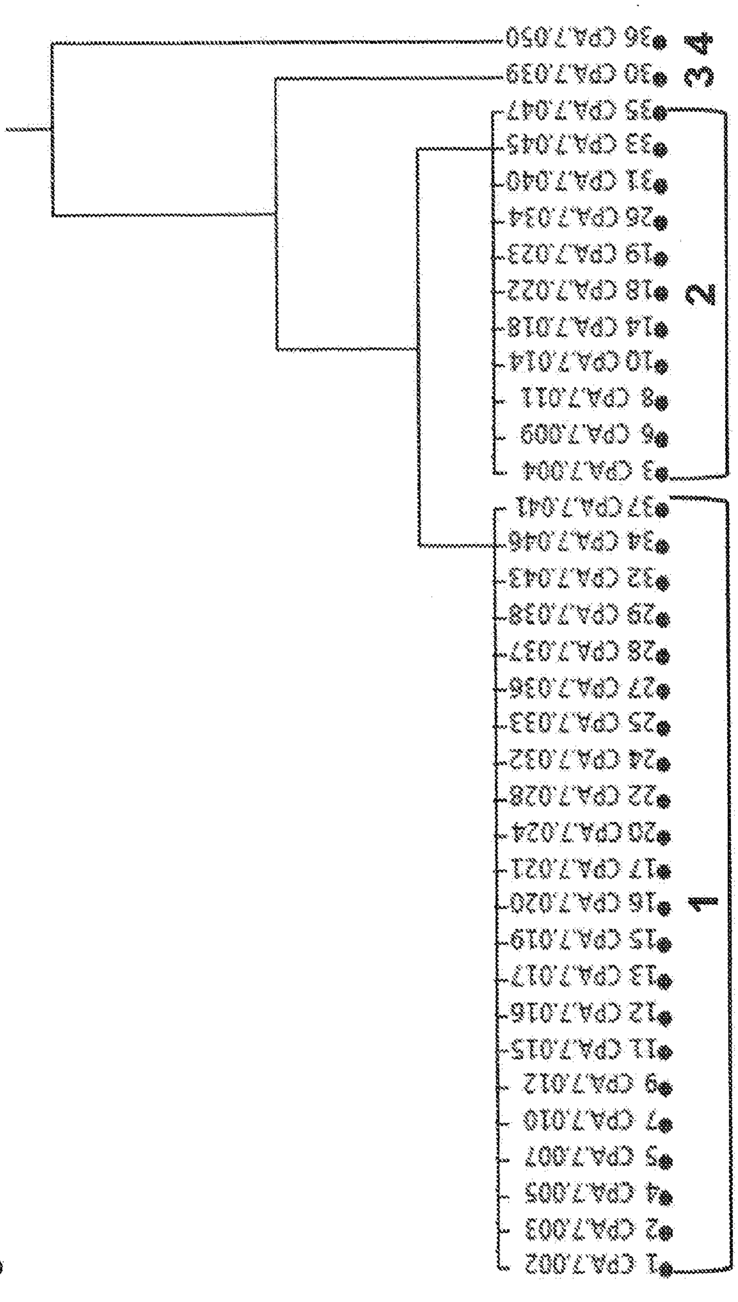
What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGTELVKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKAALTVDKSSSTAYMQLSSLTSEDSAVYYCARRGLPYFFDYWGQGTTLTVSS	1052
vhCDR1	GYTFTSYW	1053
vhCDR2	INPSNGGT	1054
vhCDR3	ARRGLPYFFDY	1055
Variable light (vl) domain	DIVMTQSQKFMSTSVGDRVSVTCKGSQNVGYNVAWYQQKPGQSPKALVYSASDRHSGVPDRFAGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPLTFGAGTKLELK	1056
vlCDR1	QNVGYN	1057
vlCDR2	SAS	1058
vlCDR3	QQYNSYPLT	1059

Figure 42

1	2	3	4
CPA.7.002	CPA.7.004	CPA.7.039	CPA.7.050
CPA.7.003	CPA.7.009		
CPA.7.005	CPA.7.011		
CPA.7.007	CPA.7.014		
CPA.7.010	CPA.7.018		
CPA.7.012	CPA.7.022		
CPA.7.015	CPA.7.023		
CPA.7.016	CPA.7.034		
CPA.7.017	CPA.7.040		
CPA.7.019	CPA.7.045		
CPA.7.020	CPA.7.047		
CPA.7.021			
CPA.7.024			
CPA.7.028			
CPA.7.032			
CPA.7.033			
CPA.7.036			
CPA.7.037			
CPA.7.038			
CPA.7.043			
CPA.7.046			
CPA.7.041			

Figure 43

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2 CPA.7.003
4 CPA.7.005
5 CPA.7.007
7 CPA.7.010
9 CPA.7.012
11 CPA.7.015
12 CPA.7.016
13 CPA.7.017
15 CPA.7.019
16 CPA.7.020
17 CPA.7.021
20 CPA.7.024
22 CPA.7.028
24 CPA.7.032
25 CPA.7.033
27 CPA.7.036
28 CPA.7.037
29 CPA.7.038
32 CPA.7.043
34 CPA.7.046
37 CPA.7.041
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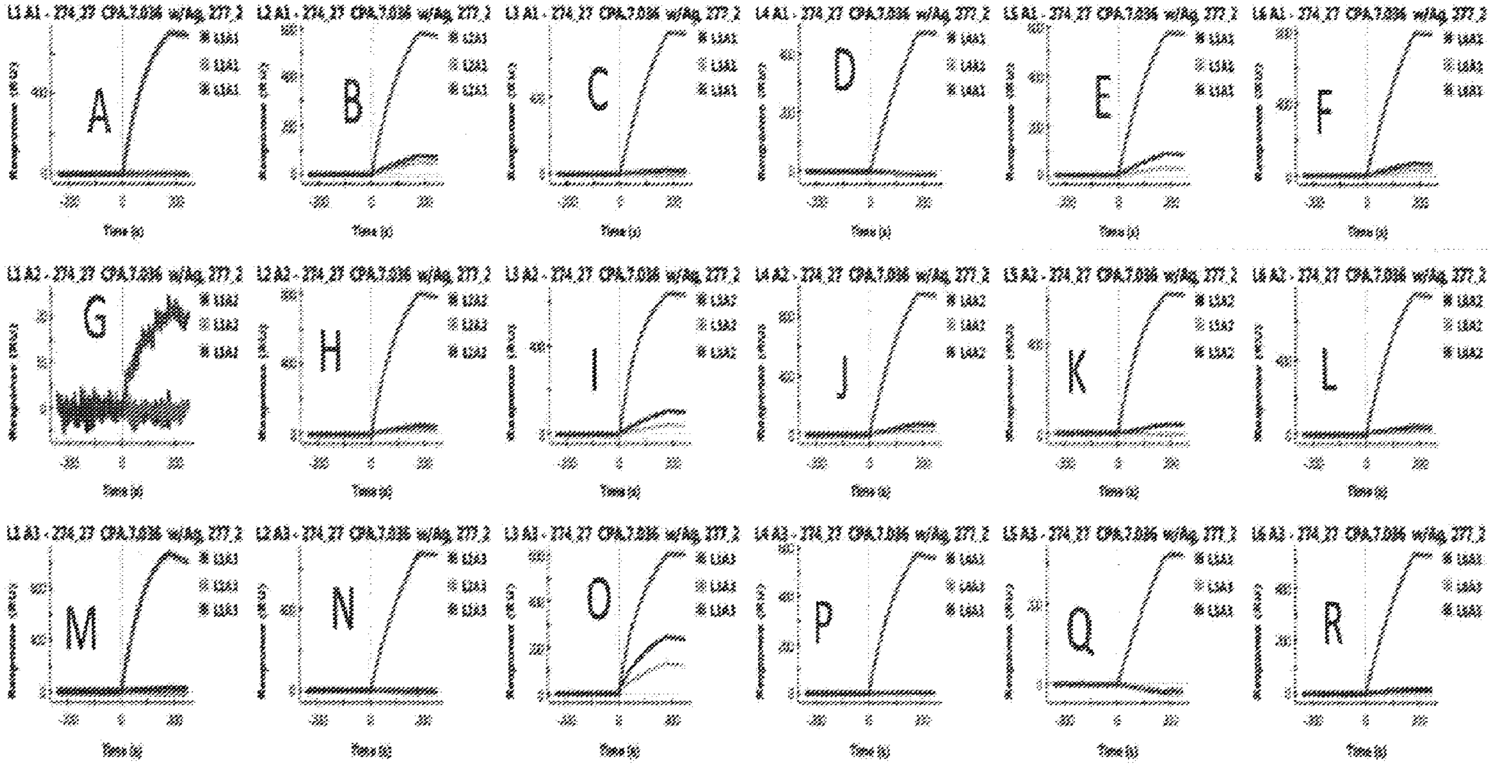
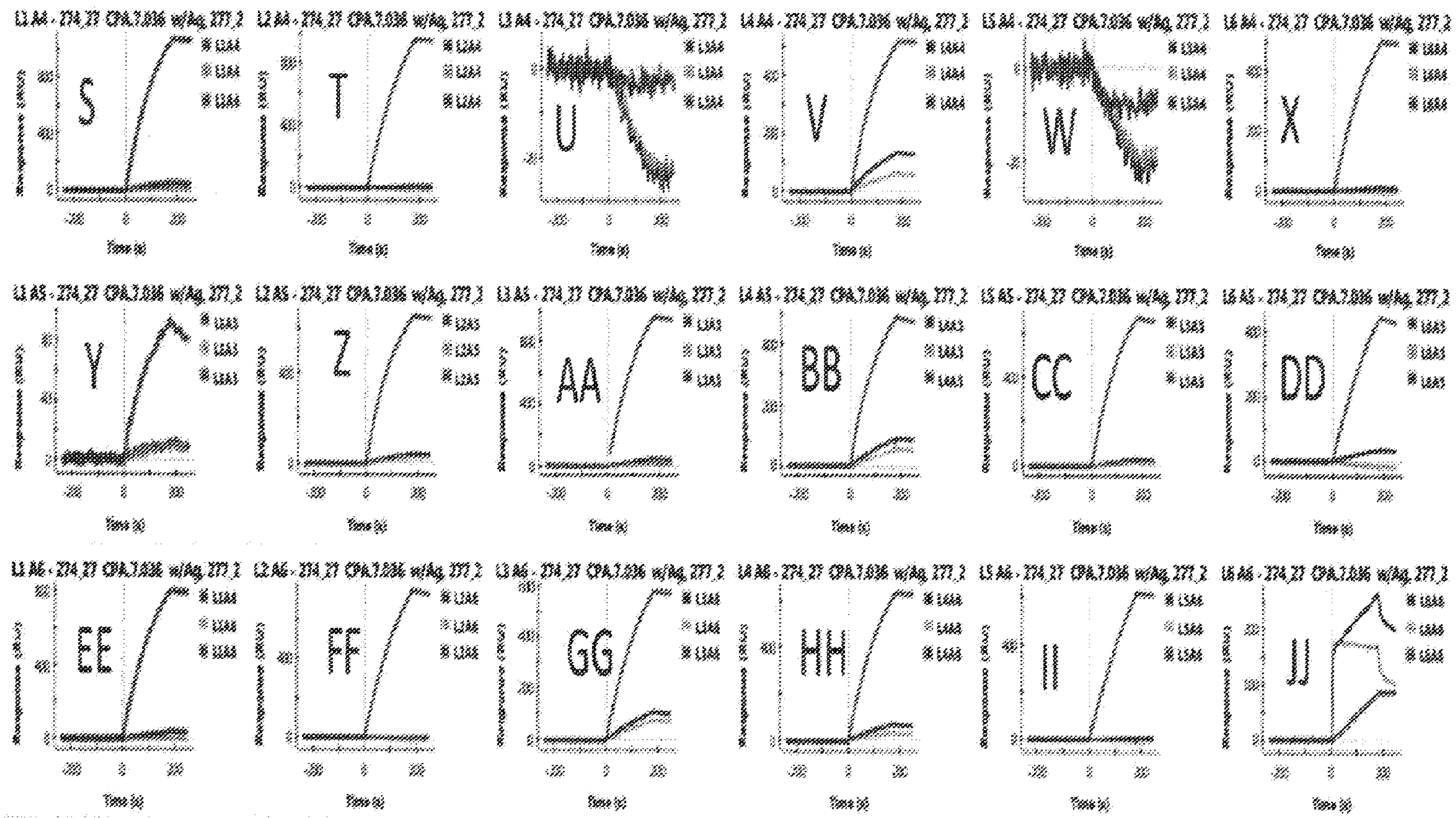
10 CPA.7.014
14 CPA.7.018
18 CPA.7.022
19 CPA.7.023
26 CPA.7.034
31 CPA.7.040
33 CPA.7.045
35 CPA.7.047
30 CPA.7.039
36 CPA.7.050
1
2
3
4

Figure 44

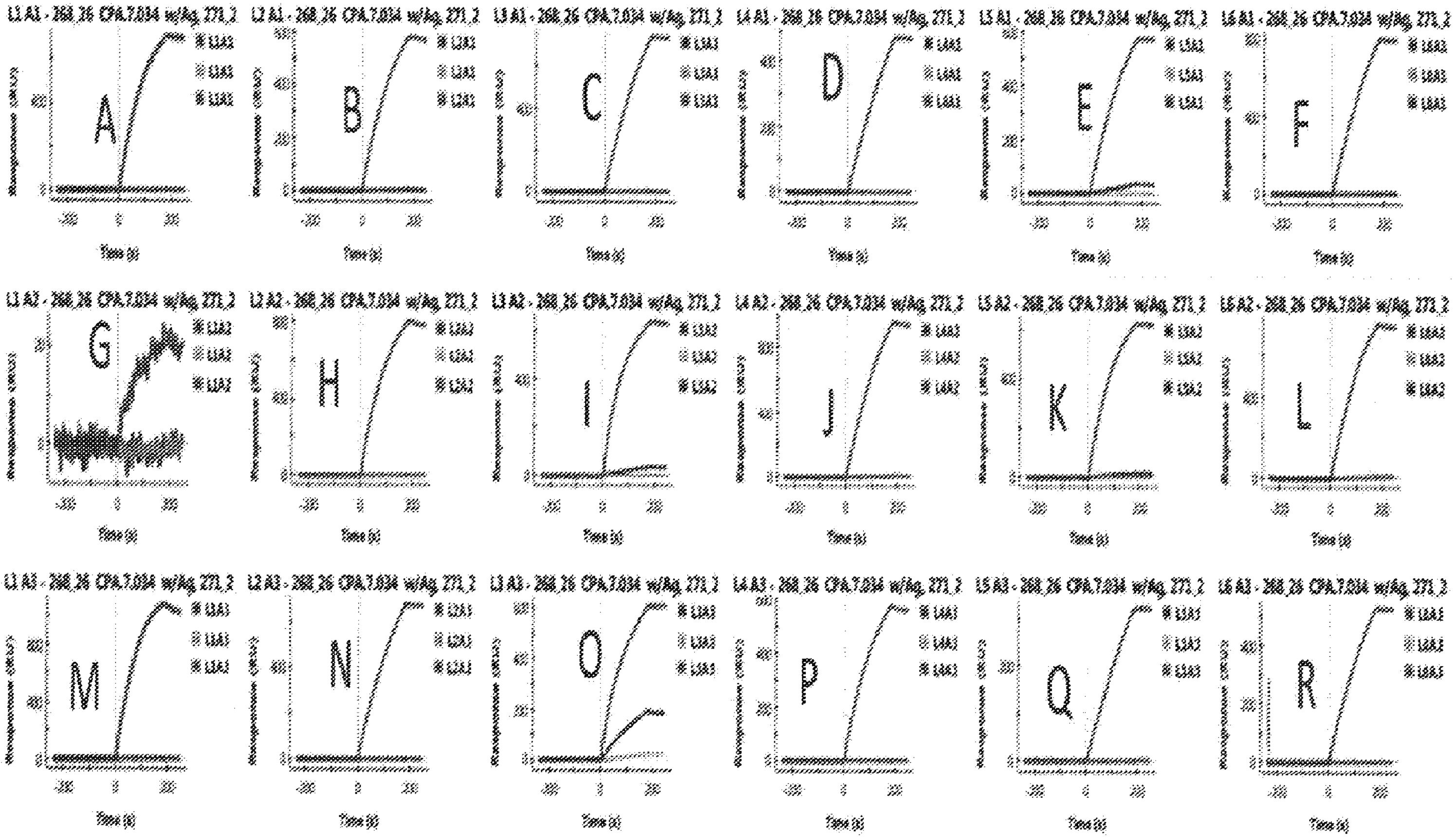
Figures 45A - 45R



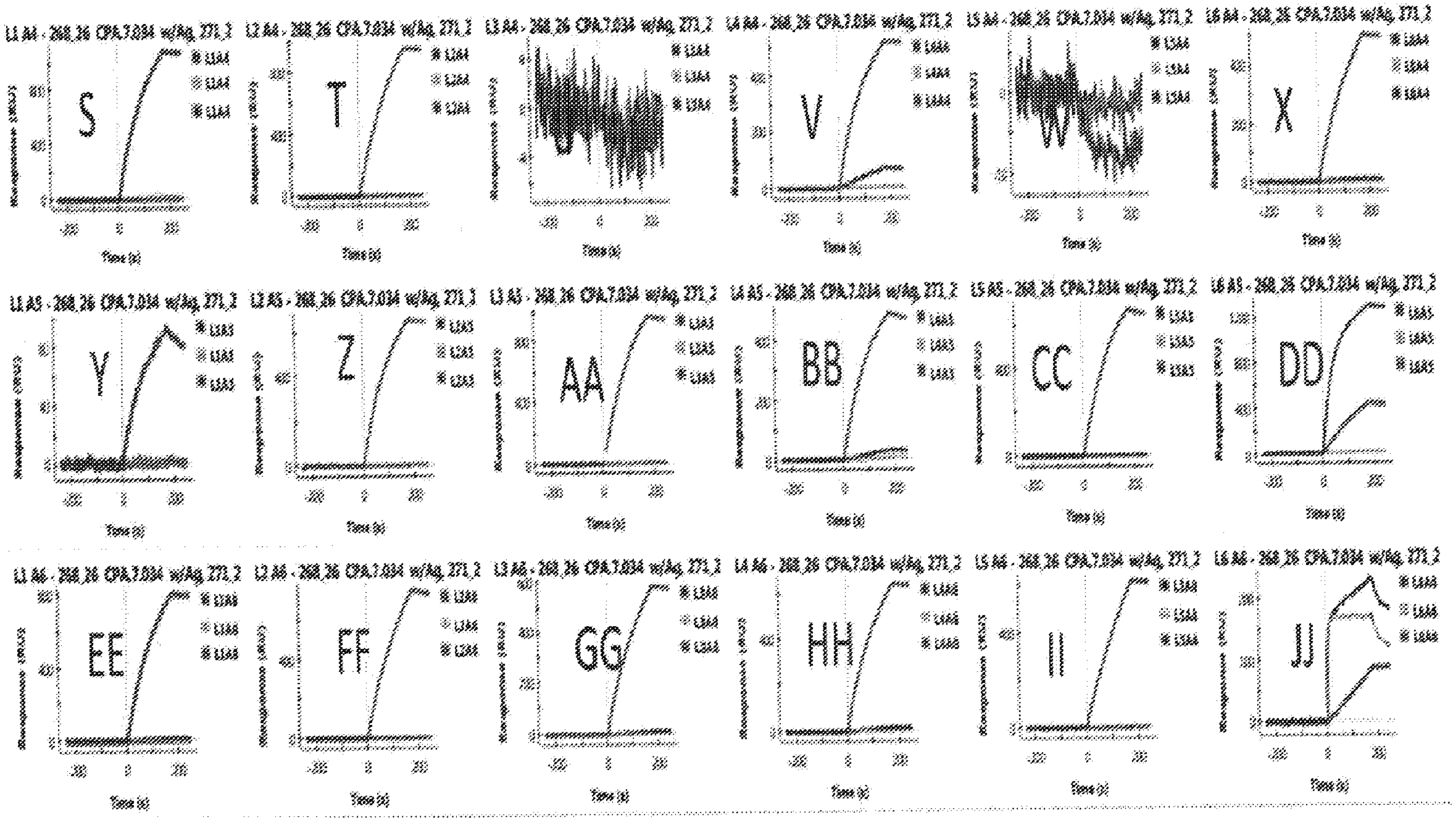
Figures 45S - 45JJ



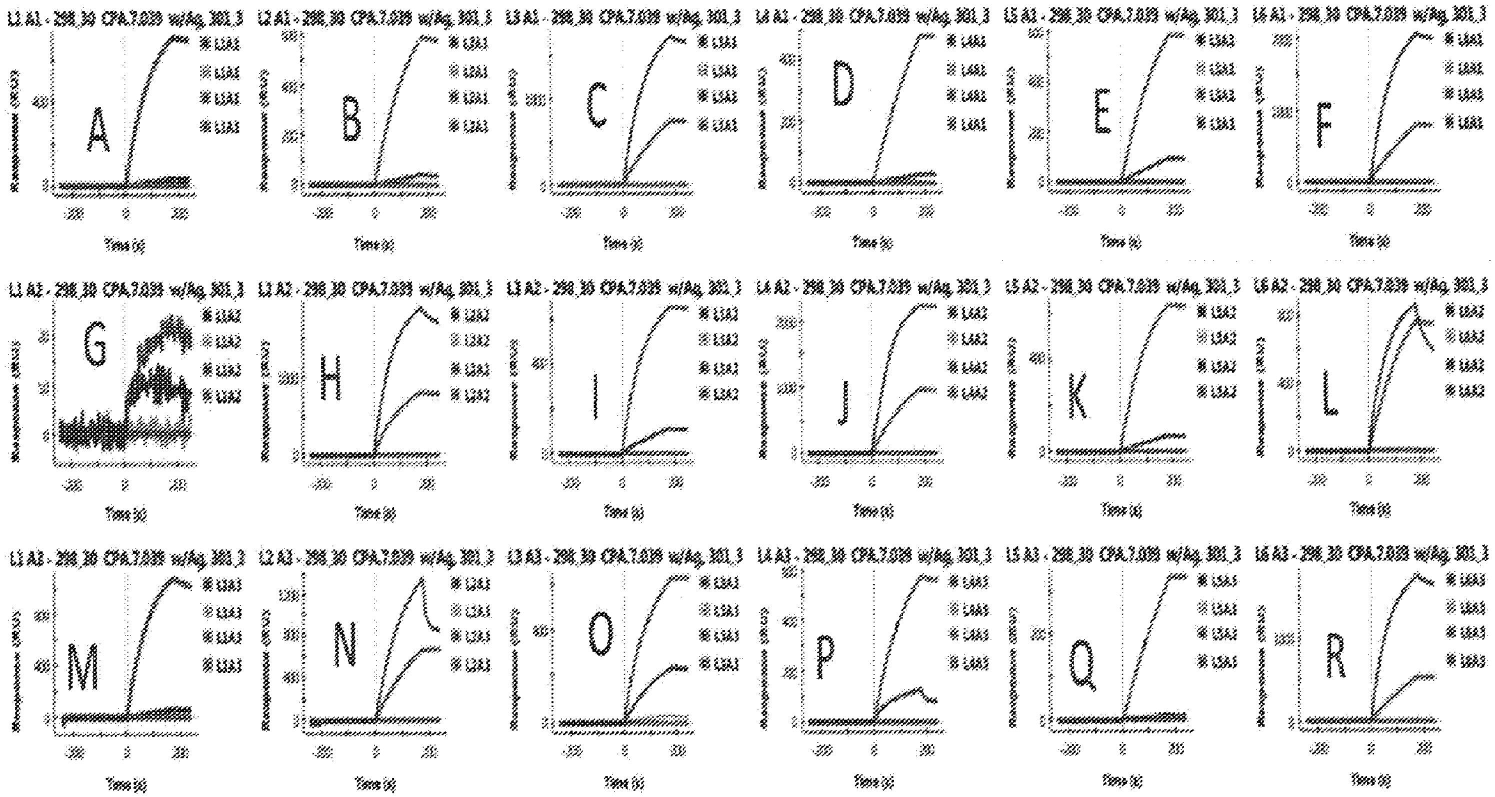
Figures 46A - 46R



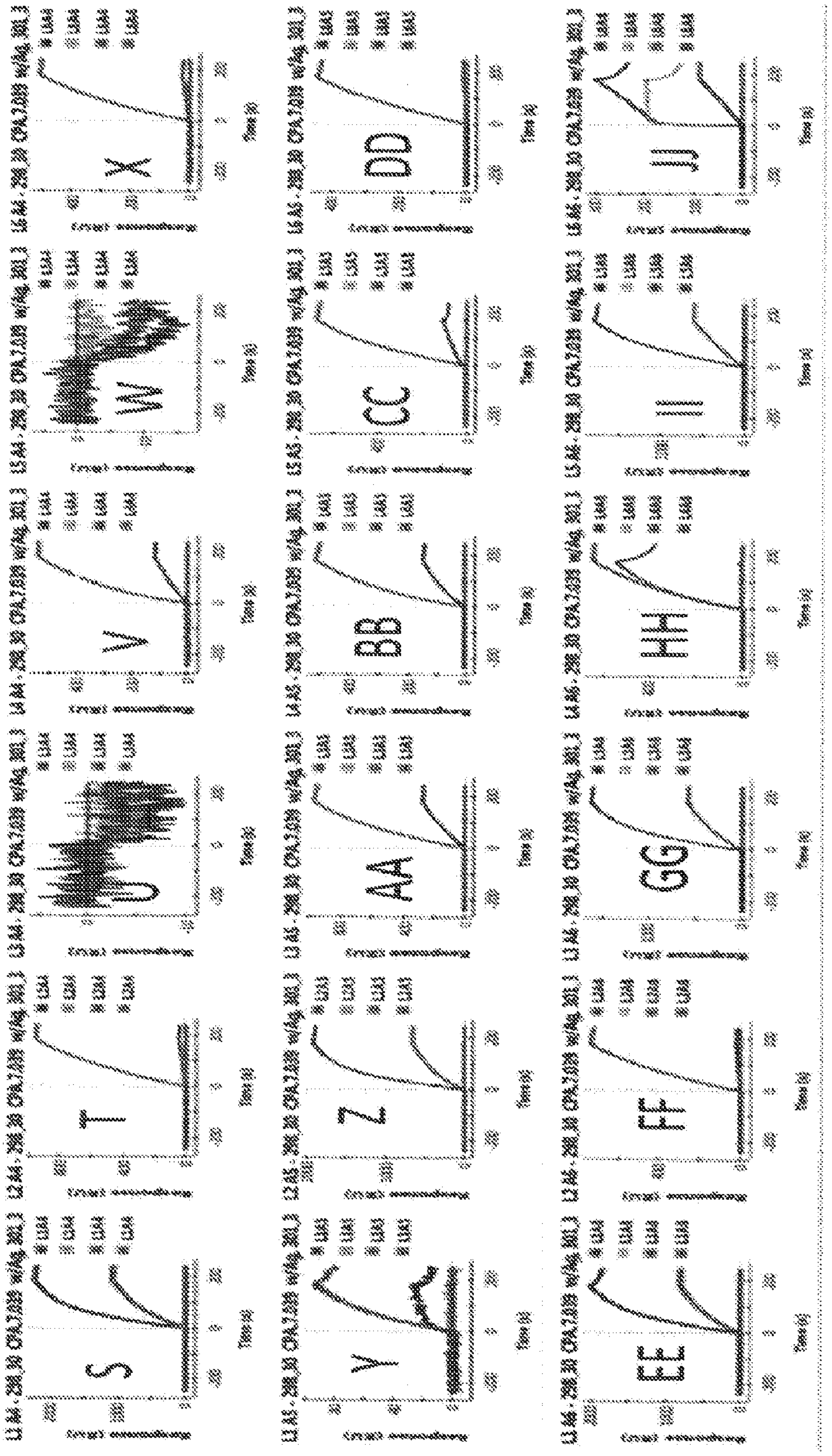
Figures 46S - 46JJ



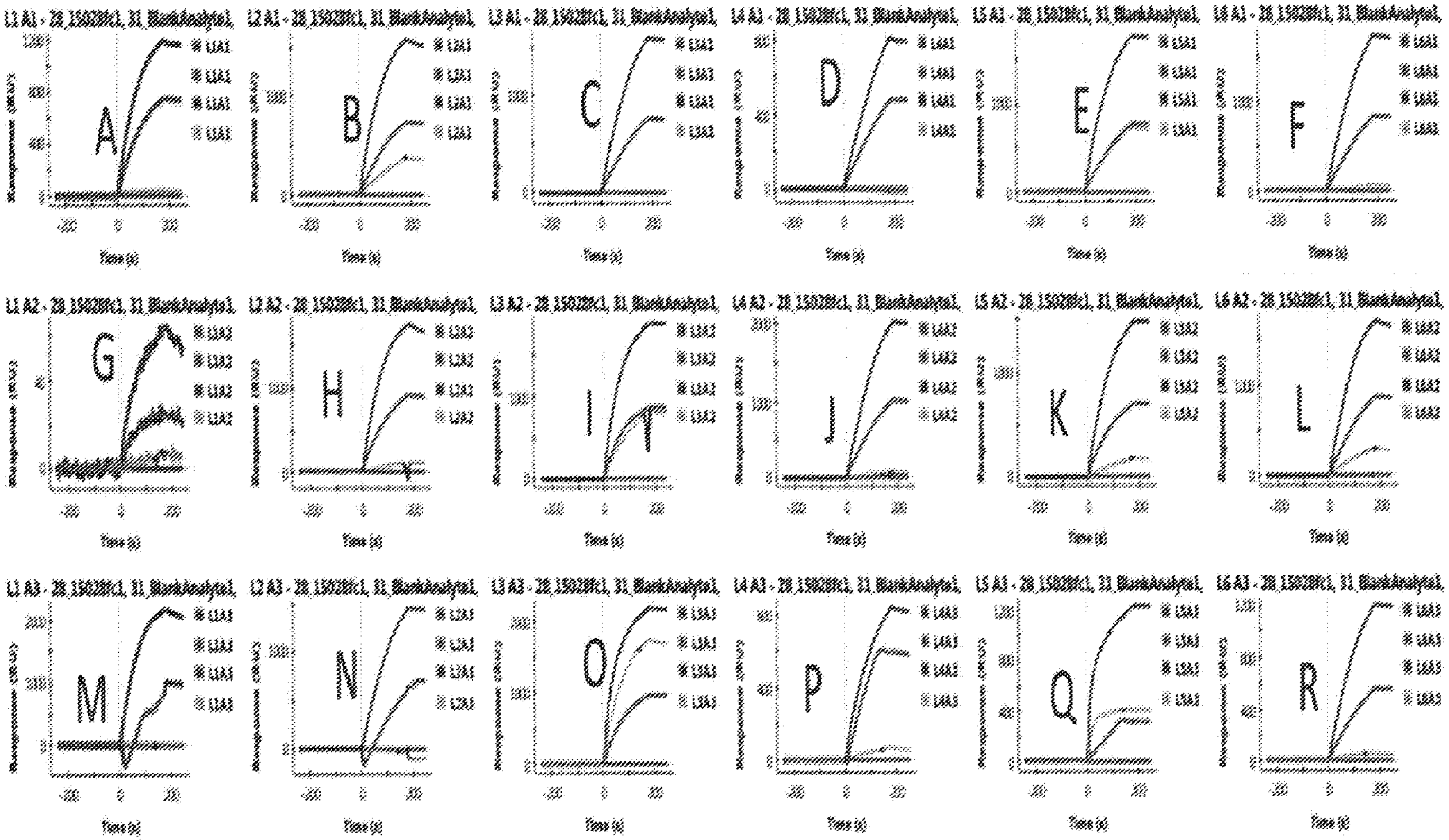
Figures 47A - 47R



Figures 47S - 47JJ



Figures 48A - 48R



Figures 48S - 48JJ

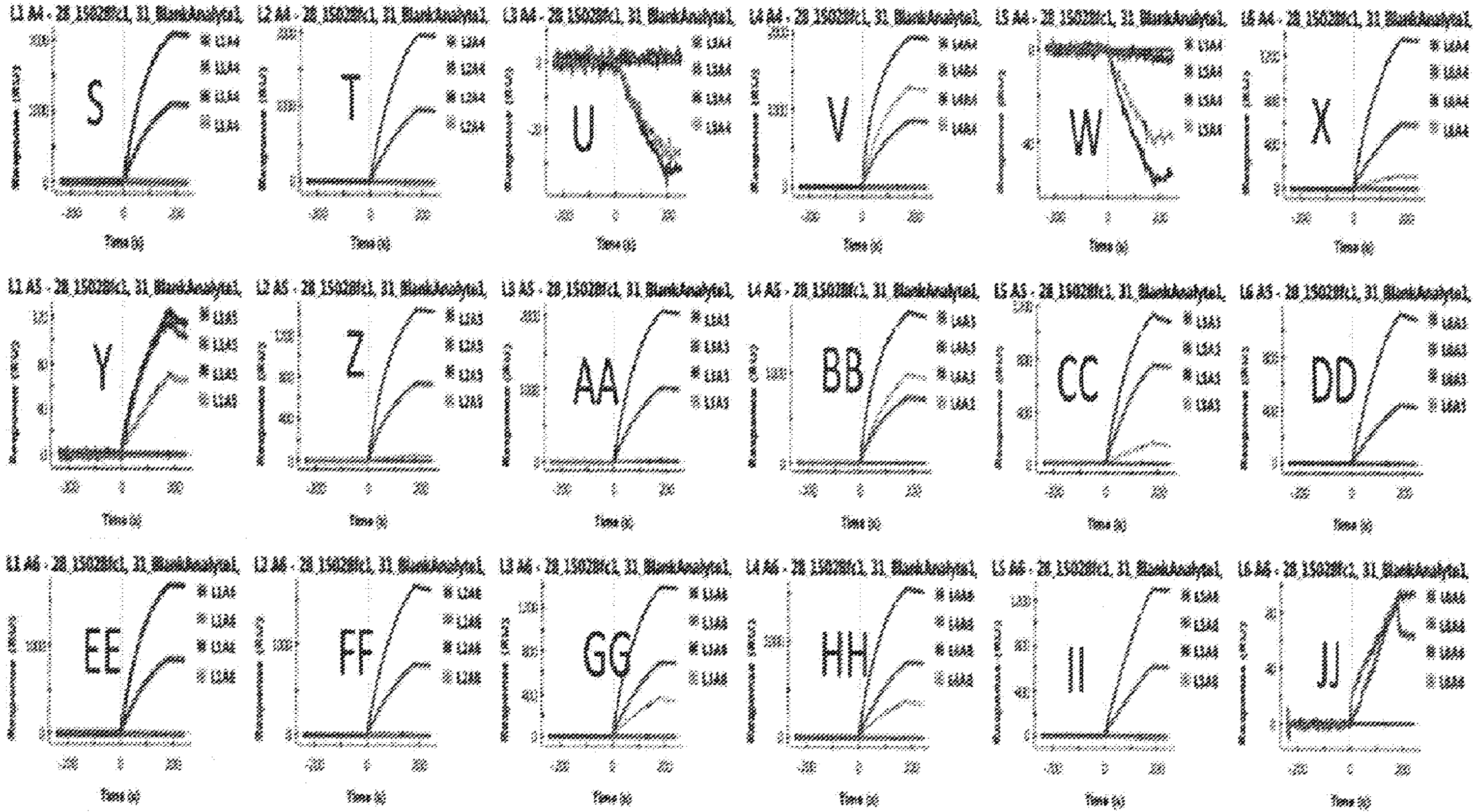


Figure 49

Table 1		k_a	k_d	K_D
Fab Clone	**Figure**	**1/M-s**	**1/s**	**M**
CPA.7.021	50A	2.20E+05	2.90E-05	1.32E-10
CPA.7.028	50B	3.08E+06	4.33E-04	1.41E-10
CPA.7.019	50C	8.59E+05	1.87E-04	2.18E-10
CPA.7.012	50D	1.51E+06	9.54E-04	6.32E-10
CPA.7.007	50E	3.24E+05	4.55E-04	1.41E-09
CPA.7.015	50F	3.13E+05	1.11E-03	3.55E-09
CPA.7.050	50G	7.08E+04	4.00E-04	5.65E-09
CPA.7.048	50H	1.35E+05	2.57E-03	1.90E-08
CPA.7.049	50I	1.51E+05	3.67E-03	2.44E-08
CPA.7.040	50J	1.33E+05	3.69E-03	2.77E-08
CPA.7.020	50K	1.34E+05	3.86E-03	2.88E-08
CPA.7.002	50L	4.59E+04	2.83E-03	6.16E-08
CPA.7.022	50M	3.55E+06	2.96E-01	8.33E-08
CPA.7.005	50N	5.22E+04	4.39E-03	8.41E-08
CPA.7.004	50O	2.12E+06	4.00E-01	1.89E-07
CPA.7.010	50P	9.06E+04	1.72E-02	1.89E-07
CPA.7.008	50Q	2.46E+04	2.03E-02	8.23E-07

Figure 50A-50Q

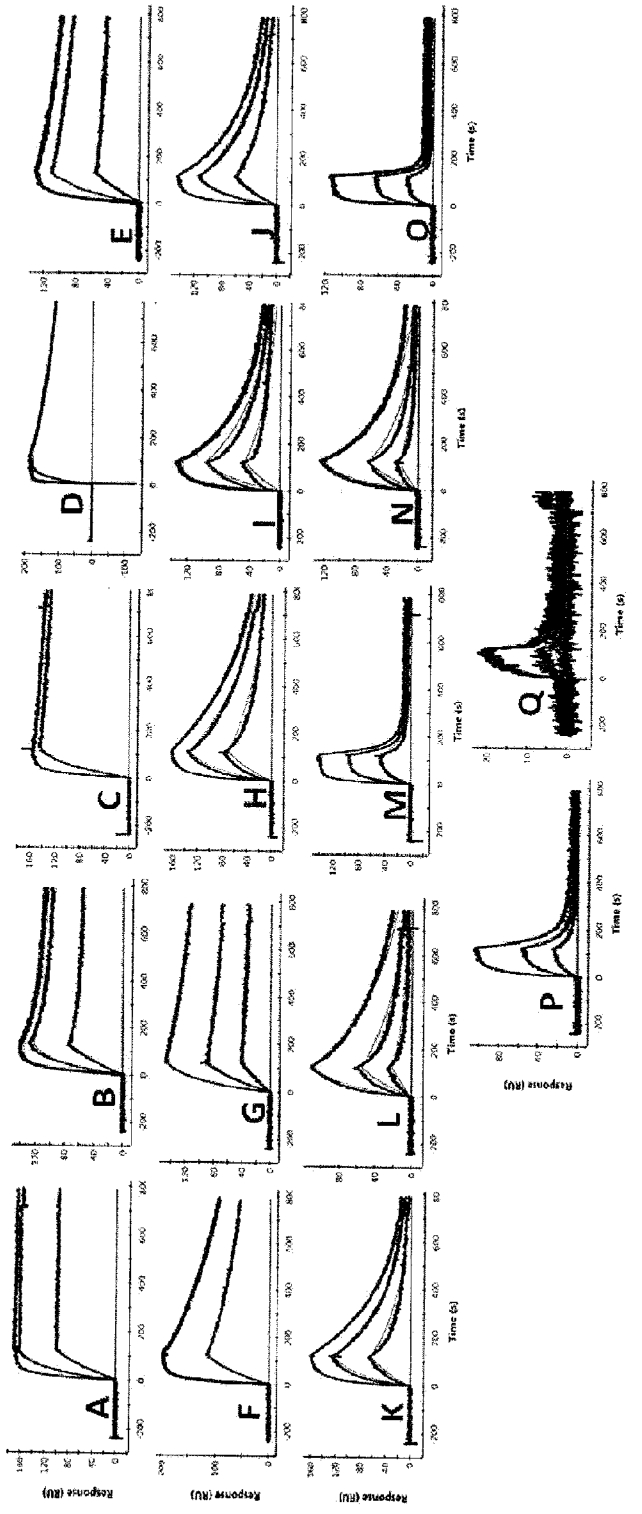


FIGURE 51A

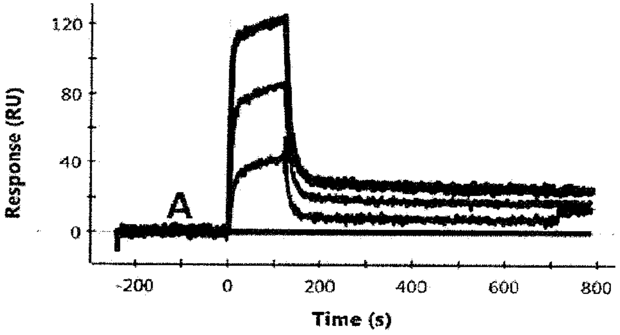
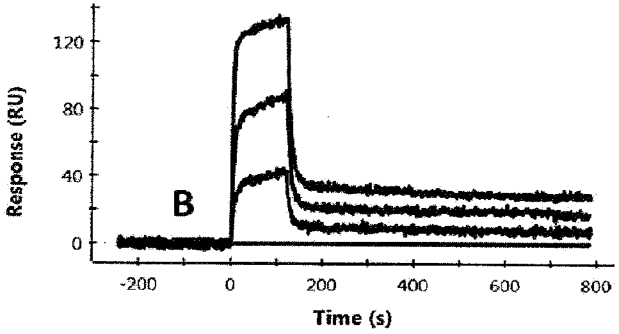


FIGURE 51B



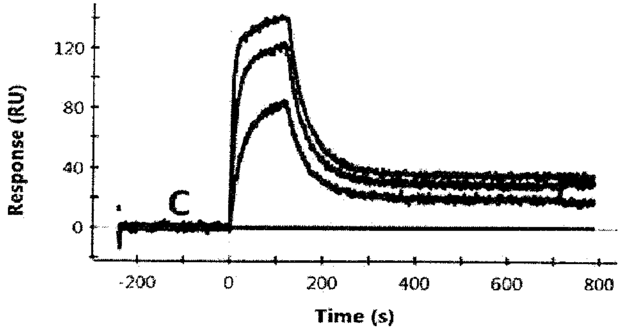


FIGURE 51C

Figure 52A

Antibody	**ELISA Signal**	**% Blocking**	**> 80% blocking**
No mab control	1.376	0	NO
CPA.7.001	0.099	93	YES
CPA.7.002	0.558	59	NO
CPA.7.003	0.153	89	YES
CPA.7.004	0.166	88	YES
CPA.7.005	1.551	-13	NO
CPA.7.006	0.134	90	YES
CPA.7.007	0.529	62	NO
CPA.7.008	0.281	80	YES
CPA.7.009	0.05	96	YES
CPA.7.010	0.141	90	YES
CPA.7.011	0.067	95	YES
CPA.7.012	0.045	97	YES
CPA.7.013	0.045	97	YES
CPA.7.014	0.044	97	YES
CPA.7.015	0.142	90	YES
CPA.7.016	1.553	-13	NO
CPA.7.017	0.197	86	YES
CPA.7.018	0.156	89	YES
CPA.7.019	0.146	89	YES
CPA.7.020	0.728	47	NO
CPA.7.021	0.122	91	YES
CPA.7.022	0.081	94	YES
CPA.7.023	0.047	97	YES
CPA.7.024	0.048	97	YES
CPA.7.025	1.159	16	NO
CPA.7.026	1.12	19	NO
CPA.7.027	1.181	14	NO
CPA.7.028	0.702	49	NO
CPA.7.029	1.272	8	NO
CPA.7.030	1.176	15	NO
CPA.7.031	0.673	51	NO
CPA.7.032	0.427	69	NO
CPA.7.033	0.186	86	YES

Figure 52B

Antibody	ELISA Signal	% Blocking	> 80% blocking
CPA.7.034	0.26	81	YES
CPA.7.035	1.16	16	NO
CPA.7.036	0.184	87	YES
CPA.7.037	0.915	34	NO
CPA.7.038	0.301	78	NO
CPA.7.039	0.541	61	NO
CPA.7.040	0.048	97	YES
CPA.7.041	0.356	74	NO
CPA.7.042	0.307	78	NO
CPA.7.043	0.773	44	NO
CPA.7.044	1.025	26	NO
CPA.7.045	0.93	32	NO
CPA.7.046	0.104	92	YES
CPA.7.047	0.223	84	YES
CPA.7.049	0.077	94	YES
CPA.7.050	0.174	87	YES

Figure 53

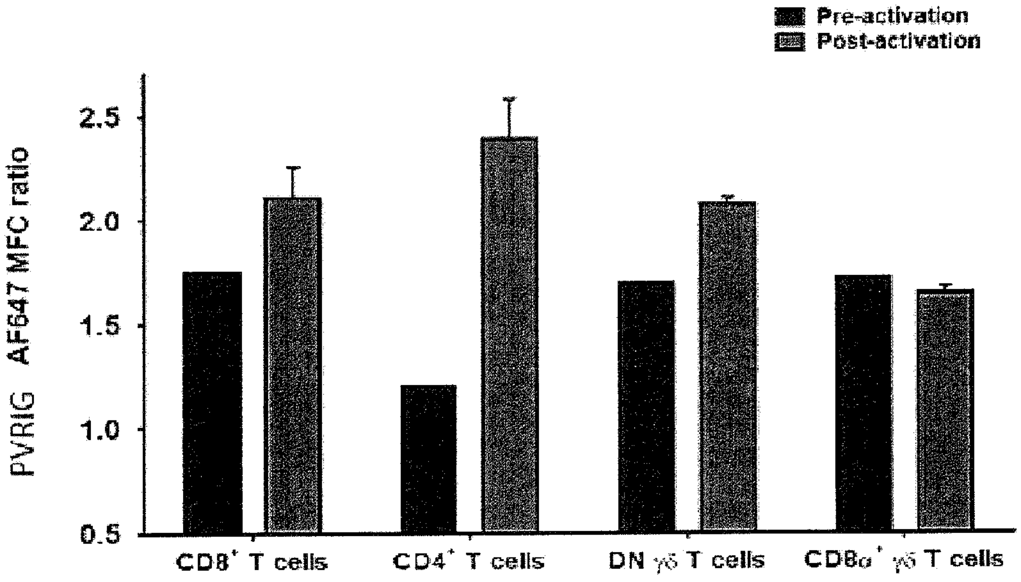

Pre-activation
Post-activation
PVRIG AF647 MFC ratio
2.5
2.0
1.5
1.0
0.5
CD8⁺ T cells
CD4⁺ T cells
DN γδ T cells
CD8α⁺ γδ T cells

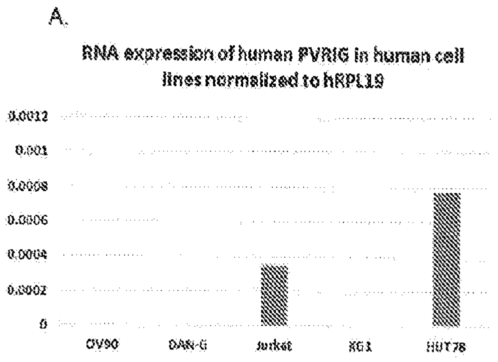
Figure 54

Cell line	ATCC No./CLS /ECACC No.	Morphology	Source
HL-60	ATCC, CCL-240	Leukemia	Blood
THP1	ATCC, TIB-202	Monocyte	Blood
KG-1	ATCC, CCL-246	Macrophage	Bone marrow
Jurkat	ATCC, TIB-152	T Cell	Blood
RPMI8226	ATCC, CCL-155	B lymphocyte	Blood
DAN-G	CLS, 300162	Epithelial	Pancreas
OV90	ATCC, CRL-11732	Epithelial	Ovary
NCI-H441	ATCC, CRL-174	Epithelial	Lung
TF1	ATCC, CRL-2003	Erythroblast	Bone Marrow
Capan2	ATCC, HTB-80	polygonal	Pancreas
ZR75-1	ATCC, CRL-1500	Epithelial	Mammary gland
Karpas299	ECACC, 06072604-1VL	Lymphoma	Blood
NK-YTS	ATCC, CL-273	Lymphoblast	Blood
H9	ATCC, HTB-176	Lymphoblast	Blood
K562	ATCC, CCL-243	Lymphoblast	Bone Marrow
NCI-H929	ATCC, CRL-9068	Lymphoblast	B Lymphocytes
BCP1	ATCC, CRL-2294	Lymphoblast	B Lymphoblast
HUT78	ATCC, TIB-161	Lymphoblast	Blood
HEK293	ATCC, CRL-3216	Epithelial	Kidney

Figure 55

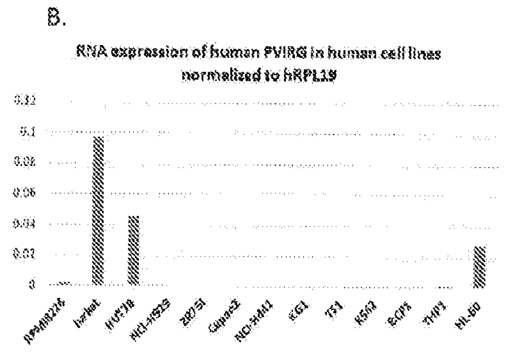
Cell line	ATCC No.	Morphology	Source
4T1	CRL-2539	Epithelial	Mammary gland
B16-F1	CRL-6323	Spindle-shaped+epithelial-like cells	Skin
EL4	TIB-39	T-lymphoblast	blood
E.G7-OVA	CRL-2113	T-lymphoblast	blood
YAC-1	TIB-160	T-lymphoblast	blood
A20	TIB-208	B-lymphoblast	blood
P815	TIB-64	Mast cells	blood
NIH/3T3	CRL-1658	fibroblast	embryo
Sal/N	CRL-2544	fibroblast	fibrosarcoma
J774A.1	TIB-67	Macrophage	blood
LL/2	CRL-1642	Epithelial	Lung
B104-1-1	CRL-1887	Fibroblast	Glioblastoma
RAW264.7	TIB-71	Macrophage	Abelson murine leukemia virus-induced tumor
P388D1	CCL-46	Macrophage	Lymphoblast
KLN205	CRL-1453	Epithelial	Lung
CT26	CRL-2638	Fibroblast	Colon
Renca	CRL-2947	Epithelial	Kidney

Figure 56A



Human cell line	Ct value	
	Human PVRIG	hRPL19
OV90	Undetermined	24.582
DAN-G	Undetermined	23.184
Jurkat	31.552	24.107
KG1	Undetermined	23.197
HUT78	29.626	23.192

Figure 56B



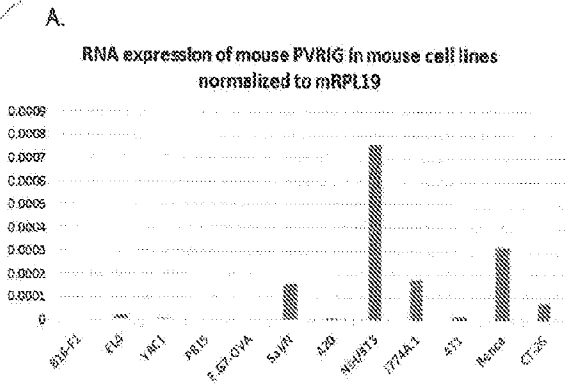
Human cell line	Ct value	
	Human PVIRG	hRPL19
RPMI8226	33.995	24.887
Jurkat	30.591	27.235
HUT78	29.688	25.225
NCI-H929	Undetermined	25.228
ZR751	Undetermined	23.889
Capan2	Undetermined	28.714
NCI-H441	Undetermined	24.886
KG1	Undetermined	25.835
TF1	38.133	25.988
K562	36.400	26.160
BCP1	Undetermined	25.860
THP1	34.678	24.615
HL-60	31.133	25.955

Figure 56C

C.

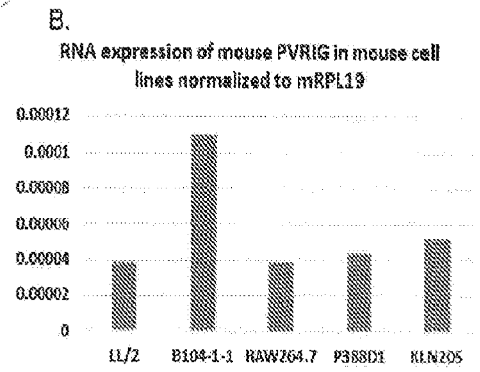
Human cell line	Ct value					
	Human PVIRG	hTATA box	hRPL19	hPBGD	hSDHA	hHPRT1
DAN-G	39.92	30.14	27.4	27.4	26.59	27.99
NCI-H441	39.46	28.34	26.71	26.71	25.2	27.36
KG1	38.95	28.39	26.74	28.28	26.39	26.39
TF1	39.22	29.25	27.61	33.74	36.25	35.25

Figure 57A



Mouse cell line	Ct value	
	Mouse PVRIG	mRPL19
B16-F1	38.531	18.549
EL4	34.033	18.740
YAC1	36.058	19.648
P815	38.159	19.417
E.G7-OVA	38.219	19.477
Sal/N	31.389	18.789
A20	35.520	18.740
NIH/3T3	30.862	20.506
J774A.1	31.457	18.996
4T1	34.722	19.013
Renca	31.237	19.632
CT-26	32.859	19.237

Figure 57B



Mouse cell line	Ct value	
	Mouse PVRIG	mRPL19
LL/2	33.401	18.732
B104-1-1	32.839	19.686
RAW264.7	34.116	19.445
P388D1	33.421	18.947
KLN205	33.698	19.472

Figure 58

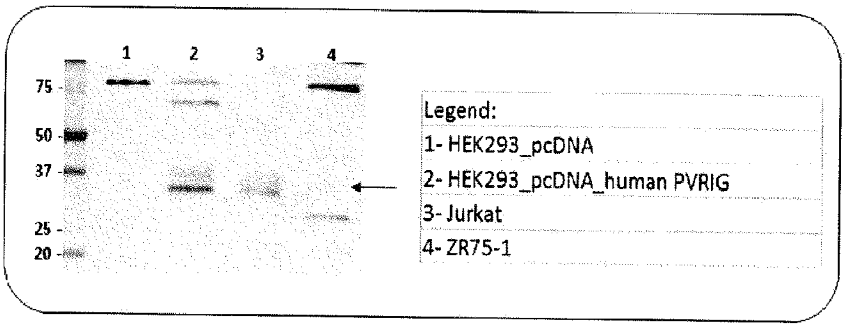
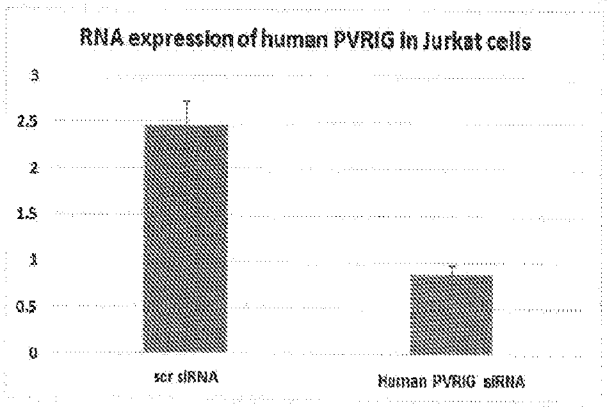

1
2
3
4
75
50
37
25
20
Legend:
1- HEK293_pcDNA
2- HEK293_pcDNA_human PVRIG
3- Jurkat
4- ZR75-1

Figure 59



Sample Name	Ct value		
	Human PVRIG	hHPRT1	hRPL19
Scr siRNA#1	30.622	21.608	21.924
Scr siRNA#2	30.526	21.553	21.915
Scr siRNA#3	30.820	22.227	22.156
hPVRIG siRNA#1	32.923	22.658	22.462
hPVRIG siRNA#2	32.678	22.859	22.542
hPVRIG siRNA#3	32.927	22.598	22.779

Figure 60

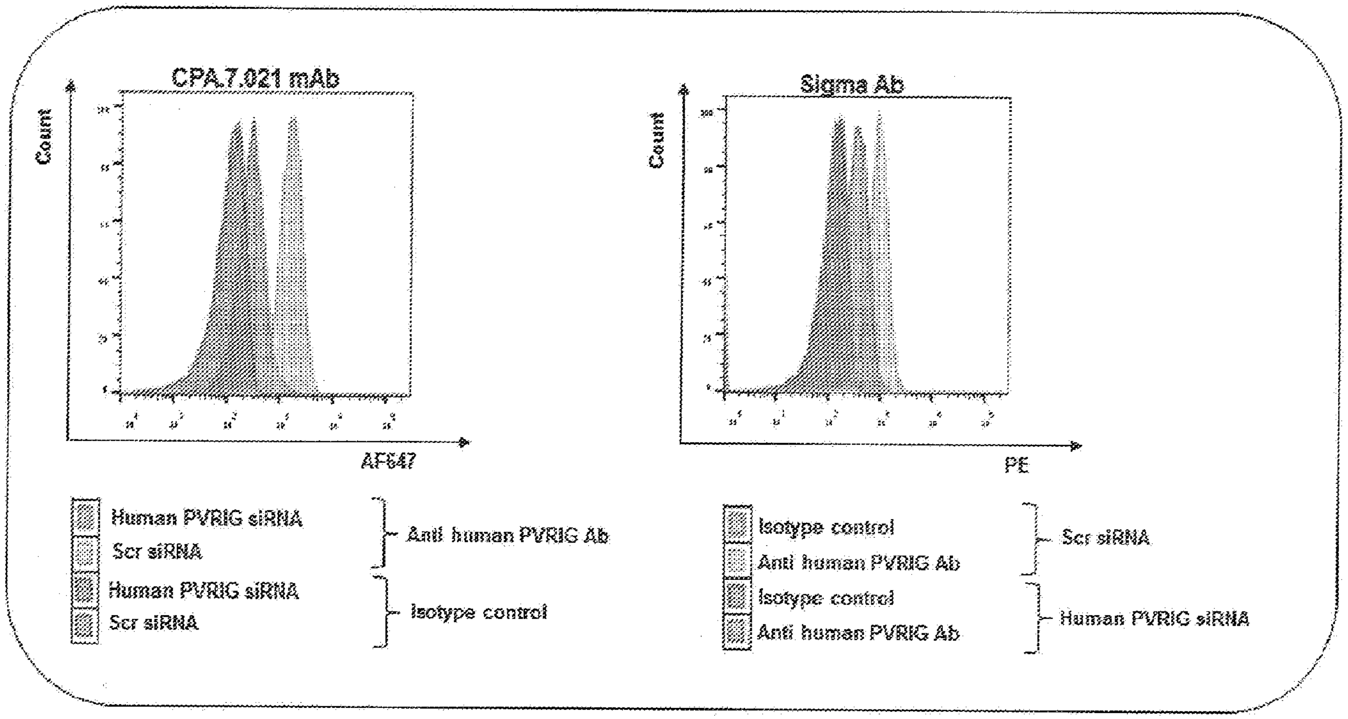

CPA.7.021 mAb
Count
AF647
Human PVRIG siRNA
Scr siRNA
Human PVRIG siRNA
Scr siRNA
Anti human PVRIG Ab
Isotype control
Sigma Ab
Count
PE
Isotype control
Anti human PVRIG Ab
Isotype control
Anti human PVRIG Ab
Scr siRNA
Human PVRIG siRNA

Figure 61

Cell line	qRT-PCR	FACS	WB	%KD-RNA	%KD-FACS
	(PVRIG Ct / HSKG Ct)	(fold change from isotype)			
OV90	Undetermined/24.582	NT	NT		
DAN-G	Undetermined/23.184	NT	NT		
Jurkat	31.552/24.107	14	+	65	82
KG1	Undetermined/23.197	NT	NT		
HUT78	29.626/23.192	X	NT		
RPMI8226	33.995/24.887	NT	NT		
NCI-H929	Undetermined/25.228	NT	NT		
ZR75-1	Undetermined/23.889	X	X		
Capan2	Undetermined/28.714	X	NT		
NCI-H441	Undetermined/24.886	NT	NT		
TF1	38.133/25.988	NT	NT		
K562	36.400/26.160	NT	NT		
BCP1	Undetermined/25.860	NT	NT		
THP1	34.678/24.615	NT	NT		
HL60	31.133/25.955	NT	NT		
Karpas299	NT	X	NT		
NK-YTS	NT	X	NT		

Figure 62

Cell line	qRT-PCR (PVRIG Ct / HSKG Ct)	FACS (fold change from isotype)	WB	%KD-RNA	%KD-FACS
4T1	34.722/19.013	NT	NT		
B16-F1	38.531/18.549	NT	NT		
EL4	34.033/18.740	NT	NT		
YAC-1	36.058/19.646	NT	NT		
P815	38.159/19.417	NT	NT		
E.G7-OVA	38.219/19.477	NT	NT		
NIH3T3	29.94 / 21.25	X	NT		
SAI/N	31.389/18.789	X	NT		
J774A.1	31.487/18.996	X	NT		
A20	35.520/18.740	NT	NT		
LL/2	33.401/18.732	NT	NT		
B104-1-1	32.839/19.686	NT	NT		
RAW264.7	34.116/19.445	NT	NT		
P388D1	33.421/18.947	NT	NT		
KLN205	33.698/19.472	NT	NT		
Renca	31.237/19.632	X	NT		

Figure 63A

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.001	GGTFSSYA	1060	IIPIFGTA	1061	AREEVSSPYGMDV	1062	TGAVTSGHY	1063	DTG	1064	LLSYSGASWV	1065
CPA.7.002	GGTFSSSA	1066	IIPIYGIT	1067	ARDDTARRVRGVPYYYYYAMDV	1068	QGIGNY	1069	SAS	1070	QQLKDYPIT	1071
CPA.7.003	GFSLSHFS	1072	FDPEEGGT	1073	ATGIWYSSGWPVDY	1074	QSLLDSSGYNY	1075	LGS	1076	MQALQTPIT	1077
CPA.7.004	GYTLTELS	1078	FDPEDGET	1079	ATVSRVRGVINYYYYMDV	1080	QSLLYRNGNNY	1081	LGS	1082	MQALQTPPT	1083
CPA.7.005	GGTFSSSA	1084	IIPIYGIT	1085	ARDDTARRVRGVPYYYYYAMDV	1086	QSVDSS	1087	DAS	1088	QQYKDWPFT	1089
CPA.7.006	GGTFGTYA	1090	ITPISATI	1091	ARGFEYSDGLLDD	1092	QSLFYSDDGNTY	1093	RLS	1094	MQHMEFPLT	1095
CPA.7.007	GGSISSSSYY	1096	IYYSGST	1097	ARGAWELRLGDWFDP	1098	SSNIGAGYD	1099	GNN	1100	QSYDSSLSIYVV	1101
CPA.7.008	SGSISSTNW	1102	IYHSGST	1103	ARVGPAAIYY	1104	SNNVGYEG	1105	RNN	1106	SAWDSSLNAVV	1107
CPA.7.009	GYTLTELS	1108	FDPEDGET	1109	ATAKPGIAVAGQNYYYYYMDV	1110	QSLLYRNGNNY	1111	LGS	1112	MQALQTPPT	1113
CPA.7.010	GFTFSSYA	1114	ISYDGSNK	1115	ASSPIGYSYGYWGGMDV	1116	SGIDVRTNK	1117	FQSDSDK	1118	LIWHTSGWV	1119
CPA.7.011	GYTLTELS	1120	FDPEDGET	1121	ATGPAAAGVGYYYYMDV	1122	QSLLYRNGYNY	1123	LGS	1124	MQALQTPPT	1125
CPA.7.012	GFTFSSYA	1126	ISYDGSNK	1127	ARDVMVYCSSTSCYFYGMDV	1128	QDIRDY	1129	DAS	1130	QQFENLPIT	1131
CPA.7.013	GYTLTELS	1132	FDPEDGET	1133	ATGGYSSGFNYYYYYMDV	1134	QSLLYRNGNNY	1135	LGS	1136	MQALQTPPT	1137
CPA.7.014	GYTLTELS	1138	FDPEDGET	1139	ATGVTTYYYYGMDV	1140	QSLLYSNGNNF	1141	LGS	1142	MQALQTPPT	1143
CPA.7.015	GFTFSSYG	1144	IRYDGSNK	1145	ARDLFDFWWDGMDV	1146	QSVSSMY	1147	GAS	1148	QQYVSSPMYT	1149

Figure 63B

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.016	GGTFSSSA	1150	IIPIYGIT	1151	ARDDTARRVRGVPYYYYYAMDV	1152	QSVSSY	1153	DAS	1154	QQYDDWPQT	1155
CPA.7.017	GGTFNNYG	1156	IIPLFGTT	1157	ARDRMAADGMAVFDY	1158	SSNIGRHF	1159	KND	1160	SSWDAALNGVV	1161
CPA.7.018	GYTLTELS	1162	FDPEDGET	1163	ATEVPMVRGARRYYYYMDV	1164	QTLLYINENNY	1165	LGS	1166	MQGLQTPPT	1167
CPA.7.019	GGSISNSNYY	1168	IYYSGST	1169	ARGAWELSLGDWFDP	1170	SSNIGAGYD	1171	GNN	1172	QSYDSSLSVYVV	1173
CPA.7.020	GGTFSSSA	1174	IIPIYGIT	1175	ARDDTARRVRGVPYYYYYAMDV	1176	QNVYGE	1177	DTF	1178	QQRRDWPIT	1179
CPA.7.021	GFTFGTSS	1180	ISFDGTEI	1181	AKGSGNIYFYSGMDV	1182	QSISGW	1183	ETS	1184	QQYYSYPLT	1185
CPA.7.022	GYTLTELS	1186	FDPEDGET	1187	ATGVPAAIGVYYYYYYMDV	1188	QSLLYSNGYNY	1189	LGS	1190	MQALQSPVT	1191
CPA.7.023	GYTLTELS	1192	FDPEDGET	1193	ATDSRDGPAARGGYYYYMDV	1194	QSLLYINGYNY	1195	LGS	1196	MQALQTPPT	1197
CPA.7.024	GGTFSSYA	1198	IIPIFGTA	1199	ARDAYYYDSSGYYNPDAFDI	1200	QSLLHSNGYNY	1201	LGS	1202	MQGLQTPRT	1203
CPA.7.025	GFSLTSGGMS	1204	IDWNDDK	1205	ARIRGMTWGFDS	1206	QSVSSSY	1207	GAS	1208	QQYGTTPFA	1209
CPA.7.026	GYTFTAYY	1210	INPNSGGT	1211	ARDGAFYYGSENYYNAGWFDP	1212	QSLLSGNGYNY	1213	LGS	1214	MQALKSPLT	1215
CPA.7.027	GYTFTNYY	1216	INPSGGIT	1217	ARAGLGYNWNYAPSGMDV	1218	SSDVGGYNY	1219	EVS	1220	SSYAGSNNLV	1221
CPA.7.028	GGSISSSSYY	1222	IYYSGST	1223	ARGAWELRLGDWFDP	1224	SSNIGAGYD	1225	GYS	1226	QSYDSSLSVYVV	1227
CPA.7.029	GFTFDDYA	1228	ISWNSGSI	1229	AKDVNRILVAGMVDY	1230	QSLLHSNGYNY	1231	LGS	1232	MQGLQTPIT	1233
CPA.7.030	RFTFEDYA	1234	ISWKSGGI	1235	VKDPTLVATDRAFNI	1236	QSLLHSNGYNY	1237	LGS	1238	MQYLQTPDT	1239

Figure 63C

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.031	GGTFSSSA	1240	IIPIYGIT	1241	ARDDTARRVRGVPYYYYYAMDV	1242	QSLLDSDDGNIH	1243	TLS	1244	MQRKEFPLT	1245
CPA.7.032	GFTFSSYG	1246	ISYDGSNK	1247	AKEDRLRFLEWLFYGMDV	1248	HDIYTY	1249	DAS	1250	QQYDNHPPEVT	1251
CPA.7.033	GGTFSSSA	1252	IIPIYGIT	1253	ARDDTARRVRGVPYYYYYAMDV	1254	QDIDDD	1255	EAS	1256	LQHDNLPLT	1257
CPA.7.034	GYTLTELS	1258	FDPEDGET	1259	ATEDPGPVAGPYYYYGMDV	1260	QSLLYINGYHY	1261	LGS	1262	MQALQTPPT	1263
CPA.7.035	GYMFTNYP	1264	INAGTGNT	1265	AREGMFYYGLESYYKGGWFDP	1266	QSLLHRNGYNY	1267	LAS	1268	MQALQTPLT	1269
CPA.7.036	GGTFSSSA	1270	IIPIYGIT	1271	ARDDTARRVRGVPYYYYYAMDV	1272	QSLLDSDDGNTY	1273	TLS	1274	MQRLQFPLT	1275
CPA.7.037	TYTFTTYY	1276	IYPSGGNT	1277	VRDQNYYYSAMDV	1278	QDIRNY	1279	DAS	1280	QQFENLPIT	1281
CPA.7.038	GGTFSSSA	1282	IIPIYGIT	1283	ARDDTARRVRGVPYYYYYAMDV	1284	RDISDS	1285	DAS	1286	HQYDNLPLT	1287
CPA.7.039	GFNFRGYA	1288	ISGSGGTT	1289	AQSYAQIGYGGHIDH	1290	SGIDVATYM	1291	YKSDSDK	1292	LIWHGSHYV	1293
CPA.7.040	GYTLTELS	1294	FDPEDGET	1295	ATGVPAAIGVYYYYYYMDV	1296	QSLLYRNGYNY	1297	WGS	1298	MQAVQNPPT	1299
CPA.7.041	GGTFSSSA	1300	IIPIYGIT	1301	ARDDTARRVRGVPYYYYYAMDV	1302	QNIDSS	1303	YAS	1304	HQSSSLPLT	1305
CPA.7.042	GGTFSSSA	1306	IIPIYGIT	1307	ARDDTARRVRGVPYYYYYAMDV	1308	QSVYNNY	1309	DAS	1310	QQYNSWPPYT	1311
CPA.7.043	GGTFSSYA	1312	IIPIFGTA	1313	ARDAYYYDSSGYYNPDAFDI	1314	QSLLYSNGYNY	1315	LGS	1316	MQARQTPYT	1317
CPA.7.044	GGTFSSSA	1318	IIPIYGIT	1319	ARDDTARRVRGVPYYYYYAMDV	1320	ESVTTF	1321	DAS	1322	HQHTNWPLT	1323
CPA.7.045	GYTLTELS	1324	FDPEDGET	1325	ATEVGYCSGGSCYISYYYGMDV	1326	QSLLYRNGHNF	1327	LGS	1328	MQALQTPPT	1329
CPA.7.046	GGTFSSSA	1330	IIPIYGIT	1331	ARDDTARRVRGVPYYYYYAMDV	1332	QTMNNY	1333	DAS	1334	QQYGDWLPIT	1335

Figure 63D

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.047	GYTLTELS	1336	FDPEDGET	1337	ATAFPEATISYYYYMDV	1338	QSLLYRNGYNY	1339	WGS	1340	MQAVQNPPT	1341
CPA.7.049	GGTFSSSA	1342	IIPIYGIT	1343	ARDDTARRVRGVPYYYYYAMDV	1344	RSLLDSDDGNTH	1345	SLS	1346	MQRKEFPLT	1347
CPA.7.050	GGTFSSYA	1348	IIPIFGTA	1349	ARGPWYYDSSGYSSYAYYMDV	1350	QSLLHSDGYNY	1351	LGS	1352	MQALHTPGVT	1353

Figure 64A

Disease	**CD8A**	**CD4**	**CD3G**	**PD-1**
Kidney renal clear cell carcinoma	0.89	0.75	0.83	0.9
Sarcoma	0.87	0.64	0.88	0.82
Liver hepatocellular carcinoma	0.86	0.6	0.78	0.63
Skin Cutaneous Melanoma	0.86	0.77	0.87	0.89
Head and Neck squamous cell carcinoma	0.85	0.72	0.82	0.86
Breast invasive carcinoma	0.84	0.67	0.81	0.85
Testicular Germ Cell Tumors	0.84	0.66	0.88	0.84
Stomach adenocarcinoma	0.8	0.66	0.78	0.78
Mesothelioma	0.79	0.63	0.8	0.67
Thyroid carcinoma	0.79	0.72	0.87	0.83
Lung squamous cell carcinoma	0.78	0.65	0.77	0.81
Kidney Chromophobe	0.77	0.6	0.7	0.71
Pancreatic adenocarcinoma	0.77	0.57	0.76	0.8
Rectum adenocarcinoma	0.77	0.69	0.68	0.72
Uterine Corpus Endometrial Carcinoma	0.77	0.68	0.75	0.7

Figure 64B

Bladder Urothelial Carcinoma	0.76	0.68	0.81	0.82
Lung adenocarcinoma	0.76	0.52	0.74	0.77
Prostate adenocarcinoma	0.76	0.73	0.72	0.76
Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.74	0.64	0.76	0.7
Colon adenocarcinoma	0.74	0.71	0.75	0.67
Kidney renal papillary cell carcinoma	0.69	0.51	0.66	0.61
Uterine Carcinosarcoma	0.69	0.4	0.59	0.58
Ovarian serous cystadenocarcinoma	0.67	0.64	0.66	0.6
Esophageal carcinoma	0.63	0.57	0.66	0.65
Acute Myeloid Leukemia	0.58	-0.15	0.56	0.29
Pheochromocytoma and Paraganglioma	0.57	0.33	0.42	0.49
Adrenocortical carcinoma	0.55	0.49	0.66	0.64

Figure 65

Antibodies	Isotype	Conjugated to	Manufacturer	Catalog number	concentration (ug/ul)	Staining concentration
Anti-human PVRIG - CPA.7.021	Human IgG2	AF-647	Compugen	CPA.7.021	0.2	7.5 ug/ml
Human IgG2 isotype control	Human IgG2	AF-647	Compugen		0.2	7.5 ug/ml
Anti-CD4	mIgG1	FITC	Biolegend	300506	0.4	4 ug/ml
Anti-CD8	mIgG1	FITC	Biolegend	300906	0.15	1.5 ug/ml
Anti-CD137	mIgG1	PE	Biolegend	309804	0.2	2 ug/ml
Anti-PD1	mIgG1	APC-cy7	Biolegend	329922	0.2	4 ug/ml
isotype control	mIgG1	PE	Biolegend	400112	0.4	2 ug/ml
isotype control	mIgG1	APC-cy7	Biolegend	400128	0.2	4 ug/ml

Figure 66A

SEQ ID NO:
1354 (IgG1)
1355 (IgG2)
1356 (IgG3)
1357 (IgG4)

CH1

EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S

EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N

EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y

EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N

EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K	S	C
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G

Hinge								Fc	>												
EU Index	221			222	223	224	225	226	227	228											
IgG1	D			K	T	H	T	C	P	P											
IgG2				V		E		C	P	P											
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P
IgG4						P	P	C	P	S											

EU Index																					
IgG1																					
IgG2																					
IgG3	P	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P
IgG4																					

															Fc	>					
EU Index														229	230	231	232	233	234	235	236
IgG1														C	P	A	P	E	L	L	G
IgG2														C	P	A	P	P	V	A	
IgG3	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P	A	P	E	L	L	G
IgG4														C	P	A	P	E	F	L	G

Figure 66B

SEQ ID NO:
1354 (IgG1)
1355 (IgG2)
1356 (IgG3)
1357 (IgG4)

CH2

EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P

EU Index	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278
IgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y
IgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y
IgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y
IgG4	E	V	T	C	V	V	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y

EU Index	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299
IgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG2	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T
IgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T

EU Index	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320
IgG1	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG2	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K
IgG3	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG4	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K

EU Index	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG4	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K

CH3

EU Index	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N

EU Index	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382
IgG1	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG2	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG3	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG4	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E

EU Index	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403
IgG1	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S
IgG2	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S
IgG3	S	S	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S
IgG4	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S

Figure 66C

SEQ ID NO:	EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424
1354 (IgG1)	IgG1	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
1355 (IgG2)	IgG2	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
1356 (IgG3)	IgG3	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	I	F	S
1357 (IgG4)	IgG4	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S

EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445
IgG1	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG3	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P
IgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L

EU Index	446	447
IgG1	G	K
IgG2	G	K
IgG3	G	K
IgG4	G	K

Figure 67

PVRIG ECD Fragment A TTLAVLHPERGIRQWAPARQA (SEQ ID NO: 1358)

PVRIG ECD Fragment B TTLAVLHPERGIRQWAPARQARWETQSSISLILE (SEQ ID NO: 1359)

PVRIG ECD Fragment C ARQARWETQSSISLILE (SEQ ID NO: 1360)

PVRIG ECD Fragment D TFCCKFASFPEGSWEA (SEQ ID NO: 1361)

PVRIG ECD Fragment E TFCCKFASFPEGSWEACGSLPPSS (SEQ ID NO: 1362)

PVRIG ECD Fragment F EATELSSFTIRCGFL (SEQ ID NO: 1363)

PVRIG ECD Fragment G EVWVQVRMEATELSSFTIRCGF (SEQ ID NO: 1364)

PVRIG ECD Fragment H FTIRCGFLGSGSISLVTVS (SEQ ID NO: 1365)

PVRIG ECD Fragment I SISLVTVSWGGPNGAGGTTLAVLH (SEQ ID NO: 1366)

PVRIG ECD Fragment J SISLILEGSGASSPCANTTFCCKFAS (SEQ ID NO: 1367)

Figure 68

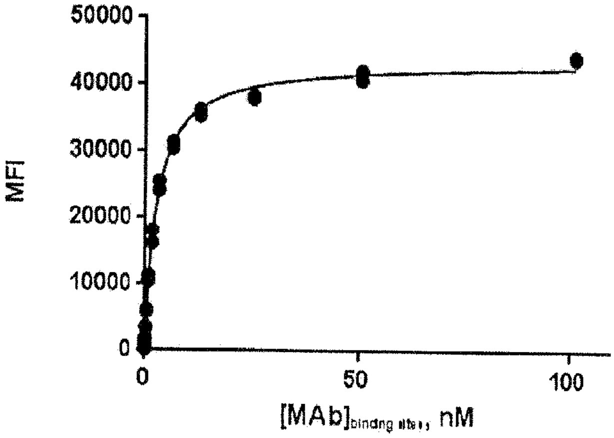

50000
40000
30000
20000
10000
0
MFI
0
50
100
[MAb]binding sites, nM

Figure 69A

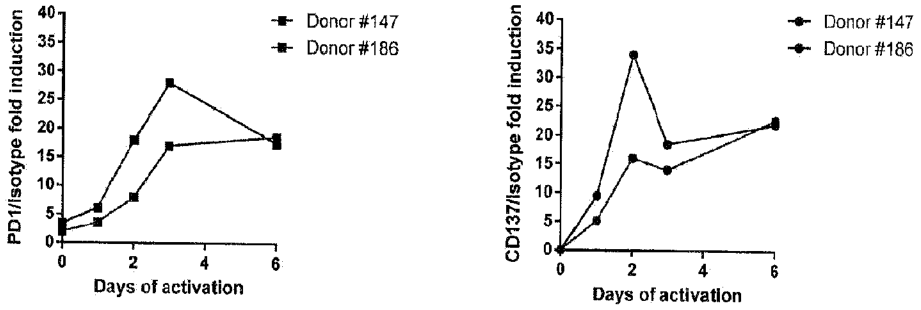


Figure 69B

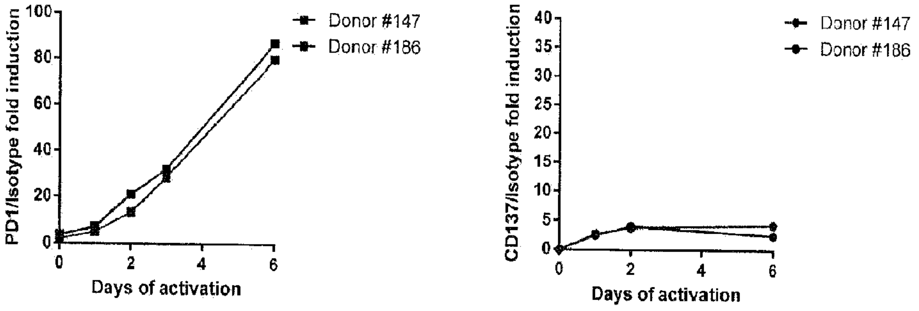


Figure 69C

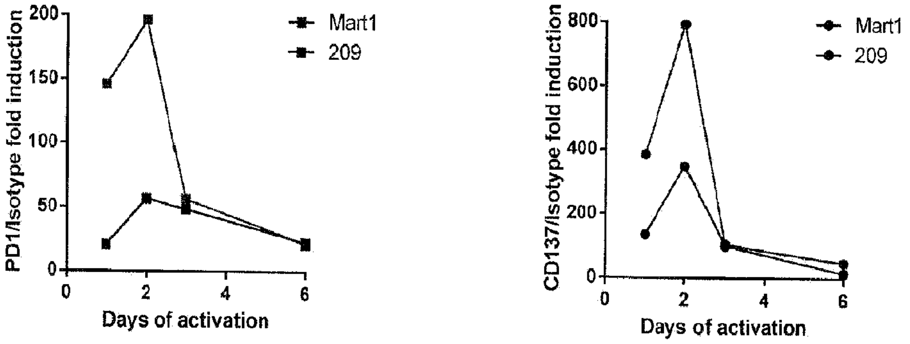


Figure 70A

A

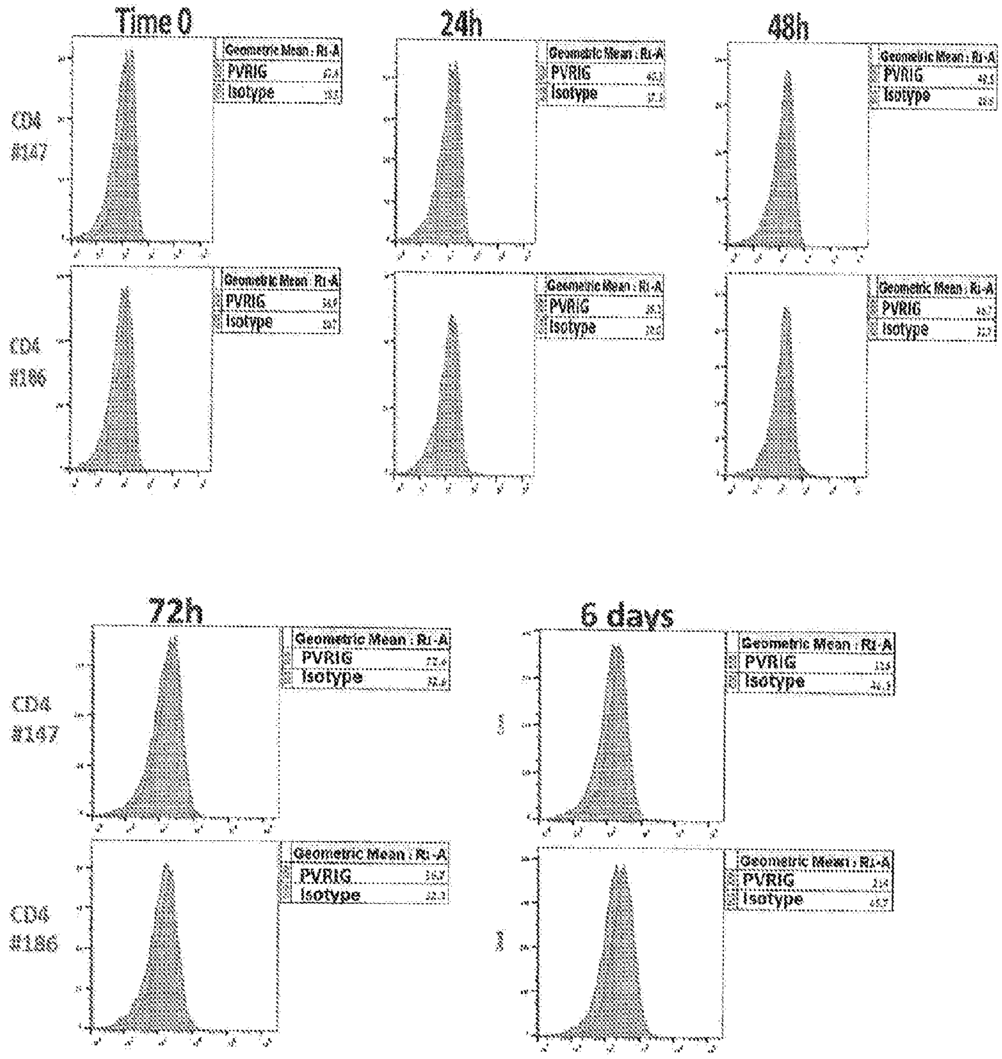

Time 0
24h
48h
CD4 #147
CD4 #186
Geometric Mean : R1-A
PVRIG
Isotype
72h
6 days
CD4 #147
CD4 #186
Geometric Mean : R1-A
PVRIG
Isotype

Figure 70B

B

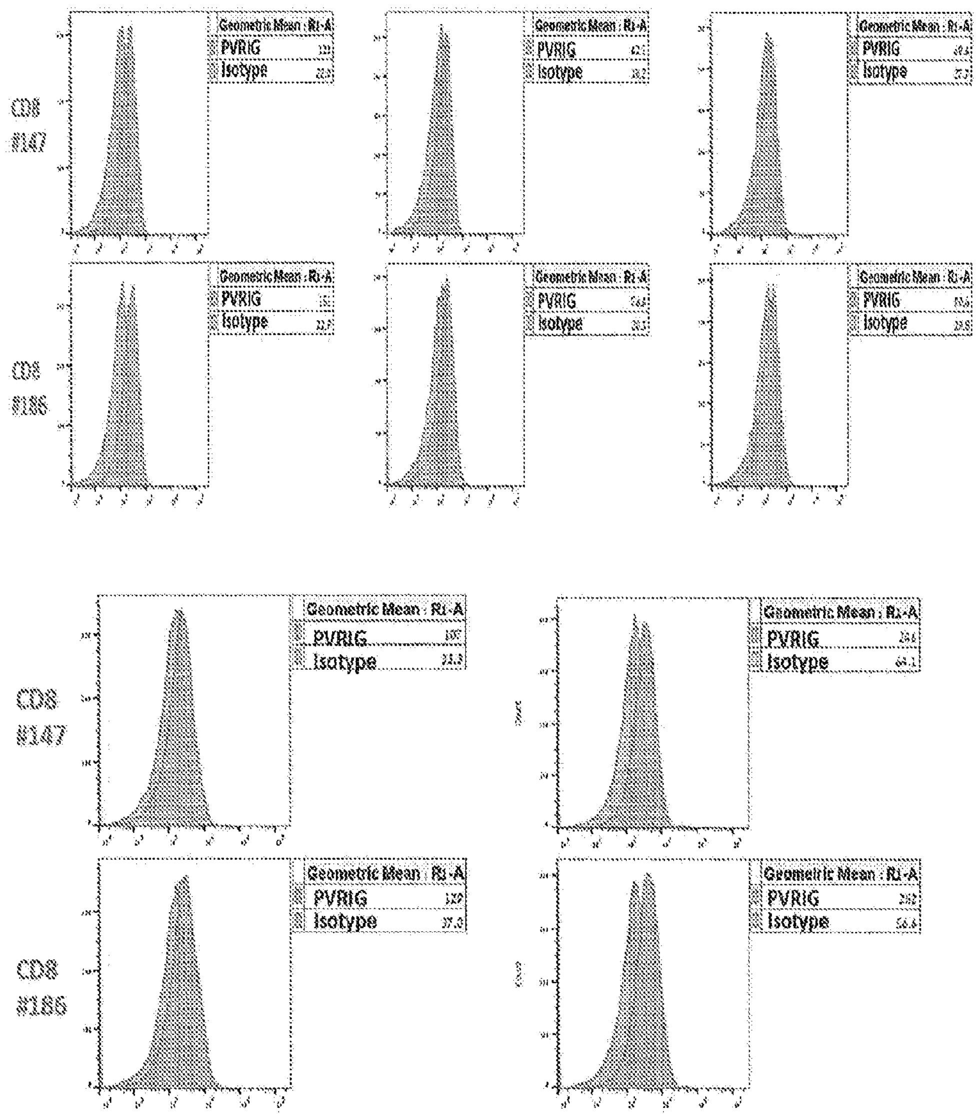

CD8
#147
CD8
#186
Geometric Mean : R1-A
PVRIG
Isotype
CD8
#147
CD8
#186
Geometric Mean : R1-A
PVRIG
Isotype 33.3
Geometric Mean : R1-A
PVRIG
Isotype 64.1
Geometric Mean : R1-A
PVRIG
Isotype 37.0
Geometric Mean : R1-A
PVRIG
Isotype 56.6

Figure 70C

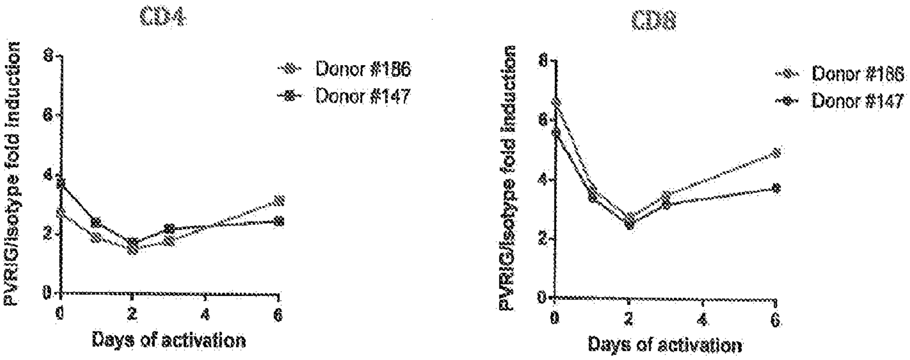

CD4
PVRIG/isotype fold induction
8
6
4
2
0
0
2
4
6
Days of activation
Donor #186
Donor #147
CD8
PVRIG/isotype fold induction
8
6
4
2
0
0
2
4
6
Days of activation
Donor #186
Donor #147

Figure 71A

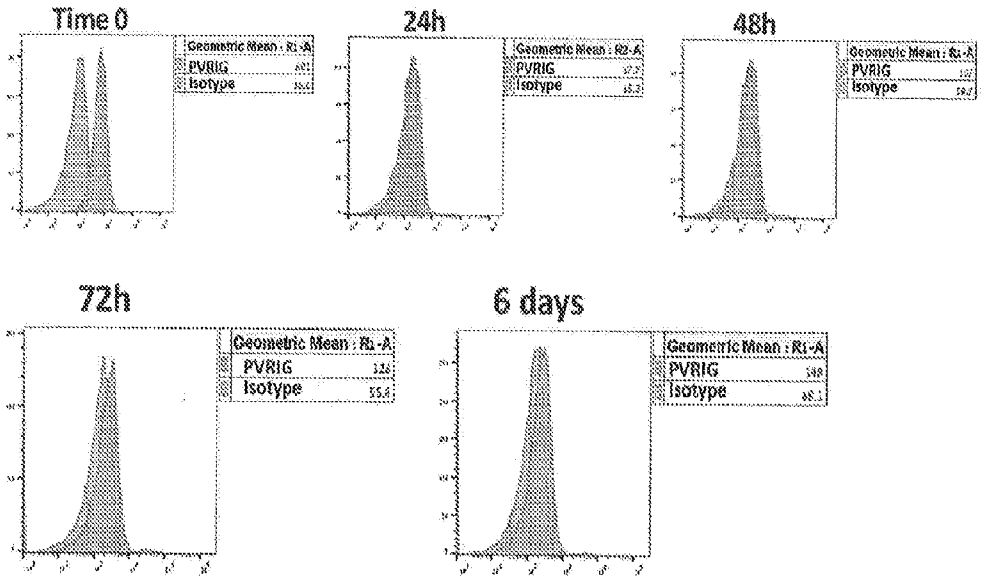


Figure 71B

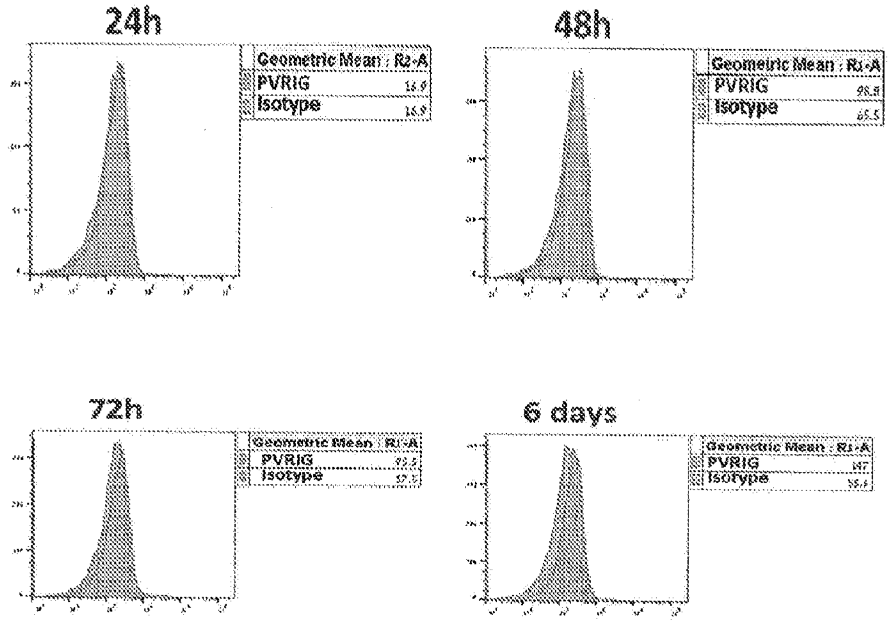


Figure 71C

C

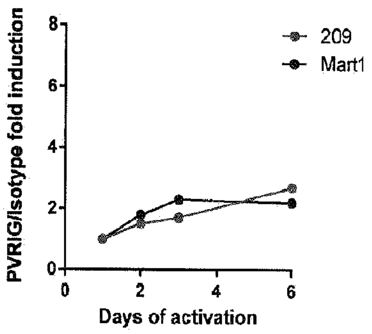

PVRIG/Isotype fold induction
8
6
4
2
0
0
2
4
6
Days of activation
209
Mart1

Figure 72

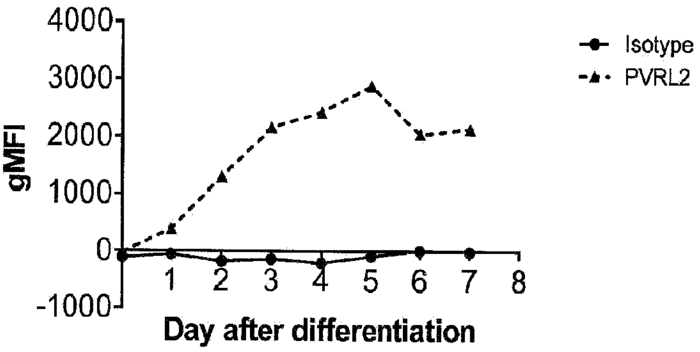

4000
3000
2000
1000
0
-1000
gMFI
1
2
3
4
5
6
7
8
Day after differentiation
Isotype
PVRL2

Figure 73A

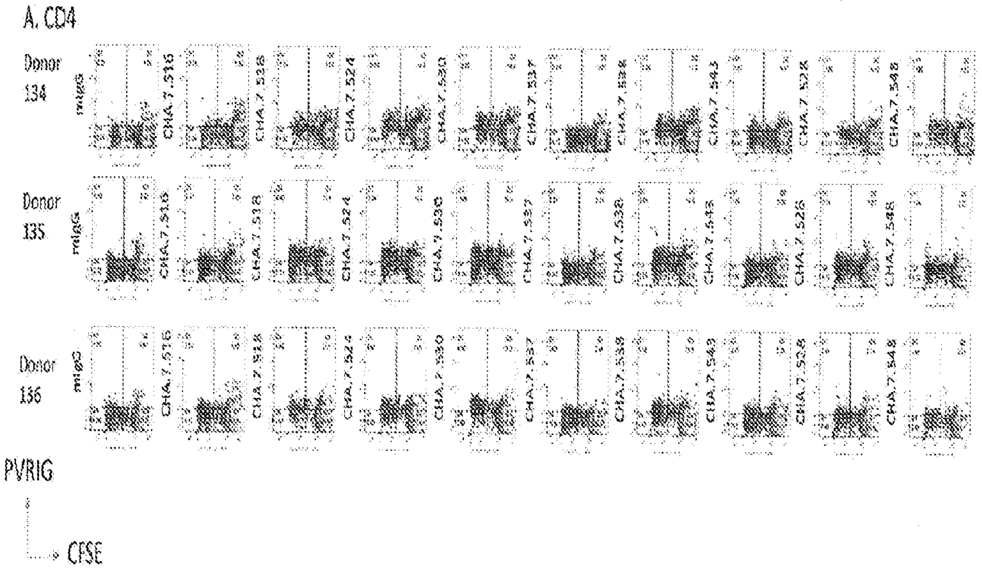


Figure 73B

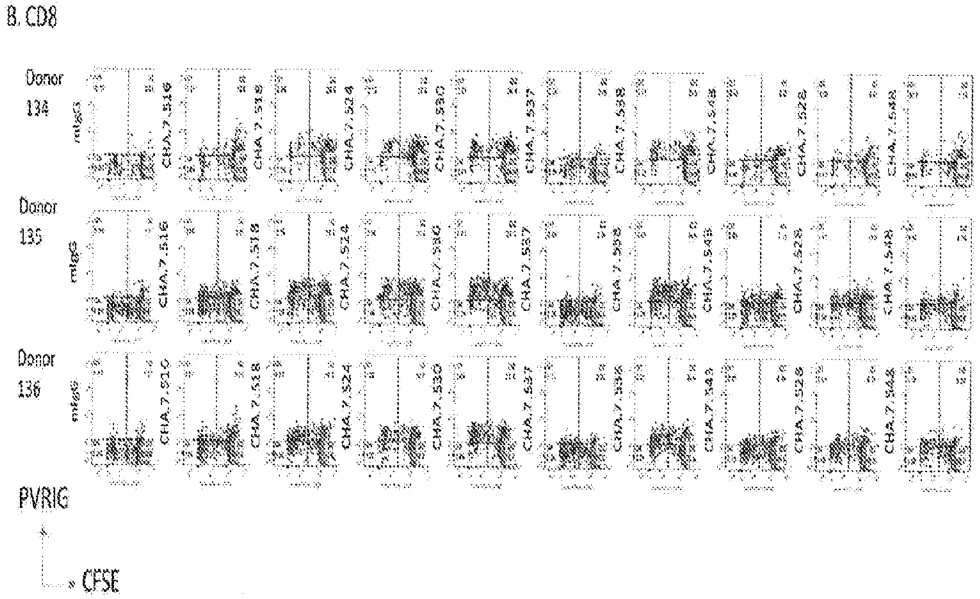


Figure 74A

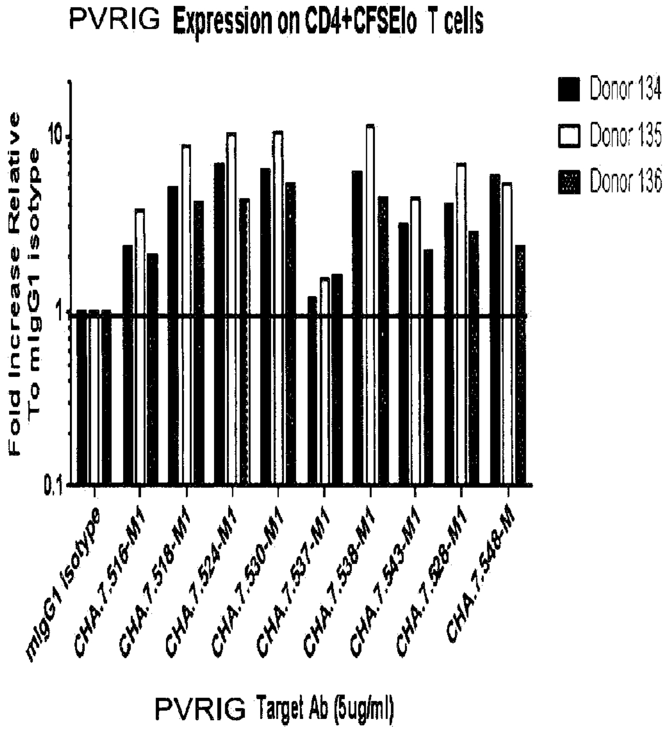


Figure 74B

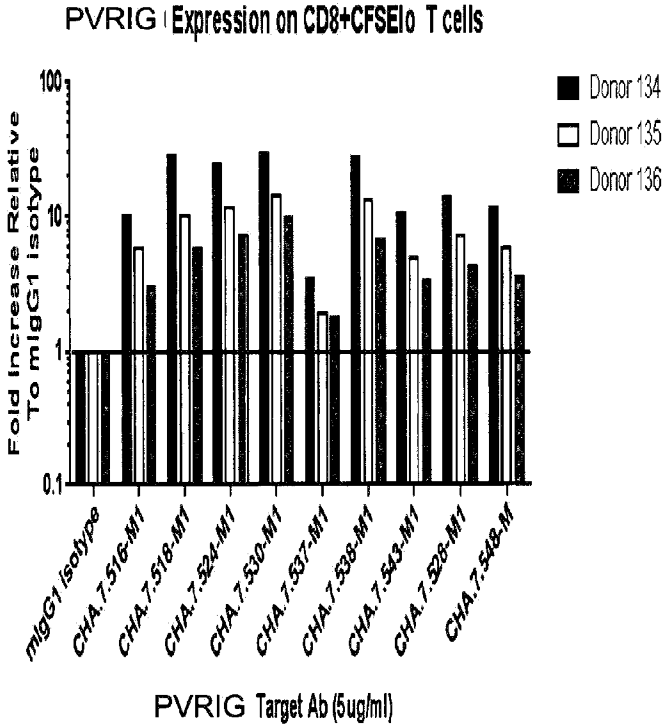


Figure 75A

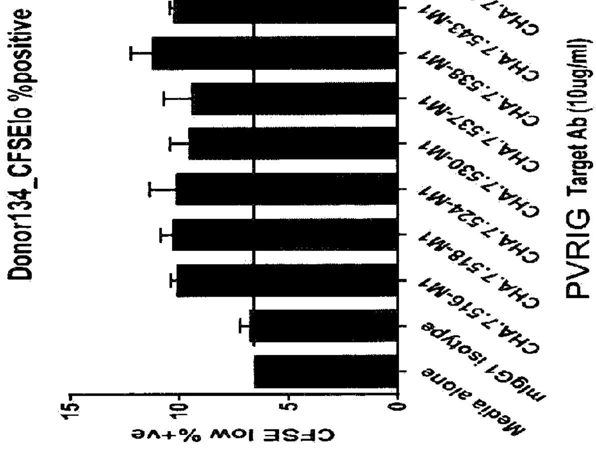


Figure 75B

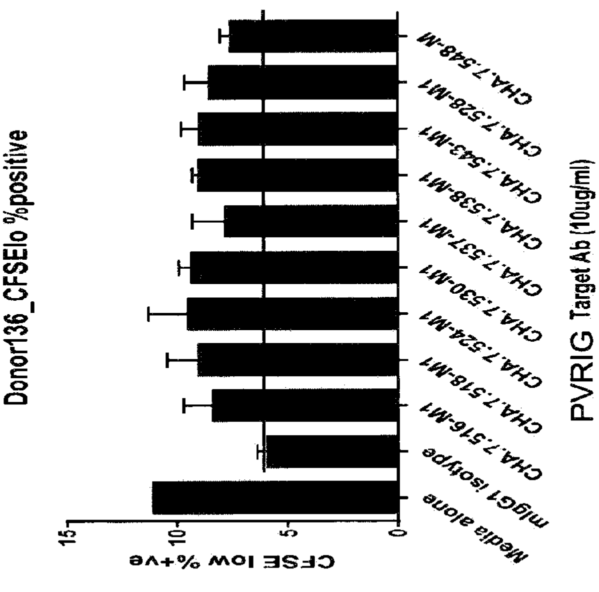


Figure 76

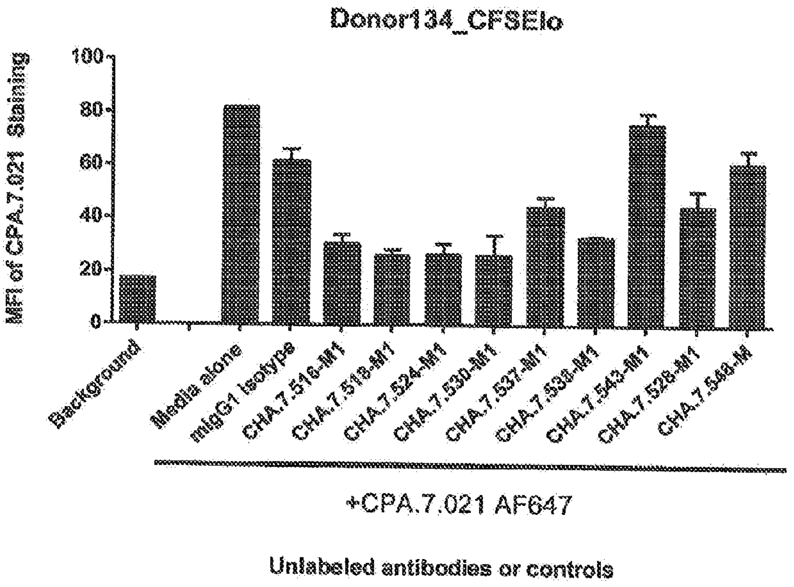

Donor134_CFSElo
MFI of CPA.7.021 Staining
100
80
60
40
20
0
Background
Media alone
migG1 isotype
CHA.7.516-M1
CHA.7.518-M1
CHA.7.524-M1
CHA.7.530-M1
CHA.7.537-M1
CHA.7.538-M1
CHA.7.543-M1
CHA.7.528-M1
CHA.7.548-M
+CPA.7.021 AF647
Unlabeled antibodies or controls

Figure 77

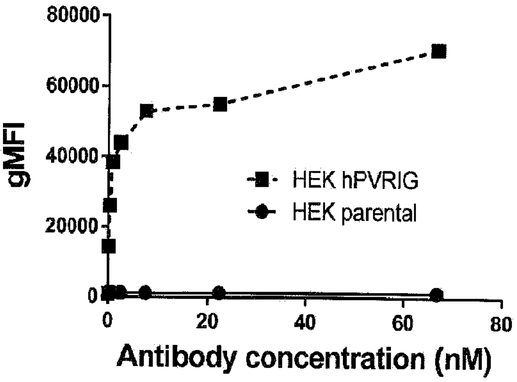

80000
60000
40000
20000
0
gMFI
0
20
40
60
80
Antibody concentration (nM)
HEK hPVRIG
HEK parental

Figure 78

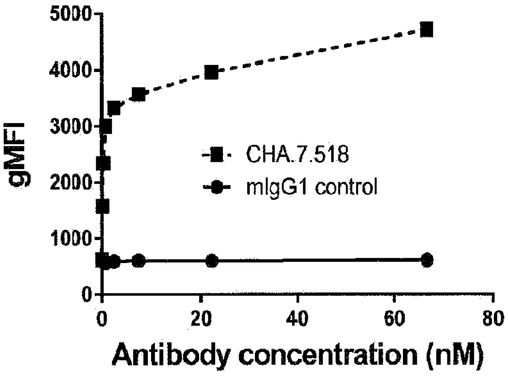

gMFI
5000
4000
3000
2000
1000
0
0
20
40
60
80
Antibody concentration (nM)
CHA.7.518
mIgG1 control

Figure 79A

Antibody (mIgG)	**EC_{50} (HEK OE, nM)**	**HEK OE/par (3.3ug/ml, gMFIr)**	**Jurkat (3.3ug/ml, gMFIr)**
CHA.7.502	40.18	71.99	6.21
CHA.7.503	1.05	260.98	23.59
CHA.7.506	No binding	0.76	No binding
CHA.7.508	3.30	45.86	6.50
CHA.7.510	92.81	16.32	4.19
CHA.7.512	52.99	5.12	1.47
CHA.7.514	5.31	49.82	7.67
CHA.7.516	0.79	37.90	5.73
CHA.7.518	0.36	42.24	6.58
CHA.7.520	No binding	1.01	No binding
CHA.7.522	91.44	10.12	2.99
CHA.7.524	0.46	48.33	7.87
CHA.7.525	3.05	41.86	4.30
CHA.7.526	2.99	47.28	3.98
CHA.7.527	No binding	0.98	No binding
CHA.7.528	7.31	44.88	6.17
CHA.7.530	0.33	51.14	8.04
CHA.7.534	1.87	43.72	5.05
CHA.7.535	3.67	40.44	2.79
CHA.7.537	2.47	36.61	5.53

Figure 79B

CHA.7.538	0.52	35.80	7.37
CHA.7.543	0.52	49.81	6.73
CHA.7.544	0.76	42.60	5.68
CHA.7.545	0.76	44.31	6.53
CHA.7.546	0.61	43.14	6.42
CHA.7.547	14.37	10.94	1.02
CHA.7.548	0.27	45.26	7.37
CHA.7.549	2.60	29.71	2.23
CHA.7.550	1.34	27.72	2.10

Figure 80A

Antibody (mIgG)	**Human CD56 int. NK (gMFIr, 10ug/ml)**	**Human CD8+ T cells (gMFIr, 10ug/ml)**	**Expi cyno OE/par (gMFIr, 3.3ug/ml)**	**Cyno NK cells (gMFIr, 10ug/ml)**	**Cyno CD8+ T cells (gMFIr, 10ug/ml)**
CHA.7.502	1.97	1.41	60.49	Not tested	Not tested
CHA.7.503	3.15	1.96	106.3	Not tested	Not tested
CHA.7.506	Not tested	Not tested	0.77	Not tested	Not tested
CHA.7.508	3.6	4.09	41.49	Not tested	Not tested
CHA.7.510	3.13	2.73	38.21	Not tested	Not tested
CHA.7.512	1.30	1.15	8.96	Not tested	Not tested
CHA.7.514	4.16	5.15	65.20	Not tested	Not tested
CHA.7.516	4.22	4.09	60.05	1.76	2.09
CHA.7.518	5.08	6.69	83.51	1.92	2.09
CHA.7.520	1.13	1.04	Not tested	Not tested	Not tested
CHA.7.522	2.06	1.90	27.24	Not tested	Not tested

Figure 80B

CHA.7.524	5.50	6.12	66.32	1.78	2.02
CHA.7.525	1.98	1.76	0.85	Not tested	Not tested
CHA.7.526	2.08	1.71	0.79	Not tested	Not tested
CHA.7.527	1.16	0.99	Not tested	Not tested	Not tested
CHA.7.528	3.08	3.63	12.2	1.21	1.18
CHA.7.530	6.04	6.47	60.80	1.73	1.89
CHA.7.534	2.60	1.96	46.27	Not tested	Not tested
CHA.7.535	2.24	1.28	0.82	Not tested	Not tested
CHA.7.537	3.90	3.41	1.55	1.18	1.19
CHA.7.538	6.49	6.17	15.16	1.36	1.45
CHA.7.543	4.48	4.33	0.83	1.35	1.39
CHA.7.544	2.36	2.54	61.09	Not tested	Not tested
CHA.7.545	2.54	2.82	0.91	Not tested	Not tested
CHA.7.546	2.75	2.95	0.85	Not tested	Not tested
CHA.7.547	2.21	1.13	26.65	Not tested	Not tested
CHA.7.548	3.15	3.35	4.25	1.18	1.09
CHA.7.549	3.05	1.42	1.00	Not tested	Not tested
CHA.7.550	1.60	1.29	0.90	Not tested	Not tested

Figure 81A

Antibody (mIgG)	**IC$_{50}$ (nM)**
CHA.7.502	39.90
CHA.7.503	No IC$_{50}$
CHA.7.506	31.65
CHA.7.508	37.88
CHA.7.510	55.00
CHA.7.512	839.6
CHA.7.514	38.88
CHA.7.516	33.11
CHA.7.518	23.15
CHA.7.520	619.3
CHA.7.522	50.48
CHA.7.524	30.20
CHA.7.525	85.52
CHA.7.526	58.88
CHA.7.527	No IC$_{50}$
CHA.7.528	28.88
CHA.7.530	34.56
CHA.7.534	181.4
CHA.7.535	821.1
CHA.7.537	38.95
CHA.7.538	51.87
CHA.7.543	No IC$_{50}$

Figure 81B

CHA.7.544	No IC_{50}
CHA.7.545	96.06
CHA.7.546	92.05
CHA.7.547	27.94
CHA.7.548	18.98
CHA.7.549	36.12
CHA.7.550	58.34

Figure 82

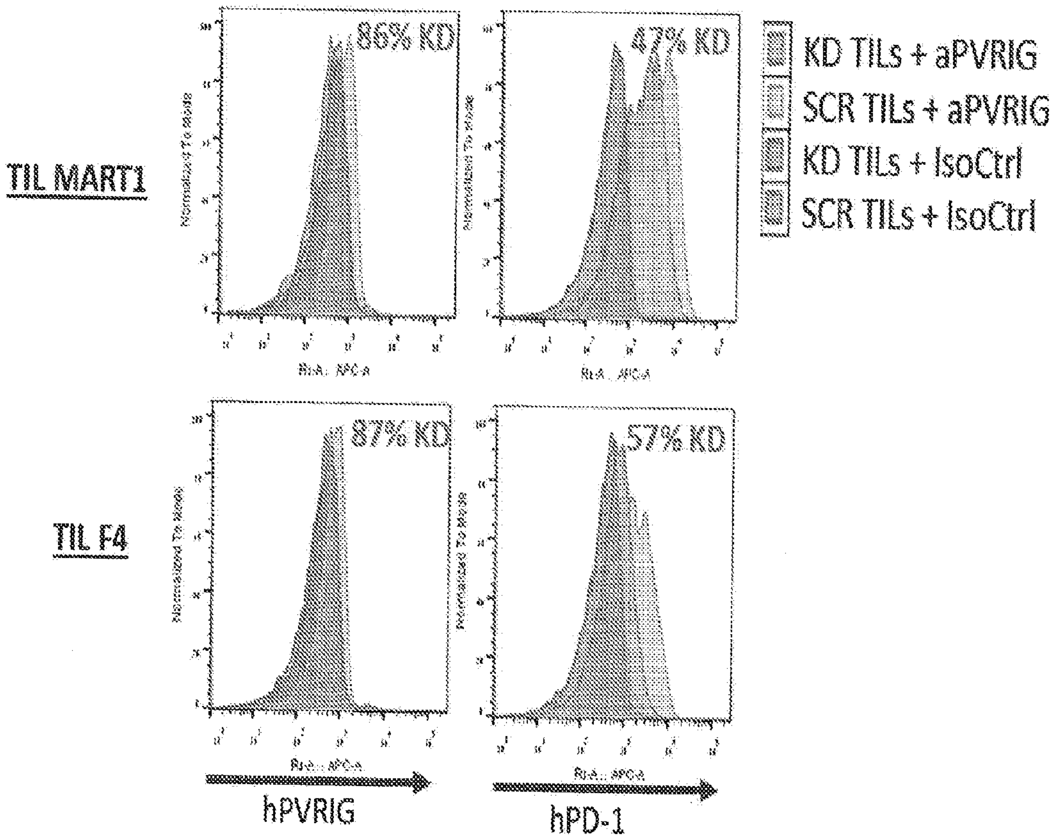

TIL MART1
86% KD
47% KD
KD TILs + aPVRIG
SCR TILs + aPVRIG
KD TILs + IsoCtrl
SCR TILs + IsoCtrl
Normalized To Mode
R1-A :: APC-A
TIL F4
87% KD
57% KD
hPVRIG
hPD-1

FIGURE 83A

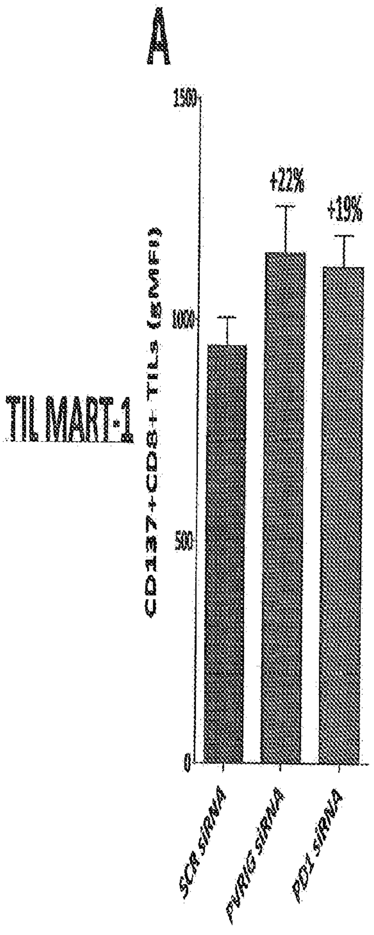


FIGURE 83B

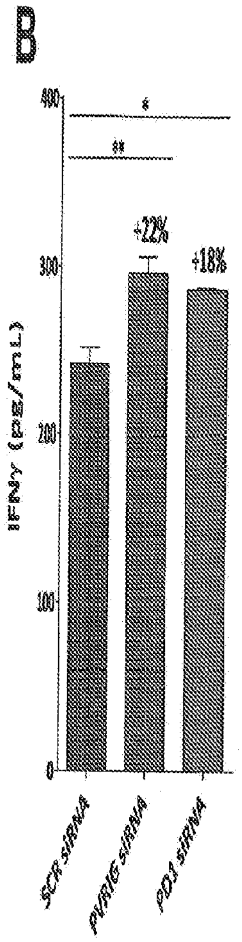
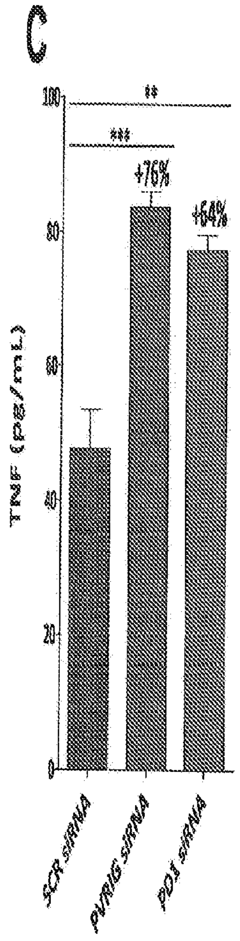


FIGURE 83C



TIL F4

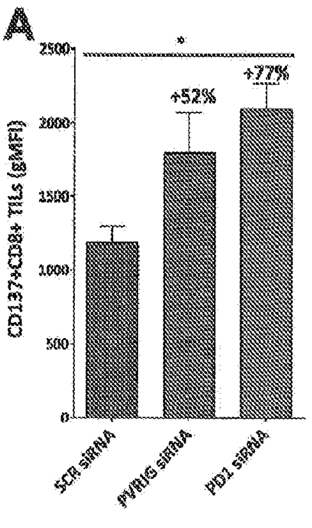


FIGURE 84A

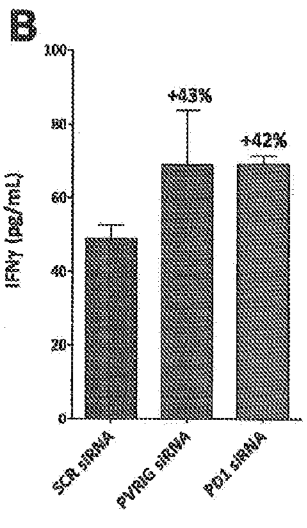


FIGURE 84B

TIL 209

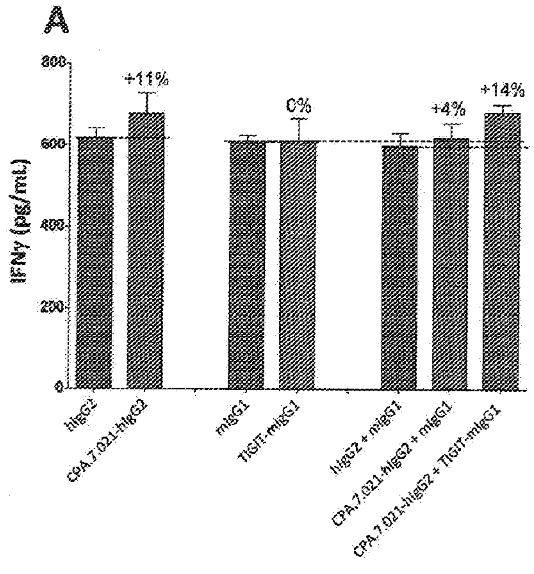


FIGURE 85A

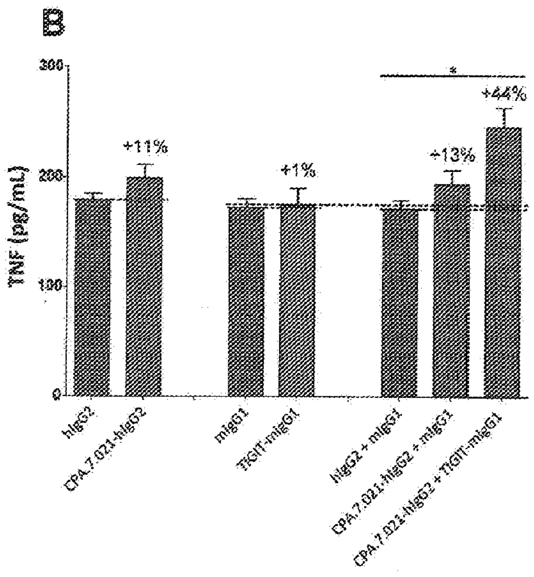


FIGURE 85B

TIL 209

FIG. 86 A

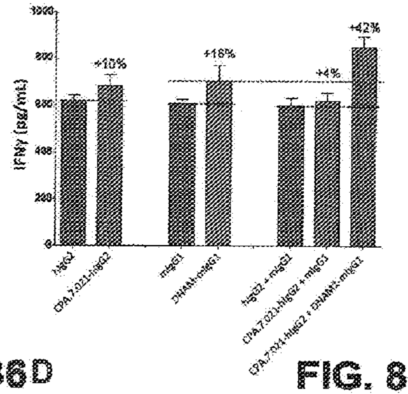


FIG. 86 B

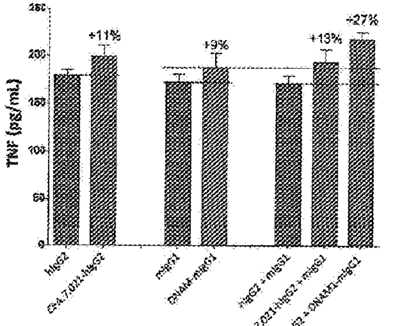
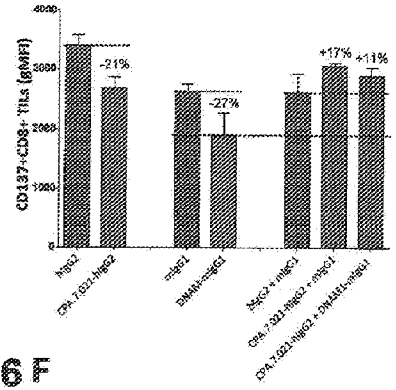


FIG. 86C



TIL MART-1

FIG. 86D

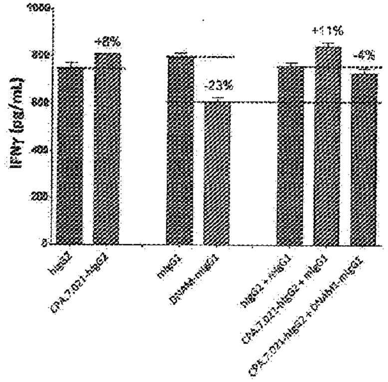


FIG. 86E

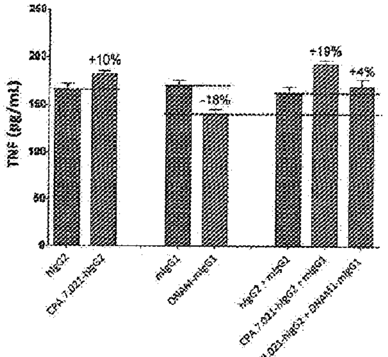
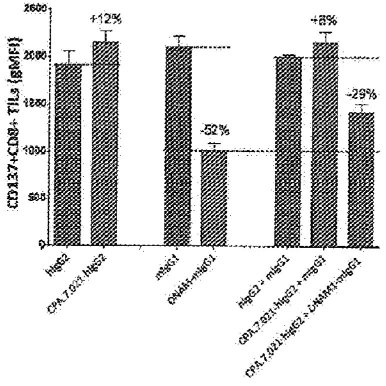


FIG. 86 F



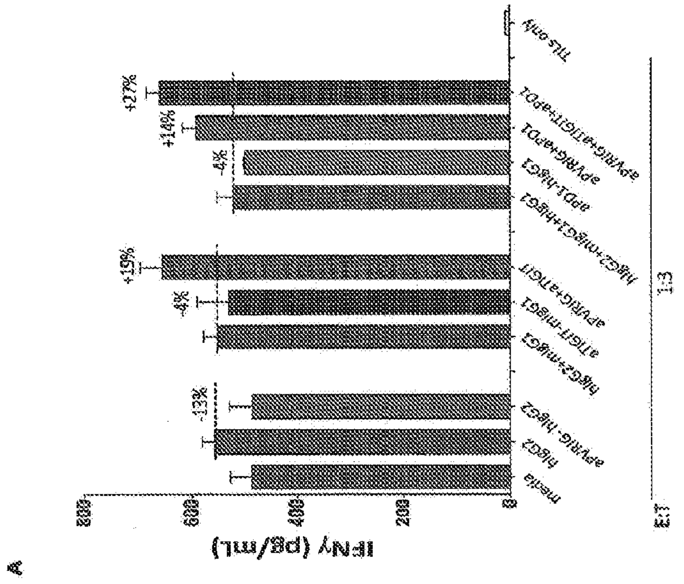


FIGURE 87A

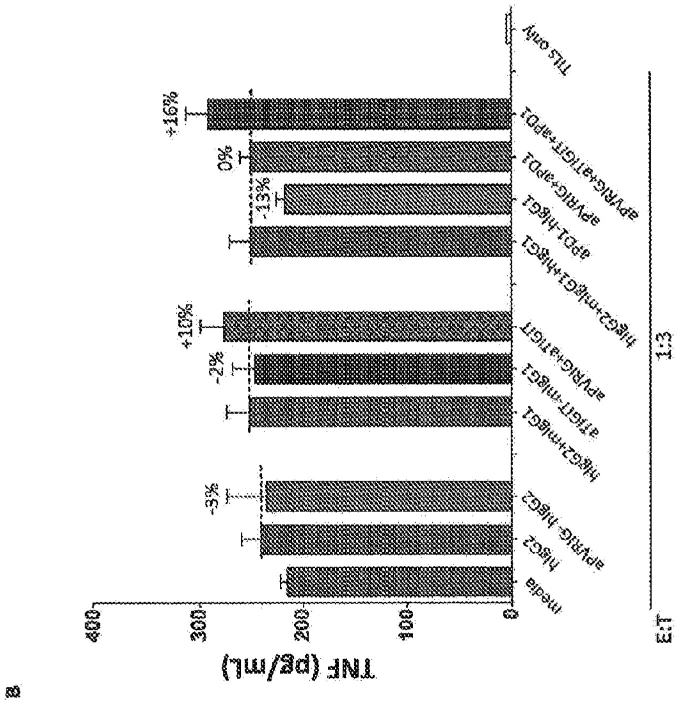


FIGURE 87B

Figure 88A
Humanized sequences of CHA.7.518 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework (AbM CDR definition)

IGHV1-46*01 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1368)

Joining region IMGT J00256|IGHJ4*01|YFDYWGQGTLVTVSS (SEQ ID NO: 1369)

```
seq             10        20         30         40         50         60        70        80        90
AbM             10        20         30         40         50  a       60        70        80  abc      90
         b b b     p     p b b b   b b    b  bi i     i ibb  b          i  b   b b b x    b b b  b     bibibb
518     EVQLQQSGPELVKPGASVKISCKAS GYTFTDYNIN WVKQSHGKSLEWIG YIYPYIGGSG YNQKFKSKATLSADNPSSTAYMELRSLTSEDSAVYYCAR
        *   *   * **       *                   * ** **   *              *   **** *** ***  *    *  *   *
1-46*01 QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYYMH WVRQAPGQGLEWMG IINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h518H1  QVQLVQSGAEVKKPGASVKVSCKAS GYTFTDYNIN WVRQAPGQGLEWMG YIYPYIGGSG YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h518H2  QVQLVQSGAEVKKPGASVKVSCKAS GYTFTDYNIN WVRQAPGQGLEWIG YIYPYIGGSG YAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCAR
h518H3  QVQLVQSGAEVKKPGASVKISCKAS GYTFTDYNIN WVRQAPGQGLEWIG YIYPYIGGSG YAQKFQGRATLTADTSTSTAYMELSSLRSEDTAVYYCAR
h518H4  QVQLVQSGAEVKKPGASVKISCKAS GYTFTDYNIN WVRQAPGQGLEWIG YIYPYIGGSG YAQKFQGRATLTADNSTSTAYMELSSLRSEDTAVYYCAR
                   V                     #                              N


seq    100        110       120
AbM                      110
                    i   b b b
518     EDKTARNAMDY WGQGTPVTVSS (SEQ ID NO: 1370)
                         *      (1-46*01 disclosed as SEQ ID NO: 1371)
h518H1  EDKTARNAMDY WGQGTLVTVSS (SEQ ID NO: 1372)
h518H2  EDKTARNAMDY WGQGTLVTVSS (SEQ ID NO: 1373)
h518H3  EDKTARNAMDY WGQGTLVTVSS (SEQ ID NO: 1374)
h518H4  EDKTARNAMDY WGQGTLVTVSS (SEQ ID NO: 1375)
              #
```

deamidation substitutions: Q/S/A

Figure 88B
Humanized sequences of CHA.7.518 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework

IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP (SEQ ID NO: 1376)

Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1377)

```
seq               10        20         30         40         50         60        70        80
AbM               10        20         30         40         50         60        70        80
           b b b    p p     p b b  b   b   bi bi i    ii ibbi      i   b   b        b b b  b    ib bib
518       DIQMTQSPASLSVSVGETVTIIC RVSENIYSNLA WYQQKQGKSPQLLVY EATNLAE GVPSRFSGSGSGTQYSLKINSLQSEDFGSYYC
                  *   *   **   *                   *  * *  *                       *** * *   *   **
IGKV1-39  DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h518L1    DIQMTQSPSSLSASVGDRVTITC RVSENIYSNLA WYQQKPGKAPKLLIY EATNLAE GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h518L2    DIQMTQSPSSLSASVGDRVTITC RVSENIYSNLA WYQQKPGKAPKLLIY EATNLAE GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC
h518L3    DIQMTQSPSSLSASVGDRVTITC RVSENIYSNLA WYQQKPGKAPKLLVY EATNLAE GVPSRFSGSGSGTDYTLTISSLQPEDFGTYYC
                      V               #   #           S          #
```

```
seq       90        100
AbM       90        100
          ibi  iib  i   b b b
518       QHFWGTPYT FGGGTKLEIK (SEQ ID NO: 1378)
                      *
IGKV1-39  QQSYSTPP (SEQ ID NO: 1379)
h518L1    QHFWGTPYT FGQGTKLEIK (SEQ ID NO: 1380)
h518L2    QHFWGTPYT FGQGTKLEIK (SEQ ID NO: 1381)
h518L3    QHFWGTPYT FGQGTKLEIK (SEQ ID NO: 1382)
             @
```

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation substitutions: Y/F/H

Figure 88C

Humanized sequences of CHA.7.524 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework
IGHV1-46*01
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1383)
Joining region IMGT J00256|IGHJ4*01|YFDYWGQGTLVTVSS (SEQ ID NO: 1384)

```
seq             10        20         30         40         50         60        70        80        90
AbM             10        20         30         40         50  a       60        70        80  abc     90
         b b b     p     p b b b   b b    b  bi i     i ibb  b          i  b   b b b x    b b b  b     bibibb
524     QVQLQQSGPEVVRPGVSVKISCKGS GYKFPDYVMH WVKQSHAKSLEWIG IISIYSGNTN YNQKFKGKATMTVDKSSSTAYMELARLTSEDSAIYYCAR
            *   *  **  *   *   *               * *****   *              *   * **   * * *  *    ** *   * *

1-46*01 QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYYMH WVRQAPGQGLEWMG IINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h524H1  QVQLVQSGAEVKKPGASVKVSCKAS GYKFPDYVMH WVRQAPGQGLEWMG IISIYSGNTN YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h524H2  QVQLVQSGAEVKKPGASVKVSCKAS GYKFPDYVMH WVRQAPGQGLEWIG IISIYSGNTN YAQKFQGRVTMTVDTSTSTVYMELSSLRSEDTAVYYCAR
h524H3  QVQLVQSGAEVKKPGASVKISCKGS GYKFPDYVMH WVRQAPGQGLEWIG IISIYSGNTN YAQKFQGRATMTVDTSTSTAYMELSSLRSEDTAVYYCAR
h524H4  QVQLVQSGAEVKKPGASVKISCKGS GYKFPDYVMH WVRQAPGQGLEWIG IISIYSGNTN YAQKFQGRATMTVDKSTSTAYMELSSLRSEDTAVYYCAR
                   V                                               # #  N                               I


seq    100        110
AbM                    110
                  i   b b b
524     EGDLPMFAY WGQGTLVTVSA (SEQ ID NO: 1385)
                            * (1-46*01 disclosed as SEQ ID NO: 1386)
h524H1  EGDLPMFAY WGQGTLVTVSS (SEQ ID NO: 1387)
h524H2  EGDLPMFAY WGQGTLVTVSS (SEQ ID NO: 1388)
h524H3  EGDLPMFAY WGQGTLVTVSS (SEQ ID NO: 1389)
h524H4  EGDLPMFAY WGQGTLVTVSS (SEQ ID NO: 1390)
             @
```

deamidation substitutions: Q/S/A
@ methionine oxidation substitutions: L/F/A

Figure 88D
humanized sequences of CHA.7.524 antibody VL

Potential humanized sequence based on IMGT IGKV3-11*01 acceptor framework
IGKV3-11*01
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPP (SEQ ID NO: 1391)
Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1392)

```
seq               10        20         30          40         50         60         70        80
AbM               10        20         30         40         50         60         70        80
           b b b    p p     b b b  b   b   bi bi i    ii ibbi      i   b   b        b b b  b    ib bib
524       QIVLTQSPAIMSASPGEKVTMTC NASSSVS-YMY WYQQKPISSPRLLIY DTSNLAS GVPVRFSGSGSGTSYSLTIGRMEAEDAATYYC
          *        ** *    ** **                    ***                * *         ***   *** *  * *
IGKV3-11  EIVLTQSPATLSLSPGERATLSC RASQSVSSYLA WYQQKPGQAPRLLIY DASNRAT GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC
h524L1    EIVLTQSPATLSLSPGERATLSC NASSSVS-YMY WYQQKPGQAPRLLIY DTSNLAS GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC
h524L2    EIVLTQSPATLSLSPGERATLSC NASSSVS-YMY WYQQKPGQAPRLLIY DTSNLAS GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC
h524L3    EIVLTQSPATLSLSPGERVTMSC NASSSVS-YMY WYQQKPGQAPRLLIY DTSNLAS GVPARFSGSGSGTDYTLTISSMEPEDFAVYYC
                    M A           #                   S          #                              A


seq        90         100
AbM       90        100
          ibi  iib  i   b b b
524       QQWSSYPLT FGAGTKVEVK (SEQ ID NO: 1393)
                      *   * *
IGKV3-11  QQRSNWPP (SEQ ID NO: 1394)
h524L1    QQWSSYPLT FGQGTKLEIK (SEQ ID NO: 1395)
h524L2    QQWSSYPLT FGQGTKLEIK (SEQ ID NO: 1396)
h524L3    QQWSSYPLT FGQGTKVEIK (SEQ ID NO: 1397)
            @               V
```

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation substitutions: Y/F/H

Figure 88E

Humanized sequences of CHA.7.530 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework
IGHV1-46*01
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1398)
Joining region IMGT J00256|IGHJ4*01|YFDYWGQGTLVTVSS (SEQ ID NO: 1399)

```
seq             10        20         30         40         50         60        70        80        90
AbM             10        20         30         40         50  a       60        70        80  abc      90
         b b b     p     p b b b   b b    b  bi i     i ibb  b          i  b   b b b x    b b b  b     bibibb
530     QVQLQQSGAELMRPGTSVKVSCKAS GYAFTNHLIE WIKQRPGQGLEWIG VINPGSDSTD YNEKFKDKATLTADKSSSTAYMQLSSLTSDDSAVYFCAR
            *     ***  *                      ** *       *              **  **** * * * *  *  *    * * *   *

1-46*01 QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYYMH WVRQAPGQGLEWMG IINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h530H1  QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNHLIE WVRQAPGQGLEWMG VINPGSDSTD YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h530H2  QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNHLIE WVRQAPGQGLEWIG VINPGSDSTD YAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCAR
h530H3  QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNHLIE WIRQAPGQGLEWIG VINPGSDSTD YAQKFQGRATLTADTSTSTAYMELSSLRSEDTAVYYCAR
h530H4  QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNHLIE WIRQAPGQGLEWIG VINPGSDSTD YAQKFQGRATLTADKSTSTAYMELSSLRSEDTAVYYCAR
                   M                   #                      #         N                                 F

seq    100        110
AbM                     110
                   i    b b b
530     SLYYNSWFVY WGQGTLVTVSA (SEQ ID NO: 1400)
                             * (1-46*01 disclosed as SEQ ID NO: 1401)
h530H1  SLYYNSWFVY WGQGTLVTVSS (SEQ ID NO: 1402)
h530H2  SLYYNSWFVY WGQGTLVTVSS (SEQ ID NO: 1403)
h530H3  SLYYNSWFVY WGQGTLVTVSS (SEQ ID NO: 1404)
h530H4  SLYYNSWFVY WGQGTLVTVSS (SEQ ID NO: 1405)
            # @
```

deamidation substitutions: Q/S/A
@ tryptophan oxidation substitutions: Y/F/H

Figure 88F
Humanized sequences of CHA.7.530 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework (AbM CDR definition)
IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP (SEQ ID NO: 1406)
Joining region   IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1407)

```
seq                 10        20         30         40         50         60        70        80
AbM                 10        20         30         40         50         60        70        80
             b b b    p p     p b b  b   b   bi bi i    ii ibbi      i   b   b        b b b  b    ib bib
530         DIQMTQSPASLSASVGETVTITC RASENIYSYLA WYQQKRGKSPQLLVY NAKTLVE GVPSRFSGSGSGTQFSLKINSLQPEDFGSYYC
                    *       **                       *  * *  *                       * * * *       **
IGKV1-39    DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h530L1      DIQMTQSPSSLSASVGDRVTITC RASENIYSYLA WYQQKPGKAPKLLIY NAKTLVE GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h530L2      DIQMTQSPSSLSASVGDRVTITC RASENIYSYLA WYQQKPGKAPKLLVY NAKTLVE GVPSRFSGSGSGTDFTLTISSLQPEDFGTYYC
                                        #               S       #


seq         90        100
AbM         90        100
            ibi  iib  i   b b b
530         QHHYGTPYT FGGGTKLEIK (SEQ ID NO: 1408)
                        *
IGKV1-39    QQSYSTPP (SEQ ID NO: 1409)
h530L1      QHHYGTPYT FGQGTKLEIK (SEQ ID NO: 1410)
h530L2      QHHYGTPYT FGQGTKLEIK (SEQ ID NO: 1411)
```

deamidation substitutions: Q/S/A/D

Figure 88G
Humanized sequences of CHA.7.538_1 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework
IGHV1-46*01
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1412)
Joining region IMGT J00256|IGHJ4*01|YFDYWGQGTLVTVSS (SEQ ID NO: 1413)

```
seq             10        20         30         40         50         60        70        80        90
AbM             10        20         30         40         50  a       60        70        80  abc      90
         b b b     p     p b b b   b b    b  bi i     i ibb  b          i  b   b b b x    b b b  b     bibibb
538_1   QVQLQQSGAELVRPGASVKVSCKTS GYAFTNYLIE WVKQRPGQGLEWIG VINPGSGGIY YNDKFKVKTTLTADKSSSTAYMQLSSLTSDDSAVYFCAR
            *     ***          *               * *       *              **  **** * * * *  *  *    * * *   *
1-46*01 QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYYMH WVRQAPGQGLEWMG IINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h5381H1 QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNYLIE WVRQAPGQGLEWMG VINPGSGGIY YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h5381H2 QVQLVQSGAEVKKPGASVKVSCKAS GYAFTNYLIE WVRQAPGQGLEWIG VINPGSGGIY YAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCAR
h5381H3 QVQLVQSGAEVKKPGASVKVSCKTS GYAFTNYLIE WVRQAPGQGLEWIG VINPGSGGIY YAQKFQGRVTLTADTSTSTAYMELSSLRSEDTAVYYCAR
h5381H4 QVQLVQSGAEVKKPGASVKVSCKTS GYAFTNYLIE WVRQAPGQGLEWIG VINPGSGGIY YAQKFQGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAR
                   V                   #                      #         N      T                          F

seq    100        110
AbM                     110
                   i   b b b
538_1   SETHDTWFAY WGQGTLVTVSA (SEQ ID NO: 1414)
                             * (1-46*01 disclosed as SEQ ID NO: 1415)
h5381H1 SETHDTWFAY WGQGTLVTVSS (SEQ ID NO: 1416)
h5381H2 SETHDTWFAY WGQGTLVTVSS (SEQ ID NO: 1417)
h5381H3 SETHDTWFAY WGQGTLVTVSS (SEQ ID NO: 1418)
h5381H4 SETHDTWFAY WGQGTLVTVSS (SEQ ID NO: 1419)
              @
```

deamidation substitutions: Q/S/A
@ tryptophan oxidation substitutions: Y/F/H

Figure 88H
humanized sequences of CHA.7.538_1 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework
IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP (SEQ ID NO: 1420)

Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1421)

IGKV1-17*02
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISNLQPEDFATYYCLQHNSYPP (SEQ ID NO: 1422)

```
seq                  10        20         30         40         50         60        70        80
AbM                  10        20         30         40         50         60        70        80
              b b b    p p     p b b  b   b   bi bi i    ii ibbi      i   b   b        b b b  b    ib bib
538          DIVMTQSQKFISTSVGDRVSITC KASQSVRIAVA WFQQKPGQSPKALIY LASTRHT GVPDRFTGSGSGTDFTLTISNVQSEDLADYFC
               *    **** *      *                 *     **  *               *  *             ** *  * * *
IGKV1-39     DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h538L1       DIQMTQSPSSLSASVGDRVTITC KASQSVRIAVA WYQQKPGKAPKLLIY LASTRHT GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
h538L2       DIQMTQSPSSLSASVGDRVTITC KASQSVRIAVA WFQQKPGKAPKALIY LASTRHT GVPSRFSGSGSGTDFTLTISSVQPEDFATYYC
                       I T                               S                                         L   F

seq          90        100
AbM          90        100
             ibi  iib  i   b b b
538          LQHWNYPYT FGGGTKLEIK (SEQ ID NO: 1423)
                         *
IGKV1-39     QQSYSTPP (SEQ ID NO: 1424)
h538L1       LQHWNYPYT FGQGTKLEIK (SEQ ID NO: 1425)
h538L2       LQHWNYPYT FGQGTKLEIK (SEQ ID NO: 1426)
                @#
```

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation substitutions: Y/F/H

Figure 88I
humanized sequences of CHA.7.538_2 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework
IGHV1-46*01
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1427)
Joining region
IMGT J00256|IGHJ4*01|YFDYWGQGTLVTVSS (SEQ ID NO: 1428)

```
seq               10        20         30         40         50         60        70        80        90
AbM               10        20         30         40         50  a       60        70        80  abc      90
           b b b     p     p b b b   b b    b  bi i     i ibb  b          i  b   b b b x    b b b  b     bibibb
538_2     QVQLQQSGAELVRPGTSVKMSCKAA GYTFTNYWIG WVKQRPGHGLEWIG DIYPGGGYTN YNEKFKGKATLTADTSSSTAYMQLSSLTSEDSAIYYCAS
              *     ***  *   *    *              * *  *    *              **  * ** * *   *  *  *    *   **     *
1-46*01   QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYYMH WVRQAPGQGLEWMG IINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h5382H1   QVQLVQSGAEVKKPGASVKVSCKAS GYTFTNYWIG WVRQAPGQGLEWMG DIYPGGGYTN YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
h5382H2   QVQLVQSGAEVKKPGASVKVSCKAS GYTFTNYWIG WVRQAPGQGLEWIG DIYPGGGYTN YAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCAS
h5382H3   QVQLVQSGAEVKKPGASVKMSCKAS GYTFTNYWIG WVRQAPGQGLEWIG DIYPGGGYTN YAQKFQGRATLTADTSTSTAYMELSSLRSEDTAVYYCAS
                     V                   # @                           #  N                              I

seq       100       110
AbM                       110
                      i   b b b
538_2     PYYGSSYGFAF WGQGTLVTVSA (SEQ ID NO: 1429)
                                * (1-46*01 disclosed as SEQ ID NO: 1430)
h5382H1   PYYGSSYGFAF WGQGTLVTVSS (SEQ ID NO: 1431)
h5382H2   PYYGSSYGFAF WGQGTLVTVSS (SEQ ID NO: 1432)
h5382H3   PYYGSSYGFAF WGQGTLVTVSS (SEQ ID NO: 1433)
```

deamidation substitutions: Q/S/A
@ tryptophan oxidation substitutions: Y/F/H

Figure 89A

humanized CHA.7.518 VH
h518HH1
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNINWVRQAPGQGLEWMGYIYPYIGGSGYAQKFQGRVTMTRDTSTSTVY
MELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1434)

h518HH2
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRVTMTADTSTSTVY
MELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1435)

h518HH3
QVQLVQSGAEVKKPGASVKISCKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRATLTADTSTSTAYM
ELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1436)

h518HH4
QVQLVQSGAEVKKPGASVKISCKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRATLTADNSTSTAYM
ELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1437)

humanized CHA.7.524 VH
h524HH1
QVQLVQSGAEVKKPGASVKVSCKASGYKFPDYVMHWVRQAPGQGLEWMGIISIYSGNTNYAQKFQGRVTMTRDTSTSTV
YMELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1438)

h524HH2
QVQLVQSGAEVKKPGASVKVSCKASGYKFPDYVMHWVRQAPGQGLEWIGIISIYSGNTNYAQKFQGRVTMTVDTSTSTVY
MELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1439)

h524HH3
QVQLVQSGAEVKKPGASVKISCKGSGYKFPDYVMHWVRQAPGQGLEWIGIISIYSGNTNYAQKFQGRATMTVDTSTSTAY
MELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1440)

Figure 89B
h524HH4
QVQLVQSGAEVKKPGASVKISCKGSGYKFPDYVMHWVRQAPGQGLEWIGIISIYSGNTNYAQKFQGRATMTVDKSTSTAY
MELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1441)

humanized CHA.7.530 VH
h530HH1
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNHLIEWVRQAPGQGLEWMGVINPGSDSTDYAQKFQGRVTMTRDTSTSTVY
MELSSLRSEDTAVYYCARSLYYNSWFVYWGQGTLVTVSS (SEQ ID NO: 1442)

h530HH2
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNHLIEWVRQAPGQGLEWIGVINPGSDSTDYAQKFQGRVTMTADTSTSTVY
MELSSLRSEDTAVYYCARSLYYNSWFVYWGQGTLVTVSS (SEQ ID NO: 1443)

h530HH3
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNHLIEWIRQAPGQGLEWIGVINPGSDSTDYAQKFQGRATLTADTSTSTAYM
ELSSLRSEDTAVYYCARSLYYNSWFVYWGQGTLVTVSS (SEQ ID NO: 1444)

h530HH4
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNHLIEWIRQAPGQGLEWIGVINPGSDSTDYAQKFQGRATLTADKSTSTAYM
ELSSLRSEDTAVYYCARSLYYNSWFVYWGQGTLVTVSS (SEQ ID NO: 1445)

humanized CHA.7.538_1 VH
h5381HH1
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIEWVRQAPGQGLEWMGVINPGSGGIYYAQKFQGRVTMTRDTSTSTVY
MELSSLRSEDTAVYYCARSETHDTWFAYWGQGTLVTVSS (SEQ ID NO: 1446)

h5381HH2
QVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTMTADTSTSTVY
MELSSLRSEDTAVYYCARSETHDTWFAYWGQGTLVTVSS (SEQ ID NO: 1447)

Figure 89C

h5381HH3
QVQLVQSGAEVKKPGASVKVSCKTSGYAFTNYLIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTLTADTSTSTAYM
ELSSLRSEDTAVYYCARSETHDTWFAYWGQGTLVTVSS (SEQ ID NO: 1448)

h5381HH4
QVQLVQSGAEVKKPGASVKVSCKTSGYAFTNYLIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTLTADKSTSTAYM
ELSSLRSEDTAVYYCARSETHDTWFAYWGQGTLVTVSS (SEQ ID NO: 1449)

humanized CHA.7.538_2 VH
h5382HH1
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIGWVRQAPGQGLEWMGDIYPGGGYTNYAQKFQGRVTMTRDTSTSTV
YMELSSLRSEDTAVYYCARPYYGSSYGFAFWGQGTLVTVSS (SEQ ID NO: 1450)

h5382HH2
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIGWVRQAPGQGLEWIGDIYPGGGYTNYAQKFQGRVTMTADTSTSTV
YMELSSLRSEDTAVYYCASPYYGSSYGFAFWGQGTLVTVSS (SEQ ID NO: 1451)

h5382HH3
QVQLVQSGAEVKKPGASVKMSCKASGYTFTNYWIGWVRQAPGQGLEWIGDIYPGGGYTNYAQKFQGRATLTADTSTSTA
YMELSSLRSEDTAVYYCASPYYGSSYGFAFWGQGTLVTVSS (SEQ ID NO: 1452)

humanized CHA.7.518 VL
h518HL1
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLIYEATNLAEGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQHFWGTPYTFGQGTKLEIK (SEQ ID NO: 1453)

h518HL2
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLIYEATNLAEGVPSRFSGSGSGTDYTLTISSLQPEDFA
TYYCQHFWGTPYTFGQGTKLEIK (SEQ ID NO: 1454)

Figure 89D
h518HL3
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLVYEATNLAEGVPSRFSGSGSGTDYTLTISSLQPEDF
GTYYCQHFWGTPYTFGQGTKLEIK (SEQ ID NO: 1455)

humanized CHA.7.524 VL
h524HL1
EIVLTQSPATLSLSPGERATLSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVY
YCQQWSSYPLTFGQGTKLEIK (SEQ ID NO: 1456)

h524HL2
EIVLTQSPATLSLSPGERATLSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLASGIPARFSGSGSGTDYTLTISSLEPEDFAVY
YCQQWSSYPLTFGQGTKLEIK (SEQ ID NO: 1457)

h524HL2
EIVLTQSPATLSLSPGERVTMSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLASGVPARFSGSGSGTDYTLTISSMEPEDFA
VYYCQQWSSYPLTFGQGTKVEIK (SEQ ID NO: 1458)

humanized CHA.7.530 VL
h530HL1
DIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPGKAPKLLIYNAKTLVEGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQHHYGTPYTFGQGTKLEIK (SEQ ID NO: 1459)

h530HL2
DIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPGKAPKLLVYNAKTLVEGVPSRFSGSGSGTDFTLTISSLQPEDFG
TYYCQHHYGTPYTFGQGTKLEIK (SEQ ID NO: 1460)

humanized CHA.7.538_1/538_2 VL
h538HL1
DIQMTQSPSSLSASVGDRVTITCKASQSVRIAVAWYQQKPGKAPKLLIYLASTRHTGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCLQHWNYPYTFGQGTKLEIK (SEQ ID NO: 1461)

Figure 89E
h538HL2
DIQMTQSPSSLSASVGDRVTITCKASQSVRIAVAWFQQKPGKAPKALIYLASTRHTGVPSRFSGSGSGTDFTLTISSVQPEDFA
TYYCLQHWNYPYTFGQGTKLEIK (SEQ ID NO: 1462)

Figure 90: humanized CHA.7 VH-VL Pairs

Antibody CHA.7.518

	VH	VL
ch518	chimVH	chimVL
h518-1	h518HH1	h518HL1 (optional)
h518-2	h518HH2	h518HL2
h518-3	h518HH3	h518HL2
h518-4	h518HH3	h518HL3
h518-5	h518HH4	h518HL3

Antibody CHA.7.524

	VH	VL
ch524	chimVH	chimVL
h524-1	h524HH1	h524HL1 (optional)
h524-2	h524HH2	h524HL2
h524-3	h524HH3	h524HL3
h524-4	h524HH4	h524HL3

Antibody CHA.7.530

	VH	VL
ch530	chimVH	chimVL
h530-1	h530HH1	h530HL1 (optional)
h530-2	h530HH2	h530HL2
h530-3	h530HH3	h530HL1
h530-4	h530HH3	h530HL2
h530-5	h530HH4	h530HL2

Antibody CHA.7.538_1

	VH	VL
ch538.1	chimVH	chimVL
h538.1-1	h538.1HH1	h538.1HL1 (optional)
h538.1-2	h538.1HH2	h538.1HL2
h538.1-3	h538.1HH3	h538.1HL2
h538.1-4	h538.1HH4	h538.1HL2

Antibody CHA.7.538_2

	VH	VL
ch538.2	chimVH	chimVL
h538.2-1	h538.2HH1	h538.1HL1 (optional)
h538.2-2	h538.2HH2	h538.1HL2
h538.2-3	h538.2HH3	h538.1HL2

Figure 91

Antibody (mIgG)	Human CD56 int. NK (gMFIr, 10ug/ml)	Human CD8+ T cells (gMFIr, 10ug/ml)	Expi cyno OE/par (gMFIr, 3.3ug/ml)	Cyno NK cells (gMFIr, 10ug/ml)	Cyno CD8+ T cells (gMFIr, 10ug/ml)
CHA.7.502	1.97	1.41	60.49	Not tested	Not tested
CHA.7.503	3.15	1.96	106.3	Not tested	Not tested
CHA.7.506	Not tested	Not tested	0.77	Not tested	Not tested
CHA.7.508	3.6	4.09	41.49	Not tested	Not tested
CHA.7.510	3.13	2.73	38.21	Not tested	Not tested
CHA.7.512	1.30	1.15	8.96	Not tested	Not tested
CHA.7.514	4.16	5.15	65.20	Not tested	Not tested
CHA.7.516	4.22	4.09	60.05	1.76	2.09
CHA.7.518	5.08	6.69	83.51	1.92	2.09
CHA.7.520	1.13	1.04	Not tested	Not tested	Not tested
CHA.7.522	2.06	1.90	27.24	Not tested	Not tested
CHA.7.524	5.50	6.12	66.32	1.78	2.02
CHA.7.525	1.98	1.76	0.85	Not tested	Not tested
CHA.7.526	2.08	1.71	0.79	Not tested	Not tested
CHA.7.527	1.16	0.99	Not tested	Not tested	Not tested
CHA.7.528	3.08	3.63	12.2	1.21	1.18
CHA.7.530	6.04	6.47	60.80	1.73	1.89
CHA.7.534	2.60	1.96	46.27	Not tested	Not tested
CHA.7.535	2.24	1.28	0.82	Not tested	Not tested
CHA.7.537	3.90	3.41	1.55	1.18	1.19
CHA.7.538	6.49	6.17	15.16	1.36	1.45
CHA.7.543	4.48	4.33	0.83	1.35	1.39
CHA.7.544	2.36	2.54	61.09	Not tested	Not tested
CHA.7.545	2.54	2.82	0.91	Not tested	Not tested
CHA.7.546	2.75	2.95	0.85	Not tested	Not tested
CHA.7.547	2.21	1.13	26.65	Not tested	Not tested
CHA.7.548	3.15	3.35	4.25	1.18	1.09
CHA.7.549	3.05	1.42	1.00	Not tested	Not tested
CHA.7.550	1.60	1.29	0.90	Not tested	Not tested

Figure 92

Antibody (mIgG)	IC_{50} (nM)
CHA.7.502	39.90
CHA.7.503	No IC_{50}
CHA.7.506	31.65
CHA.7.508	37.88
CHA.7.510	55.00
CHA.7.512	839.6
CHA.7.514	38.88
CHA.7.516	33.11
CHA.7.518	23.15
CHA.7.520	619.3
CHA.7.522	50.48
CHA.7.524	30.20
CHA.7.525	85.52
CHA.7.526	58.88
CHA.7.527	No IC_{50}
CHA.7.528	28.88
CHA.7.530	34.56
CHA.7.534	181.4
CHA.7.535	821.1
CHA.7.537	38.95
CHA.7.538	51.87
CHA.7.543	No IC_{50}
CHA.7.544	No IC_{50}
CHA.7.545	96.06
CHA.7.546	92.05
CHA.7.547	27.94
CHA.7.548	18.98
CHA.7.549	36.12
CHA.7.550	58.34

FIGURE 93A

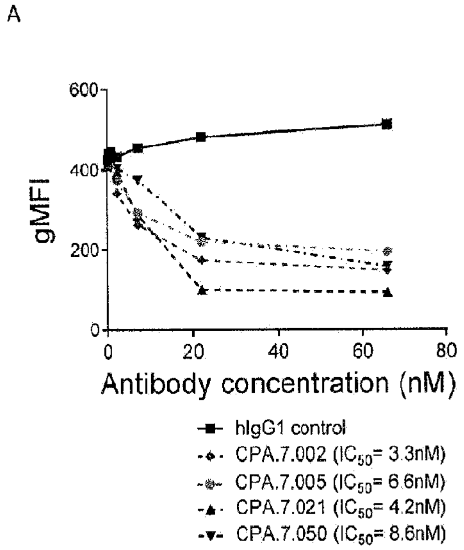
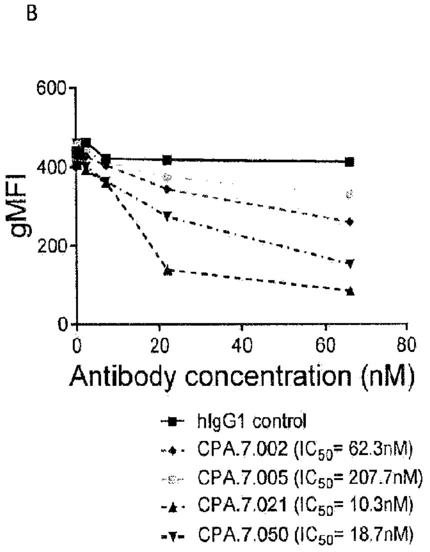


FIGURE 93B



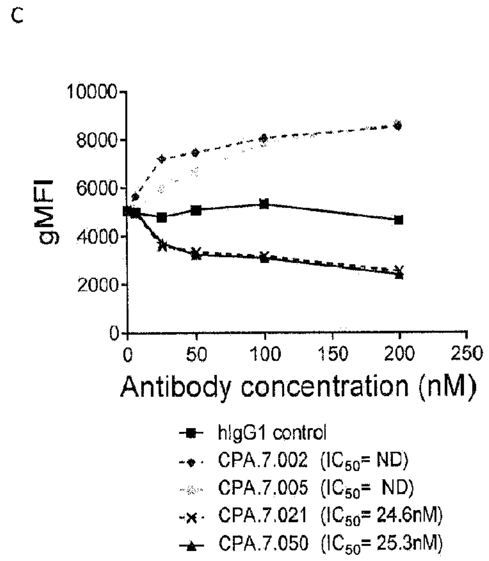


FIGURE 93C

FIG. 94A

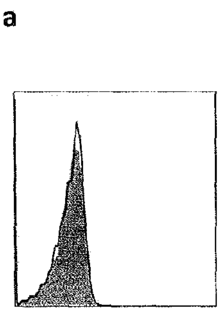


FIG. 94B

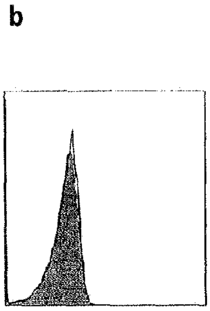


FIG. 94C

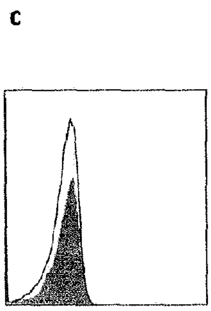
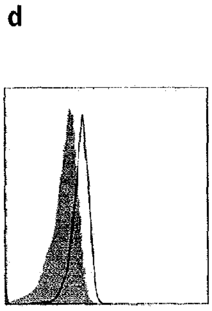


FIG. 94D



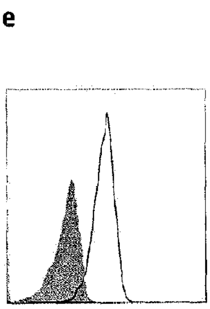


FIG. 94E

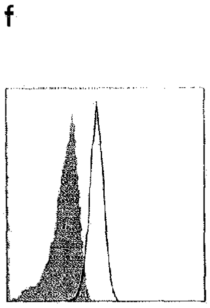


FIG. 94F

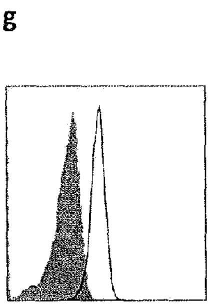


FIG. 94G

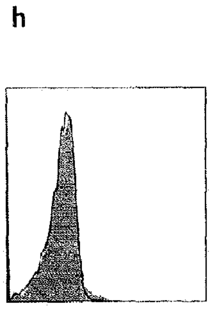


FIG. 94H

FIG. 95A

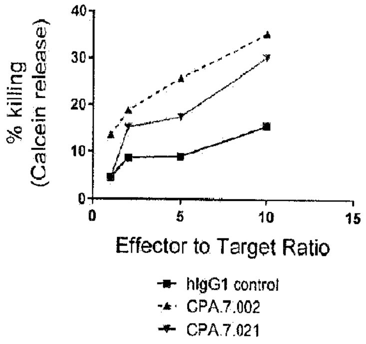


FIG. 95B

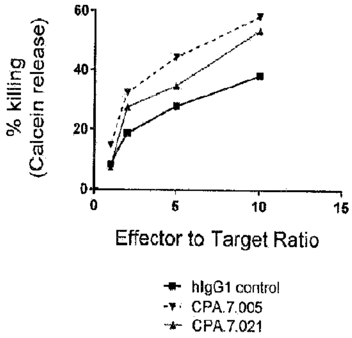


FIG. 95C

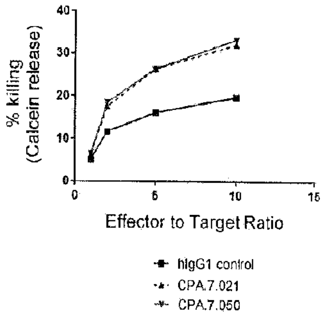


FIG. 95D

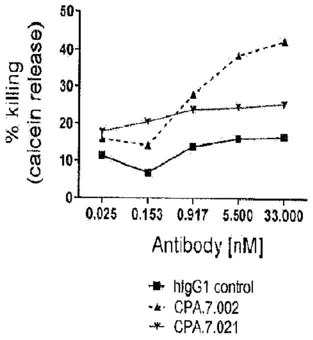


FIG. 95E

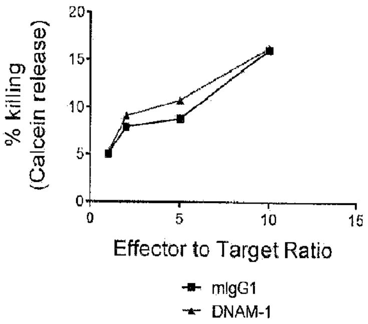


FIG. 95F

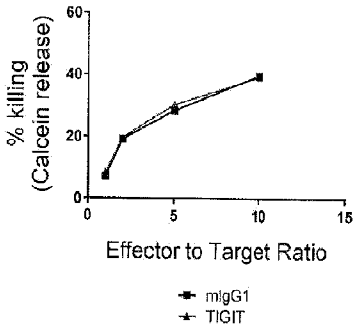


FIG. 96A

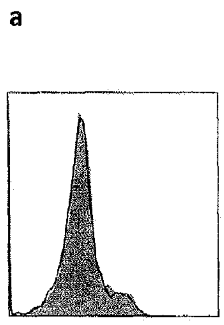


FIG. 96B

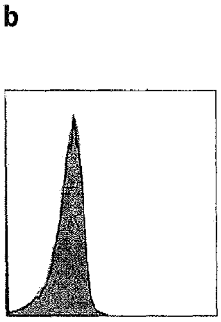


FIG. 96C

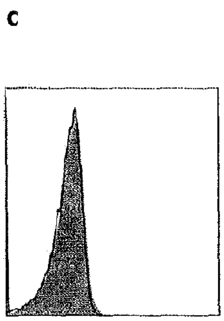
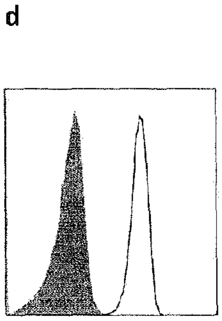


FIG. 96D



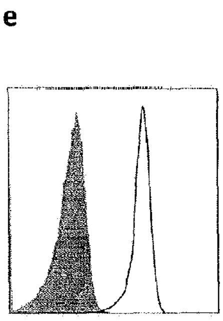


FIG. 96E

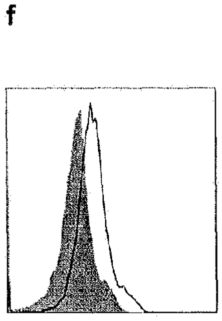


FIG. 96F

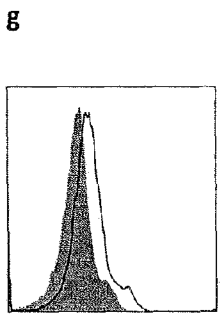


FIG. 96G

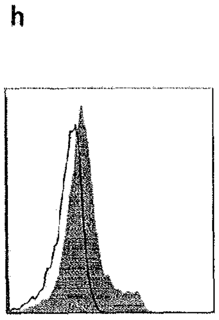
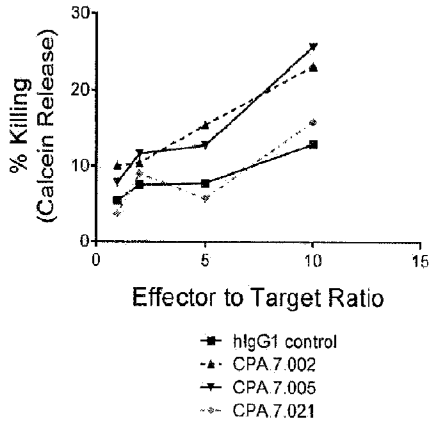


FIG. 96H

A

FIGURE 97A



B

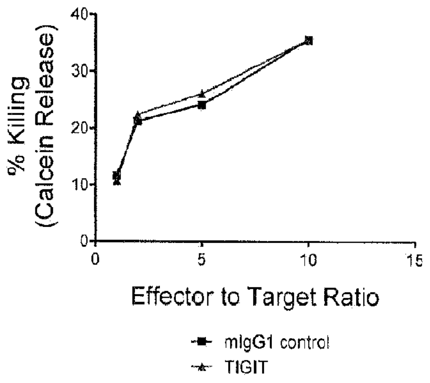


FIGURE 97B

Figure 98

Antibody (hIgG1)	Assay permutation and orientation	IC_{50} (nM)
CPA.7.002	HEK + PVRIG Fc (P)	3.3
	HEK + PVRIG Fc (NP)	62.3
	HEK hPVRIG + PVRL2 Fc	Increased binding
CPA.7.005	HEK + PVRIG Fc (P)	6.6
	HEK + PVRIG Fc (NP)	207.7
	HEK hPVRIG + PVRL2 Fc	Increased binding
CPA.7.021	HEK + PVRIG Fc (P)	4.2
	HEK + PVRIG Fc (NP)	10.3
	HEK hPVRIG + PVRL2 Fc	24.6
CPA.7.036	HEK + PVRIG Fc (P)	6.9
	HEK + PVRIG Fc (NP)	10.9
CPA.7.037	HEK + PVRIG Fc (P)	6.5
	HEK + PVRIG Fc (NP)	19.1
CPA.7.038	HEK + PVRIG Fc (P)	3.5
	HEK + PVRIG Fc (NP)	6.7
CPA.7.039	HEK + PVRIG Fc (P)	14.0
	HEK + PVRIG Fc (NP)	32.7
CPA.7.041	HEK + PVRIG Fc (P)	5.8
	HEK + PVRIG Fc (NP)	16.2
CPA.7.042	HEK + PVRIG Fc (P)	3.6
	HEK + PVRIG Fc (NP)	8.3
CPA.7.050	HEK + PVRIG Fc (P)	8.6
	HEK + PVRIG Fc (NP)	18.7
	HEKhPVRIG + PVRL2 Fc	25.3

Figure 99

Antibody (hIgG1)	Target Cell line	Fold change in cytotoxicity relative to control
CPA.7.002	Reh	2.9
	MOLM-13	1.9
CPA.7.005	Reh	1.6
	MOLM-13	1.6
CPA.7.021	Reh	1.9
	MOLM-13	0.7
CPA.7.036	Reh	0.9
CPA.7.037	Reh	1.1
CPA.7.038	Reh	0.8
CPA.7.041	Reh	0.7
CPA.7.042	Reh	0.7
CPA.7.050	Reh	1.6
TIGIT	Reh	1.1
	MOLM-13	1.1
DNAM-1	Reh	1.2

Figure 100

```
NP_076975.2-Homosapiens               TPEVWVQVKMEATELSSFTIKCGFLGSGSISLVTVSWGGPEGAGGTTLAVLHPERGIPQW
XP_005549281.1-Macacafascicularis     TPEVWVQVQMEATELSSFTVHCGFLGFGSISLVTVSWGGPDGAGGTKLAVLHPELGTRQW
XP_003732227.1-Callithrixjacchus      TPEVWVQVQMKATELSSFIVHCGFLGSGSISLVTVSWGGPDGAGGTSLAVLHPELGTRQW
XP_001103603.1-Macacamulatta          TPEVWVQVQMEATELSSFTVHCGFLGFGSISLVTVSWGGPDGAGGTKLAVLHPELGTRQW
                                      ********:*.******* ::***** *************:***** ******* *  **

NP_076975.2-Homosapiens               APARQARWETQSSISLLLEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSA
XP_005549281.1-Macacafascicularis     APARQARWETQSSISLALEDSGASSPFANTTFCCKFASFPEGSWESCGSLPPSSDPGLSA
XP_003732227.1-Callithrixjacchus      APAHQARWETQSSISLVLEPPGASSPSANTTFCCKFASFPEGSWEACGSLPPSSDPGLSA
XP_001103603.1-Macacamulatta          APARQARWETQSSISLALEDSGASSPFANTTFCCKFASFPEGSWESCGSLPPSSDPGLSA
                                      ***:************ **  ***** ******************.**************

NP_076975.2-Homosapiens               PPTPAPILRAD
XP_005549281.1-Macacafascicularis     PPTPVPILRAD
XP_003732227.1-Callithrixjacchus      PIL-----RAD
XP_001103603.1-Macacamulatta          PPTPVPILRAD
                                      *       ***
```

SEQ ID NOS 1463-1466, respectively, in order of appearance

Figure 101

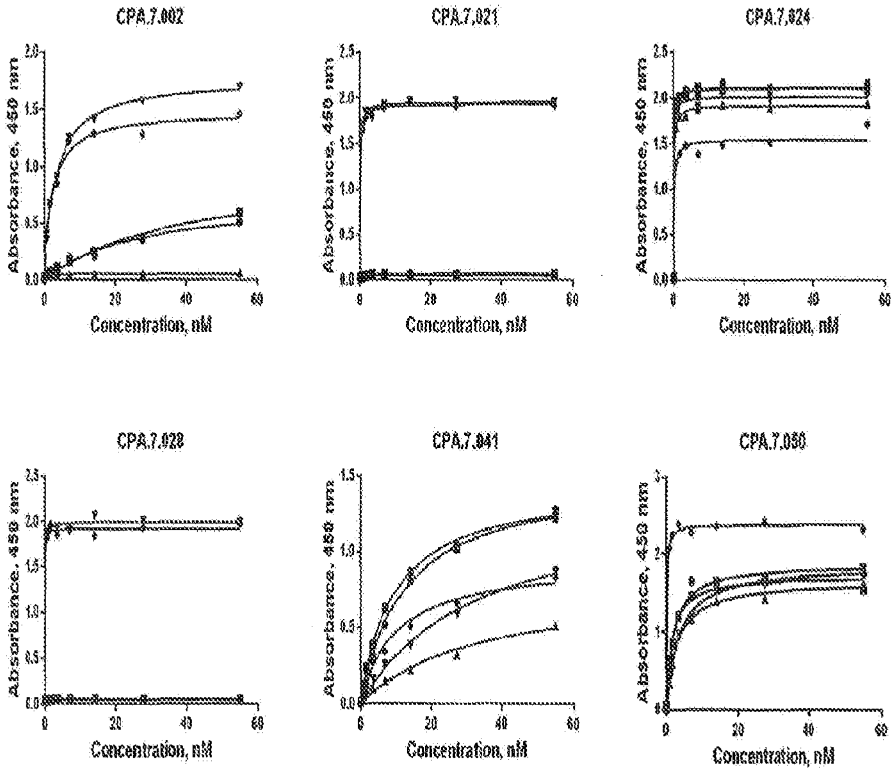


Figure 102

mAb	Cyno cross-reactivity	Epitope bin	Epitope group
CPA.7.002	+	1	2
CPA.7.021	-	1	1
CPA.7.024	+++	1	3
CPA.7.028	-	1	1
CPA.7.041	++	1	2
CPA.7.050	+++	4	3

Figure 103A

>PVRIG_NP_076975_from_41_to_171_Sequence_of_Human_WT_ECD_(without_SP) SEQ ID NO: 1467

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD

Figure 103B

>PVRIG_NP_076975_from_21_to_171_Sequence_of_Human_WT_ECD_(with_SP) SEQ ID NO: 1468

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD

Figure 103C

>PVRIG_NP_076975_from_43_to_146_Sequence_of_Ig_Domain SEQ ID NO: 1469

EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103D

>PVRIG_Splice_Variant_P6 SEQ ID NO: 1470

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHR

Figure 103E

>PVRIG_Splice_Variant_P8 SEQ ID NO: 1471

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSVPPTPAPILRADLAGILGVSGVLLFDCGYLLHLLCRQKHRPAPRLQPSHTSS

Figure 103F

>PVRIG_Splice_Variant_P4 SEQ ID NO: 1472

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRWLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQA
SQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR

Figure 103G

>PVRIG_Splice_Variant_P7 SEQ ID NO: 1473

MGHRTLVLPWVLLTLCVTAGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSCRPATLDT
AHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR

Figure 103H

>PVRIG_Splice_Variant_P14 SEQ ID NO: 1474

MGHRTLVLPWVLLTLCVTAGLSVPPTPAPILRADLAGILGVSGVLLFDCGYLLHLLCRQKHRPAPRLQPSHTSS

Figure 103I

>PVRIG_Splice_Variant_P3 SEQ ID NO: 1475

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCCKFASFPEGSWEACGSLPPSSDPGGAGARGPGGQGGARELATHLILVSVPRALCPADSCPHSAGRPGRDLGGLRSPPLWLCLPPSSAAPT

Figure 103J

>PVRIG_Splice_Variant_P10 SEQ ID NO: 1476

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGAQISPRGPKIGGPPCPRPGPAGKPRAASLSSW
KALGPAAPAPTPPSAASLRPSQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPD
PRGPRAMEGPLGVR

Figure 103K

>PVRIG_Splice_Variant_P13 SEQ ID NO: 1477

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCCKFASFPGCSSRPLCHYQHQLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHTHPCTWQLCLC

Figure 103L

>PVRIG_Splice_Variant_P3_ECD SEQ ID NO: 1478

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
LPPSSDPGGAGARGPGGQGGARELATHLILVSVPRALCPADSCPHSAGRPGRDLGGLRSPPLWLCLPPSSAAPT

Figure 103M

>PVRIG_Splice_Variant_P10_ECD SEQ ID NO: 1479

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGAQISPRGPKIGGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRPS
QAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR

Figure 103N

>PVRIG_Splice_Variant_P13_ECD SEQ ID NO: 1480

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPGCSSRPLCHY
QHQLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHTHPCTWQLCLC

Figure 103O

>PVRIG_Splice_Variant_P3_ECD_Unique SEQ ID NO: 1481

GAGARGPGGQGGARELATHLILVSVPRALCPADSCPHSAGRPGRDLGGLRSPPLWLCLPPSSAAPT

Figure 103P

>PVRIG_Splice_Variant_P10_ECD_All_Unique SEQ ID NO: 1482

AQISPRGPKIGGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRPSQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVR

Figure 103Q

>PVRIG_Splice_Variant_P10_ECD_Unique_without_intracellular SEQ ID NO: 1483

AQISPRGPKIGGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRP

Figure 103R

>PVRIG_Splice_Variant_P13_ECD_Unique SEQ ID NO: 1484

GCSSRPLCHYQHQLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHTHPCTWQLCLC

Figure 103S

>PVRIG_NP_076975_from_41_to_169_variation_of_ECD_(without_SP) SEQ ID NO: 1485

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILR

Figure 103T

>PVRIG_NP_076975_from_41_to_170_variation_of_ECD_(without_SP) SEQ ID NO: 1486

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRA

Figure 103U

>PVRIG_NP_076975_from_41_to_172_variation_of_ECD_(without_SP) SEQ ID NO: 1487

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
LPPSSDPGLSAPPTPAPILRADL

Figure 103V

>PVRIG_NP_076975_from_41_to_173_variation_of_ECD_(without_SP) SEQ ID NO: 1488

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
LPPSSDPGLSAPPTPAPILRADLA

Figure 103W

>PVRIG_NP_076975_from_41_to_144_variation_of_Ig_Domain SEQ ID NO: 1489

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103X

>PVRIG_NP_076975_from_41_to_145_variation_of_Ig_Domain SEQ ID NO: 1490

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103Y

>PVRIG_NP_076975_from_41_to_146_variation_of_Ig_Domain SEQ ID NO: 1491

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103Z

>PVRIG_NP_076975_from_41_to_147_variation_of_Ig_Domain SEQ ID NO: 1492

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AA

>PVRIG_NP_076975_from_41_to_148_variation_of_Ig_Domain SEQ ID NO: 1493

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AB

>PVRIG_NP_076975_from_42_to_144_variation_of_Ig_Domain SEQ ID NO: 1494

PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AC

>PVRIG_NP_076975_from_42_to_145_variation_of_Ig_Domain SEQ ID NO: 1495

PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AD

>PVRIG_NP_076975_from_42_to_146_variation_of_Ig_Domain SEQ ID NO: 1496

PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AE

>PVRIG_NP_076975_from_42_to_147_variation_of_Ig_Domain SEQ ID NO: 1497

PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AF

>PVRIG_NP_076975_from_42_to_148_variation_of_Ig_Domain SEQ ID NO: 1498

PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AG

>PVRIG_NP_076975_from_43_to_144_variation_of_Ig_Domain SEQ ID NO: 1499

EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AH

>PVRIG_NP_076975_from_43_to_145_variation_of_Ig_Domain SEQ ID NO: 1500

EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AI

>PVRIG_NP_076975_from_43_to_147_variation_of_Ig_Domain SEQ ID NO: 1501

EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AJ

>PVRIG_NP_076975_from_43_to_148_variation_of_Ig_Domain SEQ ID NO: 1502

EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AK

>PVRIG_NP_076975_from_44_to_144_variation_of_Ig_Domain SEQ ID NO: 1503

VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AL

>PVRIG_NP_076975_from_44_to_145_variation_of_Ig_Domain SEQ ID NO: 1504

VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AM

>PVRIG_NP_076975_from_44_to_146_variation_of_Ig_Domain SEQ ID NO: 1505

VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AN

>PVRIG_NP_076975_from_44_to_147_variation_of_Ig_Domain SEQ ID NO: 1506

VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AO

>PVRIG_NP_076975_from_44_to_148_variation_of_Ig_Domain SEQ ID NO: 1507

VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AP

>PVRIG_NP_076975_from_45_to_144_variation_of_Ig_Domain SEQ ID NO: 1508

WVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AQ

>PVRIG_NP_076975_from_45_to_145_variation_of_Ig_Domain SEQ ID NO: 1509

WVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AR

>PVRIG_NP_076975_from_45_to_146_variation_of_Ig_Domain SEQ ID NO: 1510

WVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AS

>PVRIG_NP_076975_from_45_to_147_variation_of_Ig_Domain SEQ ID NO: 1511

WVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AT

>PVRIG_NP_076975_from_45_to_148_variation_of_Ig_Domain SEQ ID NO: 1512

WVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AU

>PVRIG_XP_006544085_PREDICTED_transmembrane_protein_PVRIG-like_isoform_X2_[Mus_musculus] SEQ ID NO: 1513

MRTGNTQAAHATNMGQMQTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETP
NSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRILGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHL
ASTHSSFISMENGLYALA

Figure 103AV

>PVRIG_2nd_Met_Mouse_WT SEQ ID NO: 1514

MGQMQTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKA
RSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRILGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISMENGLY
ALA

Figure 103AW

>PVRIG_Mouse_WT_ECD_(without_SP) SEQ ID NO: 1515

SPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRV
ACRDLHRSDPGLSAPTPALNLQAD

Figure 103AX

>PVRIG_Mouse_WT_ECD_(with_SP) SEQ ID NO: 1516

MGQMQTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKA
RSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQAD

Figure 103AY

>PVRIG_HH-1_(Human_ECD_+_human_IgG1_Fc_mutated_C220S_at_hinge)_-_without_SP SEQ ID NO: 1517

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
LPPSSDPGLSAPPTPAPILRADEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103AZ

>PVRIG_HH-1-1_(Human_ECD_with_C127F_and_C147S_mutations_+_human_IgG1_Fc_mutated_C220S_at_hinge)_-_without_SP SEQ ID NO:
1518

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPFANTTFCCKFASFPEGSWEASGSL
PPSSDPGLSAPPTPAPILRADEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BA

>PVRIG_HH-2_(Human_ECD_+_Human_IgG1_Fc_mutated_at_C220S,_C226S,_C229S_of_hinge)_-_without_SP SEQ ID NO: 1519

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BB

>PVRIG_HH-2-1-1_(Human_ECD_with_C127F_mutation_+_Human_IgG1_Fc_mutated_at_C220S,_C226S,_C229S_of_hinge,_encoded_by_natural_codons_instead_of_codons_optimized_for_CHO_cells)_-_without_SP SEQ ID NO: 1520

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPFANTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BC

>PVRIG_MM-1_(Mouse_ECD_+_mouse_IgG2a_Fc)_-_without_SP SEQ ID NO: 1521

SPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BD

>PVRIG_MM-1-1_(Mouse_ECD_+_mouse_IgG2a_Fc_with_3_C_mutated_to_S_at_hinge)_-_without_SP SEQ ID NO: 1522

SPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRV
ACRDLHRSDPGLSAPTPALNLQADEPRGPTIKPSPPSKSPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLR
VVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSK
LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BE

>Human_Fc_(IgG1) SEQ ID NO: 1523

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Figure 103BF

>Human_Fc_(IgG1)_C220S SEQ ID NO: 1524

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Figure 103BG

>Human_Fc_(IgG1)_with_the_C220S_(at_hinge)_and_N297A_mutations SEQ ID NO: 1525

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Figure 103BH

>Human_Fc_(IgG1)_without_hinge SEQ ID NO: 1526

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BI

>Mouse_Fc_(IgG2a) SEQ ID NO: 1527

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVN
NKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH
NHHTTKSFSRTPGK

Figure 103BJ

>Mouse_Fc_(IgG2a)_Fc_with_the_N297A_mutation SEQ ID NO: 1528

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYASTLRVVSALPIQHQDWMSGKEFKCKVN
NKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH
NHHTTKSFSRTPGK

Figure 103BK

>Mouse_Fc_(IgG2a)_without_hinge SEQ ID NO: 1529

APNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPK
GSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BL

>Human_Fc_(IgG1)_with_the_C220S_C226S_C229S_mutations_(at_hinge) SEQ ID NO: 1530

EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Figure 103BM

>PVRIG_PRIMER_200-554_m15029_F SEQ ID NO: 1531

CCACCAACCTCTCGTCTTTC

Figure 103BN

>PVRIG_PRIMER_200-553_m15029_R SEQ ID NO: 1532

TCATGCCAGAGCATACAG

Figure 103BO

>PVRIG_PRIMER_200-571_m15029_F SEQ ID NO: 1533

CAGTGCCTCTAACTGCTGAC

Figure 103BP

>PVRIG_PRIMER_200-572_m15029_R SEQ ID NO: 1534

TCACTGTTACCAGGGAGATGAG

Figure 103BQ

>PVRIG_PRIMER_200-549_m15029_F SEQ ID NO: 1535

CACAGGCTGCCCATGCAAC

Figure 103BR

>PVRIG_PRIMER_200-551_m15029_R SEQ ID NO: 1536

TGCCTGGGTGCTAGTGAGAG

Figure 103BS

>PVRIG_PRIMER_200-554_m15029_F SEQ ID NO: 1537

CCACCAACCTCTCGTCTTTC

Figure 103BT

>PVRIG_PRIMER_200-546_m15029_R SEQ ID NO: 1538

GACCCTGTTACCTGTCATTG

Figure 103BU

>PVRIG_flag_protein SEQ ID NO: 1539

MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTS
CRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPRAMEGPLGVRDYKDDDDK

Figure 103BV

>PVRIG_Mouse_First_Methionine_signal_peptide-Flag-ECD SEQ ID NO: 1540

MRTGNTQAAHATNMGQMQTLVLFSTLLTLCVSEADYKDDDDKSPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRILGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISMENGLYALA

Figure 103BW

>PVRIG_Mouse_Third_Methionine_untagged SEQ ID NO: 1541

MQTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRILGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISMENGLYALA

Figure 103BX

>PVRIG_Mouse_Third_Methionine_signal peptide-Flag-ECD SEQ ID NO: 1542

MQTLVLFSTLLTLCVSEADYKDDDDKSPEVWVQVQMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRILGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISMENGLYALA

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