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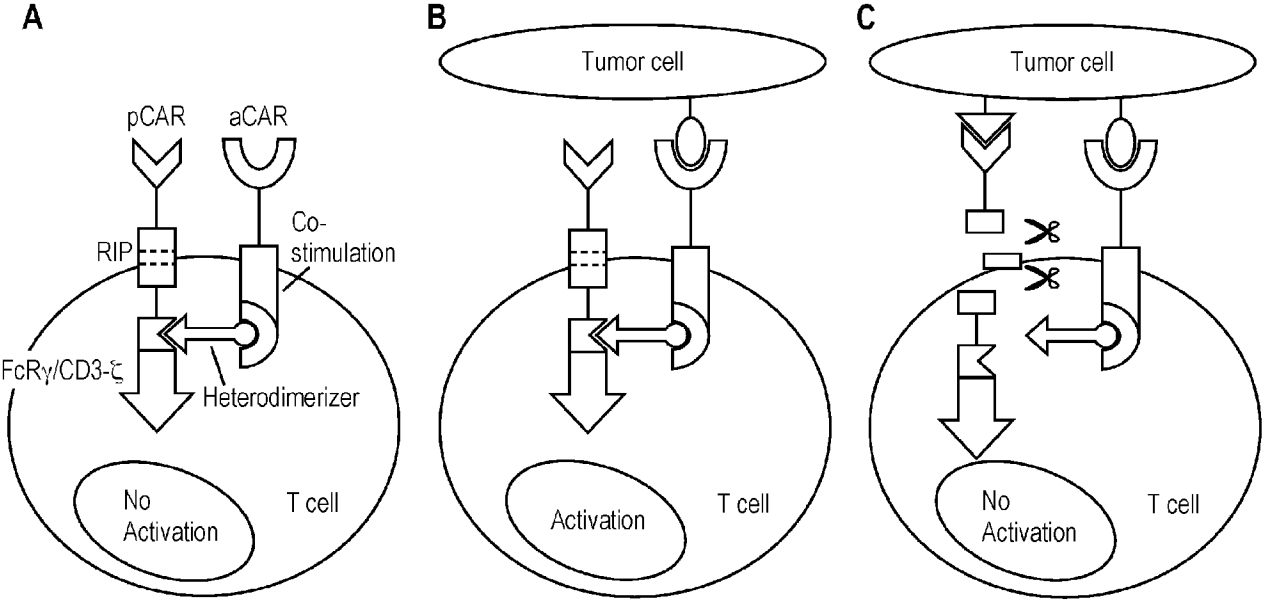
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(54) Titre : PLATE-FORME UNIVERSELLE POUR PREPARER UN RECEPTEUR D'ANTIGENE CHIMERIQUE INHIBITEUR (ICAR)

(54) Title: A UNIVERSAL PLATFORM FOR PREPARING AN INHIBITORY CHIMERIC ANTIGEN RECEPTOR (ICAR)

Figure 2



(57) Abrégé/Abstract:
The present invention provides a method of identifying a target for preparing an inhibitory chimeric antigen receptor (iCAR) or a protective chimeric antigen receptor (pCAR) capable of preventing or attenuating undesired activation of an effector immune cell. Also provided are a list of iCAR targets, as well as vectors and transduced effector immune cells comprising the nucleic acid molecule and methods for treatment of cancer comprising administering the transduced effector immune cells are further provided.

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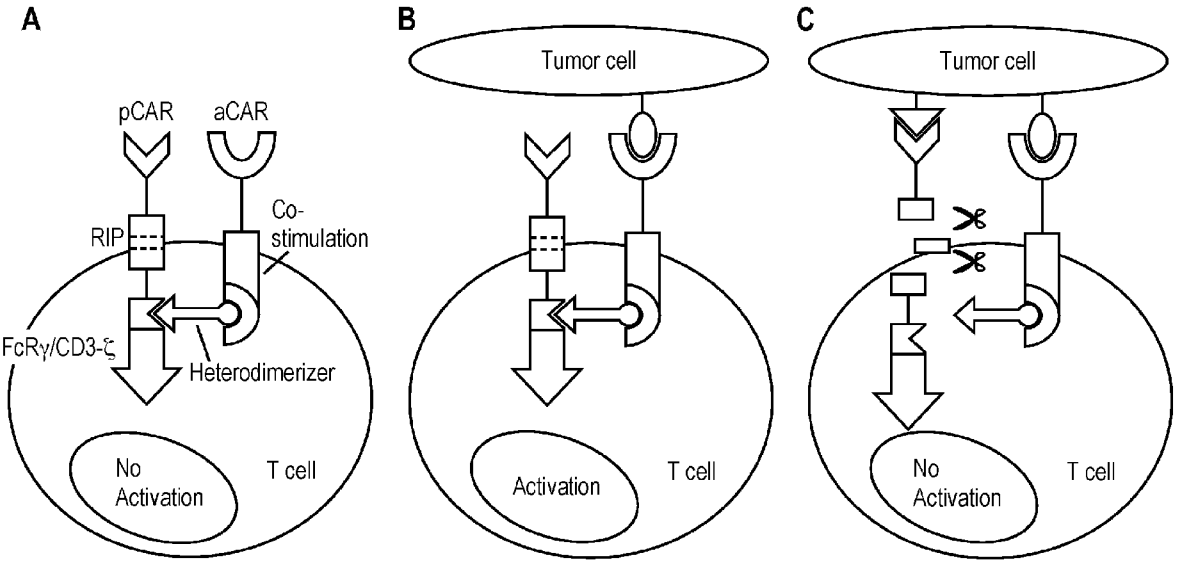
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(54) Title: A UNIVERSAL PLATFORM FOR PREPARING AN INHIBITORY CHIMERIC ANTIGEN RECEPTOR (ICAR)

Figure 2



(57) **Abstract:** The present invention provides a method of identifying a target for preparing an inhibitory chimeric antigen receptor (iCAR) or a protective chimeric antigen receptor (pCAR) capable of preventing or attenuating undesired activation of an effector immune cell. Also provided are a list of iCAR targets, as well as vectors and transduced effector immune cells comprising the nucleic acid molecule and methods for treatment of cancer comprising administering the transduced effector immune cells are further provided.

[Continued on next page]

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A UNIVERSAL PLATFORM FOR PREPARING AN INHIBITORY CHIMERIC ANTIGEN RECEPTOR (iCAR)

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/564,454, filed September 28, 2017, and U.S. Provisional Application No. 62/649,429, filed March 28, 2018, each of which is herein incorporated by reference.

SEQUENCE LISTING

[0002] This patent application contains a Sequence Listing which has been submitted electronically in ASCII format and are hereby incorporated herein by reference in its entirety. Said ASCII copy, created September 27, 2018, is named 120575-5003_ST25.txt.

ASCII TABLE

[0003] The provisional patent application to which the current application claims priority contains a lengthy table section. A copy of the table was submitted to the U.S. Patent and Trademark Office on compact disc in ASCII format with priority U.S. Provisional Application No. 62/649,429, filed March 28, 2018 and is hereby incorporated by reference, and may be employed in the practice of the invention. Said ASCII table, created March 28, 2018, is as follows: 120575-5003-PR allCandExt1167Genes_5003_PR.txt, 272,719,870 bytes.

FIELD OF THE INVENTION

[0004] The invention relates to the field of cancer immunotherapy by adoptive cell transfer, employing activating chimeric antigen receptors (aCARs) recognizing antigens expressed on the surface of tumor cells, inhibitory CARs (iCARs) and protective CARs (pCARs) directed at allelic variants of the same or other cell surface antigens expressed by normal cells but not by the tumor due to loss of heterozygosity (LOH).

BACKGROUND OF THE INVENTION

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[0005] The identification of targetable antigens that are exclusively expressed by tumor cells but not by healthy tissue is undoubtedly the major challenge in cancer immunotherapy today. Clinical evidence that T cells are capable of eradicating tumor cells comes from numerous studies evaluating highly diverse approaches for harnessing T cells to treat cancer (Rosenberg and Restifo, 2015). These approaches employ bone marrow transplantation with donor lymphocyte infusion, adoptive transfer of tumor-infiltrating lymphocytes (TILs), treatment with T cells genetically redirected at pre-selected antigens via CARs (Gross and Eshhar, 2016a) or T cell receptors (TCRs), the use of immune checkpoint inhibitors or active vaccination. Of these, the use of genetically engineered T cells and different strategies for active immunization entail pre-existing information on candidate antigens which are likely to exert a durable clinical response but minimal adverse effects. Yet, as stated in the title of a recent review by S. Rosenberg, "Finding suitable targets is the major obstacle to cancer gene therapy" (Rosenberg, 2014).

[0006] The concept of using chimeric antigen receptors (or CARs) to genetically redirect T cells (or other killer cells of the immune system such as natural killer (NK) cells and cytokine-induced killer cells) against antigens of choice in an MHC-independent manner was first introduced by Gross and Eshhar in the late 1980s (Gross et al., 1989). They are produced synthetically from chimeric genes encoding an extracellular single-chain antibody variable fragment (scFv) fused through a flexible hinge and transmembrane canonic motif to signaling components comprising immunoreceptor tyrosine-based activation motifs of CD3-ζ or FcRy chains capable of T cell activation. At present, CARs are being examined in dozens of clinical trials and have so far shown exceptionally high efficacy in B cell malignancies (Dotti et al., 2014; Gill and June, 2015; Gross and Eshhar, 2016a). The safety of CAR-T cell therapy is determined, in large, by its ability to discriminate between the tumor and healthy tissue. A major risk and the direct cause for adverse autoimmune effects that have been reported in clinical and preclinical studies is off-tumor, on-target toxicity resulting from extra-tumor expression of the target antigen (dealt with in detail in our recent review (Gross and Eshhar, 2016b) and (Klebanoff et al., 2016)). Concerning this risk, shared, non-mutated cell surface antigens which are currently tested clinically or pre-clinically for CAR therapy can be generally divided into a number of categories according to their tissue distribution and mode of expression:

- Strictly tumor-specific antigens. Perhaps the only member in this group which is already being examined clinically is variant III of the epidermal growth factor receptor (EGFRvIII) that is frequently overexpressed in glioblastoma and is also found in non-small cell lung carcinoma and prostate, breast, head and neck and ovarian cancers but not on normal tissue.
- Surface antigens expressed on the tumor and on non-vital healthy tissue. Potential CAR antigens in this group are differentiation-related molecules that are mainly restricted to the B cell lineage. Prominent among these (and a target antigen in numerous clinical trials) is CD19, a pan-B cell marker acquired very early in B cell differentiation and involved in signal transduction by the B cell receptor (BCR). Membrane prostate antigens constitute another class of antigens in this category.
- Antigens that are typically expressed by non-malignant tumor-promoting cells. One such antigen is fibroblast activation protein (FAP), a cell surface serine protease which is almost invariably expressed by tumor-associated fibroblasts in diverse primary and metastatic cancers. Another antigen is vascular endothelial growth factor (VEGF), which is highly expressed during tumor angiogenesis and is normally expressed on vascular and lymphatic endothelial cells in many vital organs.
- Tumor associated antigens (TAAs) shared with vital healthy tissue.

[0007] Most other TAAs which are presently evaluated in preclinical and clinical studies are overexpressed by tumors but are also present, usually at lower level, on essential normal tissue.

[0008] The broad spectrum of strategies devised to tackle autoimmunity in CAR T cell therapy can be divided into those which seek to eliminate, or suppress transferred T cells once damage is already evident (reactive measures) and those that aim at preventing potential damage in the first place (proactive measures) (Gross and Eshhar, 2016a). Reactive approaches often use suicide genes such as herpes simplex virus thymidine kinase (HSV-tk) and iC9, a fusion polypeptide comprising a truncated human caspase 9 and a mutated FK506-binding protein. Other approaches utilize antibodies to selectively remove engineered cells which go havoc or, as recently demonstrated, a heterodimerizing small-molecule agent which governs the coupling of the CAR recognition moiety to the intracellular signaling domain (Wu et al., 2015). While some proactive measures are designed to limit the in-vivo persistence or function of CAR T cells (for example, the use of mRNA electroporation for gene delivery), others directly

address the critical challenge of increasing antigenic selectivity of the therapeutic CARs so as to avoid damage to non-tumor tissue. Two of these raise particular interest, as they can potentially broaden the range of tumor antigens which can be safely targeted by CAR T cells:

- Combinatorial (or 'split') antigen recognition. While true tumor-specific surface antigens are rare, combinations of two different antigens, not-necessarily classified as tumor-associated antigens that are co-expressed by a given tumor, can define a new tumor-specific signature. Restricting the activity of CAR T cells to such antigen pairs provides a critical safety gauge and, consequently, extends the spectrum of tumor-specific targets and may be of substantial therapeutic value. Second and third generation CARs have been designed to provide therapeutic T cells with activation and costimulation signals upon engaging a single antigen through the tethering of two or more signaling portions at the CAR endodomain. However, if activation and costimulation are split in the same T-cell between two CARs, each specific for a different antigen, then full blown response would require the cooperation of the two complementary signals that could only be accomplished in the presence of the two antigens. This principle has been demonstrated in several preclinical studies (Kloss et al., 2013; Lanitis et al., 2013; Wilkie et al., 2012; WO 2016/126608).

[0009] While undoubtedly intriguing, this approach still faces the need in meticulous titration of the magnitude of both the activating and costimulatory signals so as to reach the optimal balance that would only allow effective on-target, on-tumor T cell reactivity. Whether such balance can be routinely attained in the clinical setting is still questionable.

[0010] An entirely new approach for limiting T cell response only to target cells that express a unique combination of two antigens was published recently (Roybal et al., 2016a). Its core element functions as a 'genetic switch' which exploits the mode of action of several cell surface receptors, including Notch. Following binding of such a receptor to its ligand it undergoes dual cleavage resulting in the liberation of its intracellular domain which translocates to the cell nucleus where it functions as a transcription factor. The implementation of this principle entails the co-introduction of two genes to the effector T cells. The first one is expressed constitutively and encodes such a chimeric cleavable receptor equipped with a recognition moiety directed at the

first antigen. Engagement with this antigen on the surface of a target cell will turn on the expression of the second gene encoding a conventional CAR which is directed at the second antigen. The target cell will be killed only if it co-expresses this second antigen as well.

[0011] Inhibitory CARs. Off-tumor reactivity occurs when the target antigen of CAR-redirected killer cells is shared with normal tissue. If this normal tissue expresses another surface antigen not present on the tumor, then co-expressing in the gene-modified cells an additional CAR targeting this non-shared antigen, which harbors an inhibitory signaling moiety, can prevent T-cell activation by the normal tissue.

[0012] Instead of an activating domain (such as FcRy or CD3-ζ), an iCAR possesses a signaling domain derived from an inhibitory receptor which can antagonize T cell activation, such as CTLA-4, PD-1 or an NK inhibitory receptor. If the normal tissue which shares the candidate aCAR antigen with the tumor expresses another surface antigen not shared with the tumor, an iCAR expressed by the same T cell which targets this non-shared antigen can protect the normal tissue (**Fig. 1**).

[0013] Unlike T cells, each of which expresses a unique two-chain TCR encoded by somatically rearranged gene segments, NK cells do not express antigen-specific receptors. Instead, NK cells express an array of germline-encoded activating and inhibitory receptors which respectively recognize multiple activating and inhibitory ligands at the cell surface of infected and healthy cells. The protective capacity of an iCAR based on NK inhibitory receptors such as KIR3DL1 has been described (US 9,745,368). KIR3DL1 and other NK inhibitory receptors function by dismantling the immunological synapse in a rapid and comprehensive manner. There is compelling evidence that a single NK cell can spare a resistant cell expressing both inhibitory and activating ligands yet kill a susceptible cell it simultaneously engages, which expresses only the activating ligands (Abeyweera et al., 2011; Eriksson et al., 1999; Treanor et al., 2006; Vyas et al., 2001). This exquisite ability is governed by the different spatial organization of signal transduction molecules formed at each of the respective immune synapses which consequently affects the exocytosis of cytolytic granules (see (Huse et al., 2013) for review). More recently, Fedorov et al. (Fedorov et al., 2013a; WO 2015/142314) successfully employed for this purpose the intracellular domains of PD-1 and CTLA-4. Unlike NK inhibitory receptors, the regulatory effects of these iCARs

affected the entire cell. Yet, these effects were temporary, allowing full T-cell activation upon subsequent encounter with target cells expressing only the aCAR antigen.

[0014] Tissue distribution of the antigens targeted by the iCAR and aCAR dictates the optimal mode of action of the iCAR required for conferring maximal safety without compromising clinical efficacy. For example, if the anatomical sites of the tumor and the normal tissue(s) to be protected do not intersect, transient inhibition (CTLA-4- or PD-1-like) will likely suffice. Yet, if these sites do overlap, only synapse-confined inhibition (*e.g.*, an NK mode of action) will prevent constant paralysis of the therapeutic cells and allow their effective tumoricidal activity. The approach of using iCARs to reduce on-target off-tumor reactivity suffers from a dire lack of antigens downregulated in tumor cells but present on normal tissue.

[0015] Next generation sequencing (NGS) allows the determination of the DNA sequence of all protein-coding genes (~1% of the entire genome) in a given tumor biopsy and the comparison of the cancer 'exome' to that of a healthy tissue (usually from white blood cells) of the same patient. Exome sequencing can be completed within several days post-biopsy removal and at relatively low cost. In parallel, transcriptome analysis (RNA-seq) can provide complementary information on the genes that are actually expressed by the same cell sample.

[0016] It is becoming increasingly clear that the mutational landscape of each individual tumor is unique (Lawrence et al., 2013; Vogelstein et al., 2013). As a result of nonsynonymous mutations the tumor cell can potentially present a private set of neopeptides to the patient's immune system on one or more of his or her HLA products. Indeed, tremendous efforts are being put in recent years into identifying tumor-specific neoepitopes which can be recognized by the patient's own CD8 or CD4 T cell repertoire and serve as targets for immunotherapy (for review see (Blankenstein et al., 2015; Van Buuren et al., 2014; Heemskerk et al., 2013; Overwijk et al., 2013; Schumacher and Schreiber, 2015)). However, cumulative findings suggest that neoantigen-based T cell immunotherapies are more likely to be effective in cancers displaying higher mutational load, such as melanoma and lung cancers, but may often fail to show benefit in most cancers with fewer mutations (Savage, 2014; Schumacher and Schreiber, 2015). Furthermore, considerable intratumoral heterogeneity (Burrell et

al., 2013) entails the simultaneous co-targeting of several antigens so as to avoid emergence of mutation-loss variants, a task which becomes increasingly demanding in view of the scarcity of useful immunogenic neopeptides.

[0017] All in all, the urgent need to identify suitable targets for cancer immunotherapy via the adoptive transfer of genetically redirected killer cells is still largely unmet.

BRIEF SUMMARY OF THE INVENTION

[0018] In some aspects, the present invention provides a method of identifying a target for preparing an inhibitory chimeric antigen receptor (iCAR) or a protective chimeric antigen receptor (pCAR) capable of preventing or attenuating undesired activation of an effector immune cell, wherein the target is identified by a method comprising:

(i) identifying a gene with at least two expressed alleles that encodes a protein comprising an extracellular polymorphic epitope;

(ii) determining that at least one of the expressed alleles exhibits an amino acid sequence change in the extracellular polymorphic epitope sequence relative to an extracellular polymorphic epitope reference sequence;

(iii) determining that the gene is located in a chromosomal region which undergoes loss of heterozygosity (LOH) in a tumor type; and

(iv) determining that the gene is expressed in the tissue-of-origin of the tumor type in which the chromosomal region was found to undergo LOH.

[0019] In some embodiments, the LOH position is selected from the group consisting of a substitution, deletion, and insertion. In some embodiments, the LOH position is a SNP. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA gene.

[0020] In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-A, HLA-B, HLA-C, HLA-G, HLA-E, HLA-F, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA_DQ, or HLA-DR gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-A gene. In some

embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-B gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-C gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-G gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-E gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-F gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-K gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-L gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-DM gene. In some embodiments, the gene comprising the extracellular polymorphic epitope is an HLA-DO gene. In some embodiments, the extracellular polymorphic epitope is an HLA-DP gene. In some embodiments, the extracellular polymorphic epitope is an HLA_DQ gene. In some embodiments, the extracellular polymorphic epitope is an HLA-DR gene.

[0021] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 1. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7,

SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[0022] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 2. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

[0023] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 3. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[0024] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 4. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[0025] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 5. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[0026] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 6. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

[0027] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 7. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL,

PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[0028] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 8. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[0029] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 9. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[0030] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 10. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[0031] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 11. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32,

MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[0032] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 12. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[0033] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 13. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[0034] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 14. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[0035] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 15. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[0036] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 16. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[0037] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 17. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[0038] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 18. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[0039] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 19. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[0040] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 20. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[0041] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 21. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of CLDN8,

DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[0042] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 22. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[0043] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome X. In some embodiments, the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[0044] In some embodiments, the tumor is selected from the group consisting of a breast tumor, a prostate tumor, an ovarian tumor, a cervical tumor, a skin tumor, a pancreatic tumor, a colorectal tumor, a renal tumor, a liver tumor, a brain tumor, a lymphoma, a leukemia, a lung tumor, and a glioma.

[0045] In some embodiments, the tumor is selected from the group consisting of an adrenal gland tumor, a kidney tumor, a melanoma, DLBC, a breast tumor, a sarcoma, an ovary tumor, a lung tumor, a bladder tumor, and a liver tumor. In some embodiments, the adrenal gland tumor is an adrenocortical carcinoma. In some embodiments, the kidney tumor is a chromophobe renal cell carcinoma. In some embodiments, the melanoma is uveal melanoma.

[0046] The present invention also provides safe effector cells. In some embodiments, the present invention provides a safe effector immune cell expressing (i) an iCAR or pCAR according to any of claims 1 through 46 and (ii) an activating chimeric antigen receptor (aCAR).

[0047] In some embodiments, the safe effector immune cell of claim 47, wherein the aCAR is directed against or specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope. In some embodiments, due to the protective effects of the iCAR or pCAR, the aCAR can be directed against any surface protein expressed on a cancer cell.

[0048] In some embodiments, the the aCAR is directed against or specifically binds to a tumor associated protein, a CAR target as listed in table 1, any cell surface protein that is expressed in a tumor tissue in which the iCAR is also expressed.

[0049] In some embodiments, the non-polymorphic cell surface epitope is selected from the group consisting of CD19, CD20, CD22, CD10, CD7, CD49f, CD56, CD74, CAIX Igκ, ROR1, ROR2, CD30, LewisY, CD33, CD34, CD38, CD123, CD28, CD44v6, CD44, CD41, CD133, CD138, NKG2D-L, CD139, BCMA, GD2,GD3, hTERT, FBP, EGP-2, EGP-40, FR-α, L1-CAM, ErbB2,3,4, EGFRvIII, VEGFR-2, IL-13Rα2, FAP, Mesothelin, c-MET, PSMA, CEA, kRas, MAGE-A1, MUC1, MUC16, PDL1, PSCA, EpCAM, FSHR, AFP, AXL, CD80, CD89, CDH17, CLD18, GPC3, TEM8, TGFB1, NY-ESO-1, WT-1 and EGFR.

[0050] 51. The safe effector immune cell of any of claims 47 to 50, wherein the safe effector immune cell is an autologous or a universal (allogeneic) effector cell.

[0051] In some embodiments, the safe effector immune cell is selected from the group consisting of a T cell, a natural killer cell and a cytokine-induced killer cell.

[0052] In some embodiments of the safe effector immune cell, the expression level of the iCAR or pCAR is greater than or equal to the expression level of the aCAR.

[0053] In some embodiments of the safe effector immune cell, the iCAR or pCAR is expressed by a first vector and the aCAR is expressed by a second vector.

[0054] In some embodiments of the safe effector immune cell, the iCAR or pCAR and the aCAR are both expressed by the same vector.

[0055] In some embodiments of the safe effector immune cell, the nucleotide sequence encoding for the aCAR is downstream of the nucleotide sequence encoding for the iCAR or pCAR.

[0056] In some embodiments of the safe effector immune cell, the nucleotide sequence comprises a viral self-cleaving 2A peptide between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR or pCAR.

[0057] In some embodiments of the safe effector immune cell, the viral self-cleaving 2A peptide is selected from the group consisting of T2A from *Thosea asigna virus* (TaV), F2A from Foot-and-mouth disease virus (FMDV), E2A from *Equine rhinitis A virus* (ERAV) and P2A from *Porcine teschovirus-1* (PTV1).

[0058] In some embodiments of the safe effector immune cell, the nucleotide sequence encoding the aCAR is linked via a flexible linker to the iCAR or pCAR.

[0059] In some embodiments of the safe effector immune cell, the aCAR comprises at least one signal transduction element that activates or co-stimulates an effector immune cell.

[0060] In some embodiments of the safe effector immune cell, the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to an immunoreceptor tyrosine-based activation motif (ITAM) of for example CD3ζ or FcRγ chains.

[0061] In some embodiments of the safe effector immune cell, the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to an activating killer cell immunoglobulin-like receptor (KIR), such as KIR2DS and KIR3DS.

[0062] In some embodiments of the safe effector immune cell, the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to or an adaptor molecule, such as DAP12.

[0063] In some embodiments of the safe effector immune cell, the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to or a co-stimulatory signal transduction element of CD27, CD28, ICOS, CD137 (4-1BB), CD134 (OX40) or GITR.

[0064] The present invention also provides a method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient a safe effector immune cell expressing an iCAR as described herein.

[0065] In some embodiments, the invention further provides a method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient a safe effector immune cell as described herein.

[0066] In one aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding an inhibitory chimeric antigen receptor (iCAR) capable of preventing or attenuating undesired activation of an effector immune cell, wherein the iCAR comprises an extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope absent from mammalian

tumor cells due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue and on vital organs ; and an intracellular domain comprising at least one signal transduction element that inhibits an effector immune cell. In some embodiments, the iCAR or pCAR target is expressed on all cells that the aCAR target is normally expressed in. In some embodiments, the iCAR or pCAR target is expressed in the vital organ cells the aCAR is expressed in.

[0067] In an additional aspect, the present invention provides a vector comprising a nucleic acid molecule of the invention as defined herein, and at least one control element, such as a promoter, operably linked to the nucleic acid molecule.

[0068] In another aspect, the present invention provides a method of preparing an inhibitory chimeric antigen receptor (iCAR) capable of preventing or attenuating undesired activation of an effector immune cell, according to the present invention as defined herein, the method comprising: (i) retrieving a list of human genomic variants of protein-encoding genes from at least one database of known variants; (ii) filtering the list of variants retrieved in (i) by: (a) selecting variants resulting in an amino acid sequence variation in the protein encoded by the respective gene as compared with its corresponding reference allele, (b) selecting variants of genes wherein the amino acid sequence variation is in an extracellular domain of the encoded protein, (c) selecting variants of genes that undergo loss of heterozygosity (LOH) at least in one tumor, and (d) selecting variants of genes that are expressed at least in a tissue of origin of the at least one tumor in which they undergo LOH according to (c), thereby obtaining a list of variants having an amino acid sequence variation in an extracellular domain in the protein encoded by the respective gene lost in the at least one tumor due to LOH and expressed at least in a tissue of origin of the at least one tumor; (iii) defining a sequence region comprising at least one single variant from the list obtained in (ii), sub-cloning and expressing the sequence region comprising the at least one single variant and a sequence region comprising the corresponding reference allele thereby obtaining the respective epitope peptides; (iv) selecting an iCAR binding domain, which specifically binds either to the epitope peptide encoded by the cloned sequence region, or to the epitope peptide encoded by the corresponding reference allele, obtained in (iii); and (vii) preparing iCARs as defined herein, each comprising an iCAR binding domain as defined in (iv).

[0069] In still another aspect, the present invention provides a method for preparing a safe effector immune cell comprising: (i) transfecting a TCR-engineered effector immune cell directed to a tumor-associated antigen with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR as defined herein or transducing the cells with a vector defined herein; or (ii) transfecting a naïve effector immune cell with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR as defined herein and a nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein; or transducing an effector immune cell with a vector as defined herein.

[0070] In yet another aspect, the present invention provides a safe effector immune cell obtained by the method of the present invention as described herein. The safe effector immune cell may be a redirected T cell expressing an exogenous T cell receptor (TCR) and an iCAR, wherein the exogenous TCR is directed to a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared at least by cells of related tumor and normal tissue, and the iCAR is as defined herein; or the safe effector immune cell is a redirected effector immune cell such as a natural killer cell or a T cell expressing an iCAR and an aCAR as defined herein.

[0071] In a further aspect, the present invention provides a method of selecting a personalized biomarker for a subject having a tumor characterized by LOH, the method comprising (i) obtaining a tumor biopsy from the subject; (ii) obtaining a sample of normal tissue from the subject, e.g., peripheral blood mononuclear cells (PBMCs); and (iii) identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, thereby identifying a personalized biomarker for the subject.

[0072] In a further aspect, the present invention provides a method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient an effector immune cell as defined herein, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

[0073] In still a further aspect, the present invention is directed to a safe effector immune cell as defined herein for use in treating a patient having a tumor characterized by LOH, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient, including the vital organs of the patient. In some embodiments, the iCAR or pCAR is expressed on all cells that the aCAR target is normally expressed in. In some embodiments, the iCAR or pCAR is expressed in vital organ cells that the aCAR is expressed in.

[0074] In yet a further aspect, the present invention is directed to a method for treating cancer in a patient having a tumor characterized by LOH comprising: (i) identifying or receiving information identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, (ii) identifying or receiving information identifying a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared by cells at least of related tumor and normal tissue in said cancer patient; (iii) selecting or receiving at least one nucleic acid molecule defining an iCAR as defined herein and at least one nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein, or at least one vector as defined herein, wherein the iCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (i) and the aCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (ii); (iv) preparing or receiving at least one population of safe redirected effector immune cells by transfecting effector immune cells with the nucleic acid molecules of (iii) or transducing effector immune cells with the vectors of (iii); and (v) administering to said cancer patient at least one population of safe redirected immune effector cells of (iv).

[0075] In a similar aspect, the present invention provides at least one population of safe redirected immune effector cells for treating cancer in a patient having a tumor characterized by LOH, wherein the safe redirected immune cells are obtained by (i) identifying or receiving information identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, (ii) identifying or receiving information

identifying a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared by cells at least of related tumor and normal tissue in said cancer patient; (iii) selecting or receiving at least one nucleic acid molecule defining an iCAR as defined herein and at least one nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein, or at least one vector as defined herein, wherein the iCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (i) and the aCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (ii); (iv) preparing or receiving at least one population of safe redirected effector immune cells by transfecting effector immune cells with the nucleic acid molecules of (iii) or transducing effector immune cells with the vectors of (iii).

[0076] In another aspect, the present invention is directed to a combination of two or more nucleic acid molecules, each one comprising a nucleotide sequence encoding a different member of a controlled effector immune cell activating system, said nucleic acid molecules being part of or forming a single continues nucleic acid molecule, or comprising two or more separate nucleic acid molecules, wherein the controlled effector immune activating system directs effector immune cells to kill tumor cells that have lost one or more chromosomes or fractions thereof due to Loss of Heterozygosity (LOH) and spares cells of related normal tissue, and wherein (a) the first member comprises an activating chimeric antigen receptor (aCAR) polypeptide comprising a first extracellular domain that specifically binds to a non-polymorphic cell surface epitope of an antigen or to a single allelic variant of a different polymorphic cell surface epitope and said non-polymorphic or polymorphic cell surface epitope is a tumor-associated antigen or is shared by cells of related abnormal and normal mammalian tissue; and (b) the second member comprises a regulatory polypeptide comprising a second extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope not expressed by an abnormal mammalian tissue due to LOH but present on all cells of related mammalian normal tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

[0077] **Fig. 1** shows the concept of iCARs (taken from (Fedorov et al., 2013a).

[0078] **Fig. 2** shows the aCAR/pCAR molecular design and mode of action. Binding of the pCAR to its antigen on normal cells, whether these express the aCAR antigen or not, is expeeted to result in rapid RIP and breaking of the polypeptide into 3 separate fragments.

[0079] **Figs. 3A-C** show the percentage of tumor samples undergoing LOH in the chromosomal region coding for the HLA class I locus. **A.** HLA-G, **B.** HLA-A, **C.** ZNRD1, in tumor types from the TCGA database. Kidney Chromophobe [KICH], Adrenocortical carcinoma [ACC], Pancreatic adenocarcinoma [PAAD], Sarcoma [SARC], Kidney renal papillary cell carcinoma [KIRP], Esophageal carcinoma [ESCA], Lung squamous cell carcinoma [LUSC], Kidney renal clear cell carcinoma [KIRC], Bladder Urothelial Carcinoma [BLCA], Ovarian serous cystadenocarcinoma [OV], Thymoma [THYM], Cervical squamous cell carcinoma and endocervical adenocarcinoma [CESC], Head and Neck squamous cell carcinoma [HNSC], Breast invasive carcinoma [BRCA], Stomach adenocarcinoma [STAD], Lymphoid Neoplasm Diffuse Large B-cell Lymphoma [DLBC], Glioblastoma multiforme [GBM], Colon adenocarcinoma [COAD], Rectum adenocarcinoma [READ], Lung adenocarcinoma [LUAD], Testicular Germ Cell Tumors [TGCT], Mesothelioma [MESO], Cholangiocarcinoma [CHOL], Uterine Carcinosarcoma [UCS], Skin Cutaneous Melanoma [SKCM], Uterine Corpus Endometrial Carcinoma [UCEC], Brain Lower Grade Glioma [LGG], Prostate adenocarcinoma [PRAD], Liver hepatocellular carcinoma [LIHC], Thyroid carcinoma [THCA], Pheochromocytoma and Paraganglioma [PCPG], Acute Myeloid Leukemia [LAML], Uveal Melanoma [UVM]

[0080] **Fig. 4** shows expression of HLA-A relative to all other protein coding genes in the genome. The value for each gene reflects the mean RPKM value of tissue medians obtained from GTEX (gtexportal.org)

[0081] **Fig. 5** shows a proposed workflow for analysis of HLA protein loss-of-heterozygosity across cancers in Example 5.

[0082] **Fig. 6** shows Frequency of LOH in the pancan12 dataset using ABSOLUTE processed copy number data. Lines represent 95% binomial confidence intervals for frequency.

[0083] **Fig. 7** shows the types of LOH observed in HLA-A. Of 588 episodes of HLA-A LOH, none involved a breakpoint within the HLA-A gene.

[0084] **Fig. 8** shows the distribution of length (in basepairs) of deletions encompassing HLA-A. A large fraction of these deletions are greater than the length of chromosome 6p.

[0085] **Fig. 9** shows the correlation between fraction of patients that have LOH of HLA-A in relative and ABSOLUTE copy number data with a threshold of -0.1.

[0086] **Fig. 10A-10C** shows the comparison of rate of LOH of HLA-A, HLA-B and HLA-C across 32 cancers reveals a nearly identical pattern of LOH.

[0087] **Fig. 11** shows the IGV screenshot of AML copy number profiles sorted for deletion of chromosome 6p. Blue indicates deletion, red indicates amplification. There are no deletions of HLA-A.

[0088] **Fig. 12** shows the proportion of uveal melanoma tumors undergoing LOH for all SNPs.

[0089] **Fig. 13** provides the TCGA Study Abbreviations (also available at https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations).

[0090] **Fig. 14** depicts the loss of a chromosomal region adjacent to the tumor suppressor protein TP53, coded on chromosome 17. Genes coded on chromosome 17 which were identified as iCAR targets can be used to treat patient RC001.

[0091] **Fig. 15** provides a schematic diagram of iCAR and aCAR constructs.

[0092] **Fig. 16** provides data regarding IL-2 secretion as measured by ELISA. iCAR specifically inhibits IL-2 secretion upon interaction with target cells expressing iCAR target.

[0093] **Fig. 17** shows that iCAR specifically inhibits IL-2 secretion upon interaction with target cells expressing iCAR target as measured by CBA.

[0094] **Fig. 18** shows specific activation of CD19 aCAR Jurkat-NFAT by CD19 expressing target cells.

[0095] **Fig. 19** shows specific inhibition of NFAT activation in CD19 aCAR/HLA-A2 iCAR Jurkat-NFAT

[0096] **Fig. 20** shows specific inhibition of NFAT activation at different E/T ratios.

[0097] **Fig. 21** provides the sequences for the iCAR and aCAR constructs of **Fig. 15**.

[0098] **Fig. 22** provides the 1167 potential iCAR, pCAR and/or aCAR targets.

[0099] **Fig. 23** provides the 3288 SNPs from the 1167 genes listed in **Fig. 22**.

DETAILED DESCRIPTION OF THE INVENTION

I. INTRODUCTION

[00100] Referring to the revolutionary concept of tumor suppressor genes (TSGs) that had been put forward in 1971 by A. G. Knudson (Knudson Jr., 1971), Devilee, Cleton-Jansen and Cornelisse stated in the opening paragraph of their essay titled 'Ever since Knudson' (Devilee et al., 2001): "Many publications have documented LOH on many different chromosomes in a wide variety of tumors, implicating the existence of multiple TSGs. Knudson's two-hit hypothesis predicts that these LOH events are the second step in the inactivation of both alleles of a TSG". In their seminal review on genetic instabilities in human cancers (Lengauer et al., 1998), Lengauer, Kinzler and Vogelstein wrote: "Karyotypic studies have shown that the majority of cancers have lost or gained chromosomes, and molecular studies indicate that karyotypic data actually underestimate the true extent of such changes. Losses of heterozygosity, that is, losses of a maternal or paternal allele in a tumor, are widespread and are often accompanied by a gain of the opposite allele. A tumor could lose the maternal chromosome 8, for example, while duplicating the paternal chromosome 8, leaving the cell with a normal chromosome 8 karyotype but an abnormal chromosome 8 `allelotype'. The 'average' cancer of the colon, breast, pancreas or prostate may lose 25% of its alleles and it is not unusual for a tumor to have lost over half of its alleles." These observations have since been reinforced and extended to almost all human cancers, including practically all carcinomas, in numerous reports (see (McGranahan et al., 2012) for review). It is now unambiguously established that nearly all individual tumors exhibit multiple losses of full chromosomes, entire chromosomal arms or sub-chromosomal regions of varying size. New algorithms are being rapidly developed (e.g., Sathirapongsasuti et al., 2011) for the determination of the LOH profile in any given cell sample based on the exome sequence data. While statistical bias may at present question the validity of some interpretations (Teo et al., 2012), such algorithms

are likely to improve and replace most other methodologies for establishing LOH profiles which had been employed for this purpose in the pre-NGS era

[00101] Early LOH events can be detected in premalignant cells of the same tissue, but not in surrounding normal cells (Barrett et al., 1999). LOH is irreversible and events can only accumulate, so that tumor heterogeneity reflects the accumulation of losses throughout tumor progression. While tumor subclones can develop which differ in later LOH events, the existence of a minimal LOH signature that is shared by premalignant cells, putative tumor stem cells and all tumor subclones in a given patient, is expected to be the rule. Branches stemming from this 'trunk' LOH pattern would still create a limited set of partially overlapping signatures which, together, cover all tumor cells in the same patient

[00102] An inevitable outcome of gross LOH events is the concomitant loss of all other genes residing on the deleted chromosomal material, and these naturally include many genes encoding transmembrane proteins. Concerning their identity, a catalog of 3,702 different human cell surface proteins (the 'surfaceome') has been compiled (Da Cunha et al., 2009). The expression of □42% of surfaceome genes display broad tissue distribution while □85 genes are expressed by all tissues examined, which is the hallmark of housekeeping genes. These genes are candidates, the different polymorphic variants of which may serve as targets for the iCARs and aCARs of the present invention

[00103] More recently, Bausch-Fluck et al. (Bausch-Fluck et al., 2015) applied their Chemoproteomic Cell Surface Capture technology to identify a combined set of 1492 cell surface glycoproteins in 41 human cell types. A large fraction of the surfaceome is expected to be expressed by any given tumor, each exhibiting a distinctive profile. Genes encoding cell surface proteins were found to be slightly enriched for single-nucleotide polymorphisms (SNPs) in their coding regions than all other genes (Da Cunha et al., 2009). Polymorphic in-frame insertions and deletions, which are rarer, further contribute to the number of variants and likely exert more robust structural effects on the polypeptide products than peptide sequence-altering (nonsynonymous) SNPs. Altogether, a typical genome contains 10,000 to 12,000 sites with nonsynonymous variants and 190–210 in-frame insertions/deletions (Abecasis et al., 2010; Auton et al., 2015). These variants are not evenly distributed throughout the

genome as highly polymorphic genes such as the HLA locus (http://www.ebi.ac.uk/imgt/hla/stats.html) or certain G-protein-coupled receptor (GPCR) genes (Lee et al., 2003; Rana et al., 2001) create distinct variant 'hotspots'. Another layer of LOH-related hotspots stems from the frequent loss of certain chromosomes, or chromosome arms in different cancers (e.g., 3p and 17p in small-cell lung carcinoma (Lindblad-Toh et al., 2000), 17p and 18q in colorectal cancer (Vogelstein et al., 1989), 17q and 19 in breast cancer (Li et al., 2014; Wang et al., 2004) 9p in melanoma (Stark and Hayward, 2007), 10q in glioblastoma (Ohgaki et al., 2004) and more)

[00104] A significant fraction of allelic variations in surface proteins would affect the extracellular portion of the respective gene products, potentially creating distinct allele-restricted epitopes which, in principle, can be recognized and distinguished from other variants by highly-specific mAbs. It is well documented that mAbs can be isolated that discriminate between two variants of the same protein which differ in a single amino acid only (see, for example, an early example of mAbs that recognize point mutation products of the Ras oncogene with exquisite specificity (Carney et al., 1986)). Interestingly, it was shown that two mAbs specific to a single amino acid interchange in a protein epitope can use structurally distinct variable regions from their heavy and light chain V gene pools (Stark and Caton, 1991). Recently, Skora et al. (Skora et al., 2015) reported the isolation of peptide-specific scFvs which can distinguish between HLA-I-bound neopeptides derived from mutated KRAS and EGFR proteins and their wild type counterparts, differing in both cases in one amino acid

[00105] All taken together, a unique antigenic signature of tumor cells emerges, that can allow their unequivocal discrimination from all other cells in the entire body of the individual patient. It comprises all transmembrane proteins encoded by allelic variants that are absent from the tumor cell surface owing to LOH but are present on normal cells of the cancer tissue of origin or other tissues expressing these genes. Naturally, each gene affected by LOH will be characterized by a distinct pattern of tissue distribution except for true housekeeping genes. The majority of these genes are not expected to be directly involved in tumorigenesis or maintenance of the transformed phenotype and, in this sense, their loss is of a 'passenger' nature

[00106] The rationale presented above argues that a unique molecular portrayal is inevitably shaped by LOH for almost all tumors, which is marked by the absence of numerous polymorphic surface structures that are present on normal cells. Converting this postulated signature of the individual tumor to a targetable set of antigenic epitopes entails a practicable immunological strategy for translating the recognition of a particular 'absence' into an activating cue capable of triggering target cell killing. Importantly, the incorporation of a safety device to assure that on-target off-tumor reactivity is strictly avoided will be highly favorable in future clinical implementation of this strategy

[00107] The present invention tackles this challenge through the co-expression in each therapeutic killer cell of a single pair of genes. One partner in this pair encodes an activating CAR (aCAR) and the other encodes a protecting CAR (pCAR) or an inhibitory CAR (iCAR)

II. SELECT DEFINITIONS

[00108] The term "nucleic acid molecule" as used herein refers to a DNA or RNA molecule.

[00109] The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*e.g.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[00110] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[00111] The term "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

[00112] The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

[00113] The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

[00114] "Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

[00115] The term "genomic variant" as used herein refers to a change of at least one nucleotide at the genomic level in a sequenced sample compared to the reference or consensus sequence at the same genomic position.

[00116] The term "corresponding reference allele" as used herein with reference to a variant means the reference or consensus sequence or nucleotide at the same genomic position as the variant.

[00117] The term "extracellular domain" as used herein with reference to a protein means a region of the protein which is outside of the cell membrane.

[00118] The term "loss of heterozygosity" or "LOH" as used herein means the loss of chromosomal materials such as a complete chromosome or a part thereof, in one copy of the two chromosomes in a somatic cell.

[00119] The term "sequence region" as used herein with reference to a variant or a reference allele means a sequence starting upstream and ending downstream from the position of the variant, which can be translated into an "epitope peptide" that can be recognized by an antibody.

[00120] The term "CAR", as that term is used herein, refers to a chimeric polypeptide that shares structural and functional properties with a cell immune-function receptor or

adaptor molecule, from *e.g.*, a T cell or a NK cell. CARs include TCARs and NKR-CARs. Upon binding to cognate antigen, a CAR can activate or inactivate the cytotoxic cell in which it is disposed, or modulate the cell's antitumor activity or otherwise modulate the cells immune response.

[00121] The term "specific binding" as used herein in the context of an extracellular domain, such as an scFv, that specifically binds to a single allelic variant of a polymorphic cell surface epitope, refers to the relative binding of the scFv to one allelic variant and its failure to bind to the corresponding different allelic variant of the same polymorphic cell surface epitope. Since this depends on the avidity (number of CAR copies on the T cell, number of antigen molecules on the surface of target cells (or cells to be protected) and the affinity of the specific CARs used, a functional definition would be that the specific scFv would provide a significant signal in an ELISA against the single allelic variant of a polymorphic cell surface epitope to which it is specific or cells transfected with a CAR displaying the scFv would be clearly labeled with the single allelic variant of a polymorphic cell surface epitope in a FACS assay, while the same assays using the corresponding different allelic variant of the same polymorphic cell surface epitope would not give any detectable signal.

[00122] The term "treating" as used herein refers to means of obtaining a desired physiological effect. The effect may be therapeutic in terms of partially or completely curing a disease and/or symptoms attributed to the disease. The term refers to inhibiting the disease, *e.g.*, arresting its development; or ameliorating the disease, *e.g.*, causing regression of the disease.

[00123] As used herein, the terms "subject" or "individual" or "animal" or "patient" or "mammal," refers to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired, for example, a human.

[00124] The phrase "safe effector immune cell" or "safe effector cell" includes those cells described by the invention that express at least one iCAR or pCAR as described herein. In some embodiments, the "safe effector immune cell" or "safe effector cell" is capable of adminsitraiton to a subject. In some embodiments, the "safe effector immune cell" or "safe effector cell" further expresses an aCAR as described herein. In some embodiments, the "safe effector immune cell" or "safe effector cell" further expresses an iCAR or a pCAR as described herein. In some embodiments, the "safe

effector immune cell" or "safe effector cell" further expresses an iCAR or a pCAR as described herein and an aCAR as described herein.

[00125] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[00126] The phrase "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

[00127] The following exemplification of carriers, modes of administration, dosage forms, etc., are listed as known possibilities from which the carriers, modes of administration, dosage forms, etc., may be selected for use with the present invention. Those of ordinary skill in the art will understand, however, that any given formulation and mode of administration selected should first be tested to determine that it achieves the desired results.

[00128] Methods of administration include, but are not limited to, parenteral, e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g., oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal, topical and intradermal routes. Administration can be systemic or local. In some embodiments, the pharmaceutical composition is adapted for oral administration.

[00129] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the active agent is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatin, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; and a glidant, such as colloidal silicon dioxide.

[00130] The term "peripheral blood mononuclear cell (PBMC)" as used herein refers to any blood cell having a round nucleus, such as a lymphocyte, a monocyte or a macrophage. Methods for isolating PBMCs from blood are readily apparent to those skilled in the art. A non-limiting example is the extraction of these cells from whole

blood using ficoll, a hydrophilic polysaccharide that separates layers of blood, with monocytes and lymphocytes forming a buffy coat under a layer of plasma or by leukapheresis, the preparation of leukocyte concentrates with the return of red cells and leukocyte-poor plasma to the donor.

[00131] The term "cancer" as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer, glioma, and the like.

III. CAR-T SYSTEM: iCARs, pCARs, and aCARs

[00132] It should be emphasized that the present invention provides a new avenue enabling specific targeting of tumor cells while keeping the normal cells secure. The concept presented herein provides for the identification of new targets for iCARs (or pCARs or protective CARs), these targets defined as comprising single allelic variants of polymorphic cell surface epitopes, which are lost from tumor cells due to LOH of the chromosomal region they reside in, while remaining expressed on normal tissue. Because of the polymorphic variation, it is possible to distinguish the two alleles and target only the allele missing in the tumor cells. Further, the target antigen may not necessarily itself be a tumor suppressor gene, or a gene predicted to be involved with cancer, since it is chosen for being in a region lost by LOH and could therefore simply be linked to such genes. This is conceptually different from the methods employed or suggested to date in cancer therapy, which target tumor associated antigens or antigens downregulated at tumors regardless of polymorphism. The present methods also provide for broadening the selection of aCAR beyond tumor associated anitgens, by conferring protection of normal cells through the co-expression of the iCAR and/or pCAR as described herein.

[00133] The distinction is crucial because the LOH, being a genomic event, results in a total loss of a specific variant from the tumor with a very rare probability of gaining back the lost allele. If the LOH event occurs very early in the development of tumors, it

ensures a uniform target signature in all tumor cells derived from the initial pre-malignant tissue including metastatic tumors. Additionally, LOH occurs in almost all types of cancer and this concept can therefore be relied upon as a universal tool for developing markers relevant to all these cancer types. Since the LOH events are to some extent random, the present invention further provides for selection of personalized tumor markers for each individual cancer patient, based on the specific LOH events which took place in that patient. The tools relied upon to execute this concept, the aCARs and the iCARs, are well-known and can be easily prepared using methods well-known in the art as taught for example, in WO 2015/142314 and in US 9,745,368, both incorporated by reference as if fully disclosed herein.

[00134] According to one strategy, the two CARs in every given pair specifically recognize the product of a different allelic variant of the same target gene for which the patient is heterozygous. The basic principle is as follows: the aCAR targets an allelic variant of a selected cell surface protein that is expressed by the given tumor cells and is not affected by LOH while the pCAR or iCAR targets the product encoded by the allelic variant of the same gene that has been lost from these tumor cells due to LOH. In other normal tissues of that individual patient that express the said gene, both alleles are present and are known to be equally functional, that is, expression is biallelic in all tissues (in contrast to other genes which may exhibit random monoallelic expression (Chess, 2012; Savova et al., 2016). In one scenario, the two CARs target two related epitopes residing at the same location on the protein product, which differ by one, or only few amino acids. In another scenario, the aCAR targets a non-polymorphic epitope on the same protein while the pCAR or iCAR is allele-specific. In this case the density of the aCAR epitope on normal cells would generally be two-fold higher than that of the iCAR or pCAR one. In some embdoiments, a single nucleic acid vector encodes both the aCAR and iCAR or pCAR.

[00135] Another strategy utilizes as the pCAR or iCAR targets the protein products of housekeeping genes. Since, by definition, these genes are expressed on all cells in the body, they are safe targets for pCAR or iCARs. That is, if the pCAR or iCAR targets a membrane product of a housekeeping gene for which the given patient is heterozygous, all cells in the body, except the tumor cells which have lost this allele due to LOH, will be protected. This strategy allows for the uncoupling of the aCAR target gene product from the pCAR or iCAR one. In fact, the aCAR target can then be any non-

polymorphic epitope expressed by the tumor. A variation of this strategy would be to utilize a known aCAR targeted to a non-polymorphic tumor-associated antigen, e.g., an aCAR in clinical use or under examination in clinical trials, in combination with an iCAR or pCAR directed against a membrane product of a gene for which the given patient is heterozygous and which is expressed in at least the tissue of origin of the tumor and preferably in additional vital normal tissues in which aCAR target antigen is expressed.

[00136] Following the same rationale which allows the uncoupling of the aCAR target antigen from the iCAR/pCAR one, the latter should not necessarily be the product of a housekeeping gene. In some embodiments, the iCAR and/or pCAR be the product of any gene the expression pattern of which is sufficiently wide so as to protect vital normal tissues expressing the aCAR target antigen in addition to the tumor. As a corollary, the aCAR antigen can be, as argued for housekeeping genes, any non-polymorphic epitope expressed by the tumor, not restricted to known 'tumor-associated antigens', a consideration which can vastly expand the list of candidate aCAR targets. In general, for both housekeeping and non-housekeeping genes, the identitiy of such normal vital tissues and level of expression would serve as important criteria in the prioritization of such candidate aCAR targets

[00137] Care must be taken to ensure that the inhibitory signal transmitted by the iCAR is strictly and permanently dominant over the aCAR signal and that no cross-recognition between the iCAR and the aCAR occurs. Dominance of the iCAR guarantees that activation of the killer cell upon encounter with normal cells expressing both alleles would be prevented. This default brake would, however, not operate upon engagement with tumor cells: in the absence of its target antigen the iCAR would not deliver inhibitory signals, thus unleashing the anticipated aCAR-mediated cellular activation and subsequent tumor cell lysis

[00138] The iCAR technology may be based on immune checkpoints. In this regard, the demonstration (Fedorov et al., 2013b; WO 2015/142314) that the regulatory elements of PD-1 and CTLA-4 possess a potent T cell inhibitory capacity when incorporated as iCAR signaling components is encouraging but the generality of these observations was recently questioned (Chicaybam and Bonamino, 2014, 2015). Furthermore, although the precise molecular pathways triggered by these checkpoint

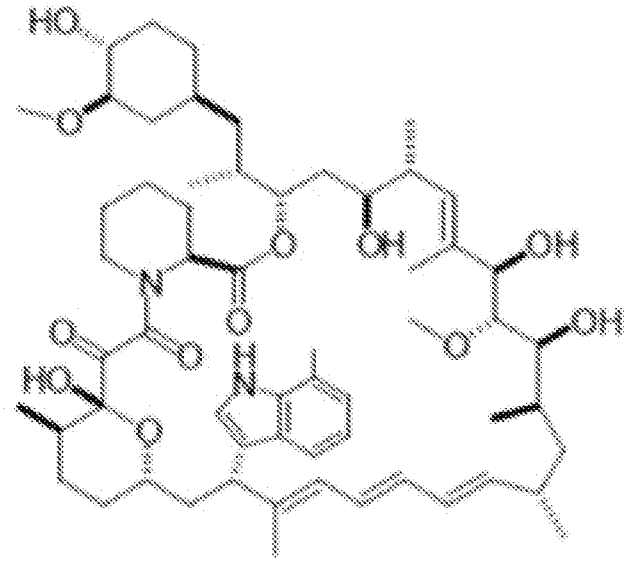
proteins are not fully understood, their engagement dampens T-cell activation through both proximal and distal mechanisms, rendering T cells unresponsive to concomitant activating stimuli (Nirschl and Drake, 2013). Hence, although the inactivation status secured by PD-1 and CTLA-4 iCARs is indeed temporary and reversible (Fedorov et al., 2013b), it would not allow T cell activation in tissues expressing both iCAR and aCAR targets. In contrast, the dominance of NK inhibitory receptors over activating receptors assures that healthy cells are spared from NK cell attack through a spatial, rather than temporal mechanism. (Long et al., 2013). There is compelling evidence that a single NK cell can spare a resistant cell expressing both inhibitory and activating ligands yet, kill a susceptible cell it simultaneously engages, which expresses only the activating ligands. This exquisite ability is governed by the different spatial organization of signal transduction molecules formed at each of the respective immune synapses which consequently affects the exocytosis of cytolytic granules (e.g., Abeyweera et al., 2011; Eriksson et al., 1999; Treanor et al., 2006; Vyas et al., 2001; US 9,745,368).

[00139] The strategy based on the control asserted by iCARs depends on the dominance of the iCAR activity over the aCAR activity as explained above. In some embodiments, the present invention provides this type of iCAR, termed here a pCAR (for 'protective CAR, see Fig. 2), designed to operate in CAR T cells in a synapse-selective manner and guarantee full dominance over the co-expressed aCAR. In some embodiments, the iCAR provided by the present invention is this particular type of iCAR referred to herein as a protective CAR (pCAR).

[00140] In some embodiments, the pCAR of the present invention integrates two technological feats. First, the pCAR allows for uncoupling the activating moiety of the aCAR (FcRγ/CD3-ζ) from the recognition unit and the co-stimulatory element (*e.g.*, CD28, 4-1BB, CD134 (OX40, GITR, IL2Rβ and STAT3 binding motif (YXXQ)) by genetically placing them on two different polypeptide products. Recoupling of these elements, which is mandatory for the aCAR function, will only take place by the addition of a heterodimerizing drug which can bridge the respective binding sites incorporated onto each of the polypeptides separately (**Fig. 2B**). The reconstruction of a fully functional CAR by bridging similarly split recognition and activating moieties by virtue of a heterodimerizing drug has recently been reported by Wu et al. (Wu et al., 2015). For this purpose, these authors used the FK506 binding protein domain (FKBP,

104 amino acids) and the T2089L mutant of FKBP-rapamycin binding domain (FRB, 89 amino acids) that heterodimerize in the presence of the rapamycin analog AP21967 (Scheme I below). This drug possess 1000-fold less immunosuppressive activity compared to rapamycin (Bayle et al., 2006; Graef et al., 1997; Liberles et al., 1997) and is commercially available (ARGENTTM, Regulated Heterodimerization Kit, ARIAD). In some emodiments, the drug is administered orally.

Scheme I. Structure of AP21967



[00141] Second, engrafting the pCAR recognition unit and the missing activating domain, respectively, onto the two surfaces of the transmembrane domain of a RIP-controlled receptor which contains the two intramembrane cleavage sites (**Fig. 2A**). Binding of the pCAR to its antigen will trigger dual cleavage of the encoded polypeptide first by a member of the extracellular disintegrin and metalloproteinase (ADAM) family which removes the ectodomain and then by intracellular γ-secretase, which liberates the intracellular domain of the pCAR. This first cleavage event is predicted to disrupt the ability of the truncated aCAR to gain access to a functional, membrane-anchored configuration of its missing activating element, thus acquiring an operative mode (**Fig. 2C**). This principle was recently exploited in the development of new genetic switches designed to limit CAR T cell activity to simultaneous recognition of two different antigens on the tumor cell, applying either the Notch receptor (Morsut et al., 2016; Roybal et al., 2016b) or Epithelial cell adhesion molecule (EpCAM, Pizem, Y., M.Sc. thesis under the supervision of the Inventor), two well-studied receptors functioning through RIP. In these studies, binding of the RIP-based CAR to one

antigen releases a genetically-engineered intracellular domain which translocates to the cell nucleus where it turns on the expression of the second CAR. Unlike the current invention which utilizes this process solely for disarming any potential aCAR activity in the presence of the protective antigen. In some embodiments, the first cleavage event disrupts the ability of the truncated aCAR to gain access to a functional, membrane-anchored configuration of its missing activating element, thus acquiring an operative mode.

[00142] The proposed mode of action described above is predicted to exert local effects so that only aCARs which reside in the same synapse are affected and are no more able to bind their antigen productively and form an immunological synapse. As a result, even when multiple interactions of the aCAR with large numbers of non-tumor cells are likely to take place, they are only expected to be transient and nonfunctional so that the cells are fully capable of further interactions.

[00143] Dominance of the pCARs over their aCARs counterparts is inherent to this system as function of the aCARs utterly depends on presence of the pCARs. Relative shortage of pCARs in a given T cell would render the aCARs non-functional due to lack of an activating domain. In some embodiments, a shortage of pCARs in a given T cell renders the aCARs non-functional due to lack of an activating domain.

[00144] It is critical that both the recognition domain and the activating one are localized to the plasma membrane (Wu et al., 2015). Therefore, the second cleavage, which detaches the activating domain from the plasma membrane, would render this domain nonfunctional and prevent unwanted cellular activation. In some embodiments, the recognition domain and the activating one are localized to the plasma membrane. In some embodiments, the second cleavage detaches the activating domain from the plasma membrane and renders this domain nonfunctional and prevents unwanted cellular activation.

[00145] The aCAR and pCAR are designed to function via mutually exclusive mechanisms. The ability of the pCAR to undergo cleavage does not depend on the strength of inhibitory signaling so no completion on signaling outcome will take place. As long as the pCARs are cleaved, the aCARs cannot function, regardless of relative avidity of their interactions with their respective antigens, a scenario which secures another crucial level of safety.

[00146] In some embodiments, the mammalian tissue is human tissue and in other embodiments the related mammalian normal tissue is normal tissue from which the tumor developed.

[00147] In some embodiments, the effector immune cell is a T cell, a natural killer cell or a cytokine-induced killer cell.

[00148] In some embodiments, the at least one signal transduction element capable of inhibiting an effector immune cell is homologous to a signal transduction element of an immune checkpoint protein, such as an immune checkpoint protein selected from the group consisting of PD1; CTLA4; BTLA; 2B4; CD160; CEACAM, such as CEACAM1; KIRs, such as KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, LIR1, LIR2, LIR3, LIR5, LIR8 and CD94–NKG2A; LAG3; TIM3; V-domain Ig suppressor of T cell activation (VISTA); STimulator of INterferon Genes (STING); immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing proteins, T cell immunoglobulin and ITIM domain (TIGIT), and adenosine receptor (*e.g.*, A2aR). In some embodiments, the immune checkpoint protein is a negative immune regulator. In some embodiments, the negative immune regulator is selected from the group consisting of 2B4, LAG-3 and BTLA-4.

[00149] In some embodiments, immune checkpoint protein is a natural killer cell inhibitory receptor, e.g., KIRs, such as KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3; or a Leukocyte Ig-like receptor, such as LIR1, LIR2, LIR3, LIR5, LIR8; and CD94–NKG2A, a C-type lectin receptor which forms heterodimers with CD94 and contains 2 ITIMs.

[00150] The methods for preparing and using killer cell receptors in iCARs has been described in US 9,745,368, incorporated by reference as if fully disclosed herein.

[00151] In some embodiments, the extracellular domain of any one of the above embodiments is fused through a flexible hinge and transmembrane canonic motif to said intracellular domain.

i. TARGET IDENTIFICATION: aCAR, iCAR and pCAR

[00152] The present invention provides methods for identification of aCAR, iCAR and/or pCAR targets based identification of candidate genes having extracellular polymorphic epitopes. In some embodiments, the aCAR can be directed at any extracellular protein expressed on the tumor tissue. In some embodiments, aCAR target is further expressed on non-tumor tissues and the iCAR taeget is also expressed on non-tumor tissues but is not expressed on tumor tissues.

[00153] In some embodiments, the method of identificaiton of candidate genes includes first determining that the gene encodes a transmembrane protein comprsing an extracellular polymorphic epitope. In some embodiments, the method of identificaiton of candidate genes further includes determining that the gene has at least two expressed alleles. In some embodiments, these alleles exhibit at least one allelic variation. In some embodiments, the allelic variation includes, for example, the presence of one or more SNPs, insertions, and/or deletions. In some embodiments, the allelic variation found for the gene causes an amino acid change relative to the reference sequence in an extracellular region of the protein. In some embodiments, the gene is located in a chromosomal region which undergoes loss of heterozygosity (LOH). In some embodiments, the gene is located in a chromosomal region which undergoes loss of heterozygosity (LOH) in cancer. In some embodiments, the gene is expressed in a tissue-of-origin of a tumor type in which the corresponding region was found to undergo LOH. In some embodiments, the gene is expressed at least in one or more tissues that the aCAR is expressed in. In some embodiments, the iCAR or pCAR target is expressed in vital organ cells the aCAR is expressed in.

[00154] In some embodiments, the target for use in the iCAR and/or pCAR is selected based on identification of a gene having at least one extracellular polymorphic epitope and wherein said gene has at least two expressed alleles. In some embodiments, the target for use in the iCAR and/or pCAR is selected based on identification of a gene having located in a chromosomal region which undergoes loss of heterozygosity. In some embodiments, the target for use in the iCAR and/or pCAR is selected based on identification of a gene having located in a chromosomal region which undergoes loss of heterozygosity in cancer. In some embodiments, the score for a theoretical SNP is calculated and a threshold limit determined. For example, if only 32% of the SNPs had

a tumor suppressor gene on the chromosome, then the percentile rank for having one would be 0.68. Further, for example, if the allele had a minor allele fraction of 0.49 (where 0.5 is the highest possible), then the percentile rank would be 0.99. If the rate of LOH was 0.10, and 75% of SNPs had more LOH than that, then the percentile rank would be 0.25. If the ratio of standard deviation of expression values across tissues to the median for the gene harboring this SNP was 1.3 and that is better than 90% of other genes, then the percentile rank is 0.9. The total score for this SNP would then be 0.68*0.99*0.25*0.9=0.15. In some embodiments, this LOH candidat score can be employed as one method for determining if a candidate gene is a suitable iCAR or pCAR target. In some embodiemtns, the target can be selected based on this LOH score. In some embodiments, the candidate gene is a determined to be suiteable as an iCAR or pCAR target. LOH candidates based on an LOH candidate score of greater than 0.4.

[00155] In some embodiments, the target for use in the iCAR and/or pCAR is selected from a gene having at least one extracellular polymorphic epitope. In some embodiments, the target is a gene is located on chromosome 1, chromosome 2, chromosome 3, chromosome 4, chromosome 5, chromosome 6, chromosome 7, chromosome 8, chromosome 9, chromosome 10, chromosome 11, chromosome 12, chromosome 13, chromosome 14, chromosome 15, chromosome 16, chromosome 17, chromosome 18, chromosome 19, chromosome 20, chromosome 21, chromosome 22, or chromosome X.

[00156] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 1. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1,

OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[00157] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 2. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

[00158] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 3. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[00159] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 4. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ANTXR2, BTC, CNGA1,

CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[00160] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 5. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[00161] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 6. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2. In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 6 and comprises an HLA target. In some embodiments, the target for use in the iCAR and/or pCAR is HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-

DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G. In some embodiments, the target for use in the iCAR and/or pCAR is HLA-A2,

[00162] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 7. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[00163] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 8. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[00164] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 9. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[00165] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 10. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8,

ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[00166] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 11. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[00167] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 12. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15,

SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[00168] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 13. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[00169] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 14. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[00170] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 15. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[00171] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 16. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[00172] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 17. In some embodiments, the target for use in the

iCAR and/or pCAR is selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[00173] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 18. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[00174] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 19. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[00175] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 20. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ABHD12, ADAM33,

ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[00176] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 21. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[00177] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome 22. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[00178] In some embodiments, the gene comprising the extracellular polymorphic epitope is located on chromosome X. In some embodiments, the target for use in the iCAR and/or pCAR is selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[00179] In some embodiments, the aCAR used to treat the cancer is directed against or specifically binds to any membrane protein which is expressed on the tumor tissue as long as the iCAR is expressed on every normal tissue in which the targeted protein is expressed. In some embodiments, the aCAR can specifically bind or be directed to a tumor associated protein, tumor associated antigen and/or antigens in clinical trials, a CAR target as listed in Table 1, as well as any cell surface protein that is expressed in a tumor tissue to which an iCAR can be matched or paired with regard to target binding, according to the criteria listed in the application. In some embodiments, the aCAR can be any expressed protein with an extracellular domain, as long as the iCAR is expressed in the same tissues as the aCAR or in any vital tissues, but is lost in the tumor cells. In some embodiments, the aCAR used to treat the cancer, such as any one of the cancer types recited above, is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as CD19. In some embodiments, the aCAR, iCAR, and/or pCAR target is any target with an extracellular

domain. In some embodiments, the aCAR used to treat the cancer, is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from but not limited to the following list of antigens: CD19, CD20, CD22,CD10, CD7, CD49f, CD56, CD74, CAIX Igκ, ROR1, ROR2, CD30, LewisY, CD33, CD34,CD38, CD123, CD28, CD44v6, CD44, CD41, CD133, CD138, NKG2D-L, CD139, BCMA, GD2,GD3 ,hTERT, FBP, EGP-2, EGP-40, FR-α, L1-CAM, ErbB2,3,4, EGFRvIII, VEGFR-2, IL-13Rα2, FAP, Mesothelin, c-MET, PSMA, CEA, kRas, MAGE-A1, MUC1MUC16, PDL1, PSCA, EpCAM, FSHR, AFP, AXL, CD80 CD89, CDH17,CLD18, GPC3, TEM8, TGFB1, NY-ESO-1 WT-1 and EGFR. In some embodiments, the aCAR, iCAR, and/or pCAR target is an antigen listed in **Table 1**.

Table 1. CAR target antigens, including some evaluated in trials registered in *ClinicalTrials.gov*

	Antigen	Key structural/functional features	Malignancy	Potential off-tumor targets
Hematologic malignancies	CD19	Pan-B cell marker involved in signal transduction by the BCR	ALL, CLL, NHL, HL, PLL	normal B cells
	CD20	Tetra-transmembrane, regulation of Ca transport and B-cell activation	CLL, NHL	normal B cells
	CD22	B-lineage specific adhesion receptor, sialic acid-binding Ig-type lectin family	ALL, NHL	normal B cells
	Igκ	Ig light chain isotype expressed by approx. 65% of normal human B cells	CLL, NHL, MM	normal B cells
	ROR1	Type I orphan-receptor tyrosine-kinase-like, survival-signaling receptor in tumors	CLL, NHL	pancreas; adipose cells
	CD30	TNFR member, pleiotropic effects on cell growth and survival involving NF-κB	NHL, TCL, HL	resting CD8 T cells; activated B and Th2 cells
	$Lewis^Y$	(CD174) a membrane oligosaccharide harboring two fucose groups	AML, MM	early myeloid progenitor cells
	CD33	Sialic acid-binding Ig-type lectin serving as adhesion molecule of the myelomonocytic lineage	AML	hematopoietic progenitors; myelo-monocytic precursors; monocytes
	CD123	The α chain of the IL-3 receptor	AML	BM myeloid progenitors; DCs, B cells; mast cells, monocytes;

				macro-phages; megakar.; endothelial cells
	NKG2D-L	Ligands for the NK and T-cell activating receptor NKG2D, bearing similarity to MHC-I molecules; upregulated during inflammation	AML, MM	gastrointestinal epithelium, endothelial cells and fibroblasts;
	CD139	Syndecan-1, cell surface heparan sulfate proteoglycan, ECM receptor	MM	precursor & plasma B cells; epithelia
	BCMA	TNFR member, binds BAFF and APRIL, involved in proliferation signaling	MM	B cells
	TACI		MM	Mono-nuclear cells, heart
Solid tumors	GD2	Disialoganglioside	NB; sarcomas; solid tumors	skin; neurons
	FR-α	GPI-linked folate receptor, functions in the uptake of reduced folate cofactors	ovarian cancer	apical surface in kidney, lung, thyroid, kidney & breast epithelia
	L1-CAM	CD171, neuronal cell adhesion molecule of the Ig superfamily	NB	CNS; sympathetic ganglia; adrenal medulla
	ErbB2	HER2, Member of the EGFR family of receptor tyrosine-protein kinases	brain, CNS, glioma, GBM, H&N, solid tumors	gastrointestinal, respiratory, reproductive & urinary tracts epithelia, skin, breast & placenta; hematopoietic cells
	EGFRvIII	Splice variant, in-frame deletion in the amplified EGFR gene encoding a truncated extracellular domain that constantly delivers pro-survival signals	brain, CNS, gliomas, GBM	none
	VEGFR-2	type III transmembrane kinase receptor of the Ig superfamily, regulates vascular endothelial function	solid tumors	vascular and lymphatic endothelia
	IL-13Rα2	The α chain of one of the two IL-13 receptors	brain, CNS, gliomas, GBM	astrocytes; brain; H&N tissue
	FAP	Cell surface serine protease	Mesothelioma	fibroblasts in chronic inflammation, wound healing, tissue remodeling
	Mesothelin	40-kDa cell surface glycoprotein with unknown function	mesothelioma, pancreatic, ovarian	peritoneal, pleural, and pericardial mesothelial surfaces
	c-MET	hepatocyte growth factor receptor (HGFR), disulfide linked α-β	TNBC	liver, gastrointestinal tract,

		heterodimeric receptor tyrosine kinase		thyroid, kidney, brain
	PSMA	type II membrane glycoprotein possessing N-Acetylated alpha-linked acidic dipeptidase and folate hydrolase activity	prostate	apical surface of normal prostate and intestinal epithelium and renal proximal tubular cells
	CEA	surface glycoprotein, member of the Ig superfamily and of the CEA-related family of cell adhesion molecules	colorectal, breast, solid tumors	apical epithelial surface: colon, stomach, esophagus & tongue
	EGFR	ErbB1, Her1, receptor tyrosine kinases signaling cell differentiation and proliferation upon ligand binding	Solid tumors	tissues of epithelial, mesenchymal & neuronal origin
	5T4	tumor-associated antigen which is expressed on the cell surface of m	Solid tumors	tissues of epithelial origin
	GPC3	heparan sulfate proteoglycan,	Solid tumors	Urine tissue
	ROR1	Receptor Tyrosine Kinase Like Orphan Receptor	Solid tumors as well as CLL	Urine, pancrease, colon, ovary, brain, monocytes
	MUC genes (MUC-1, MUC-16)	O-glycosylated protein that play an essential role in forming protective mucous barriers on epithelial surfaces	Solid tumors	Colon, kidney, lung, breast, pancrease urine
	PDL1	an immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells	lung	Spleen, breast

Table 2: Other CAR target antigens

	Antigen	Key structural/functional features	Malignancy
Hem. Malig.	CD38	a surface cyclic ADP ribose hydrolase involved in transmembrane signaling and cell adhesion	CLL, NHL, MM
	CS1	Cell surface signaling lymphocytic activation molecule (SLAM)	MM
Solid tumors	PSCA	GPI-anchored membrane glycoprotein of the Thy-1/Ly-6 family	prostate, bladder, pancreatic
	CD44v6	alternatively spliced variant 6 of the hyaluronate receptor CD44	H&N, liver, pancreatic, gastric, breast, colon; AML, NHL, MM
	CD44v7/8	alternatively spliced variant 7/8 of the hyaluronate receptor CD44	breast, cervical
	MUC1	densely glycosylated member of the mucin family of glycoproteins	colon, lung, pancreas, breast, ovarian, prostate, kidney, stomach, H&N
	L-11rα	the α subunit of the IL-11 recepto	colon, gastric, breast, prostate; osteosarcoma
	EphA2	erythropoietin-producing hepatocellular carcinoma A2 (EphA2) receptor, a member of the Eph family of receptor tyrosine kinases	Glioma; breast, colon, ovarian, prostate, pancreatic
	CAIX	transmembrane zinc metalloenzyme	RCC; tumors under hypoxia
	CSPG4	high molecular weight melanoma-associated antigen, cell surface proteoglycan	RCC; tumors under hypoxia

ii. RECOGNITION MOIETY: aCAR, iCAR and pCAR

[00180] The present invention also provides for recognition moieties designed to provide specific binding to the target. The recognition moiety allows for directing the specific and targeted binding of the aCAR, iCAR and/or pCAR. In some embodiments, the recognition moiety designed to provide specific binding to the target provides

specific binding to an extracellular polymorphic epitope. In some embodimetns, the recognition moiety is part of an extracellular domain of the aCAR, iCAR and/or pCAR. In some embodiments, the extracellular domain comprises an antibody, derivative or fragment thereof, such as a humanized antibody; a human antibody; a functional fragment of an antibody; a single-domain antibody, such as a Nanobody; a recombinant antibody; and a single chain variable fragment (ScFv). In some embodiments, the extracellular domain comprises an antibody mimetic, such as an affibody molecule; an affilin; an affimer; an affitin; an alphabody; an anticalin; an avimer; a DARPin; a fynomer; a Kunitz domain peptide; and a monobody. In some embodiments, the extracellular domain comprises an aptamer.

[00181] Generally, any relevant technology may be used to engineer a recognition moiety that confers to the aCARs and pCAR or iCARs specific binding to their targets. For example, recognition moieties comprising this iCAR-aCAR Library may be derived from a master recognition moiety pool ideally selected from a combinatorial display library, so that:

- Collectively, the selected recognition moieties target the cell-surface products of an array of genes which reside on each of the two arms of all 22 human autosomes. The shorter the distance between neighboring genes the fuller the coverage hence, the greater the universality of use.
- For each of the selected genes a set of allele-specific recognition moieties is isolated, each allowing rigorous discrimination between different allelic variants that are prevalent in the human population. The greater the number of targeted variants, the greater the number of therapeutic gene pairs that can be offered to patients.

[00182] A given allelic product can become a potential pCAR or iCAR target in one patient and a useful aCAR target in another patient harboring the same allele, depending on the particular LOH pattern in each case. Hence, as suitable recognition moiety genes are identified, each will be engrafted onto both a pCAR or an iCAR and an aCAR gene scaffold. It is therefore desirable that all recognition moieties directed at allelic variants of the same gene possess binding affinities of a similar range. Within such a given set of recognition moieties, all possible combinations of pCAR-aCAR or iCAR-aCAR pairs can be pre-assembled so as to assure the highest coverage of potential allelic compositions of that gene in the entire population.

[00183] In some embodiments, the patient is heterozygous for the major allele and a minor one, the products of which differ in a single position along the encoded polypeptide as a result of a nonsynonymous SNP or, less frequently, an indel. In some other embodiments, a patient is heterozygous for two minor alleles which differ from the major one in two separate positions. Depending on the particular LOH event involving the said gene in individual patients, a given variant epitope can serve as an iCAR target in one patient and an aCAR target in another. In some embodiments, the variant epitope that can serve as an iCAR target is not the major allele variant. In some embodiments, the variant epitope that can serve as the iCAR target is a minor allele.

[00184] The identification of a variant-specific mAb (say, a mAb specific to the epitope encoded by the minor allele 'a') is well known in the art and is similar, in principle, to the identification of a mAb against any conventional antigenic determinant, and can usually best be accomplished via high throughput screening of a recombinant antibody scFv library, utilizing, for example, phage (Barbas et al., 2004), ribosome (Hanes et al., 1997) or yeast (Chao et al., 2006) display technologies. The antigen employed for library screening can either be a synthetic peptide spanning the position of variation between the two alleles (typically 15-20 amino acid in length or more), a recombinant full-length polypeptide which can either be commercially available or tailor-synthesized by one of the many companies operating in this field, or even entire cells expressing the said allelic variant at high level by virtue of gene transfection (e.g., electroporation of mRNA encoding the full-length cDNA cloned as template for in-vitro mRNA transcription in the pGEM4Z/A64 vector (Boczkowski et al., 2000)), following a subtraction step performed on the same cells not expressing this allele. These methods are well-known and described in e.g., Molecular Cloning: A Laboratory Manual (Fourth Edition) Green and Sambrook, Cold Spring Harbor Laboratory Press; Antibodies: A Laboratory Manual (Second Edition), Edited by Edward A. Greenfield, 2012 CSH laboratory press; Using Antibodies, A laboratory manual by Ed Harlow and David Lane, 1999 CSH laboratory press.

[00185] By definition, the corresponding epitope (at the same position) which is encoded by the major allele ('A'), creates a unique antigenic determinant that differs from that created by 'a' in the identity of a single amino acid (SNP) or length (indel; for example, insertion or deletion). This determinant can, in principle, be recognized by a different set of mAbs identified by the same, or other, antibody display screening

technology. The ability of distinct members in each of the two sets of identified mAbs to distinguish between the two epitopes or variants, for example, an antibody from the first set binds the product of allele 'a' but not of 'A' and an Ab from the second set reciprocally binds 'A' but not 'a' can be determined using conventional binding assays such as ELISA or flow cytometry (Skora et al., 2015) or other technique for cell staining. Alternatively, once an 'a'-binding Ab is identified which does not bind 'A' and its protein sequence is determined, a computational method can potentially be used to predict the sequence of a 'complementary' antibody scFv which binds 'A' but not 'a'. For such a computational method see, for example (Sela-Culang et al., 2015a,b).

[00186] In some embodiments, for example with regard to the HLA-class I locus genes HLA-A, HLA-B, and HLA-C as the target genes, there are numerous allele-specific monoclonal antibodies available, for example, but not limited to, the antibodies listed in Example 3.

[00187] In some embodiments, the target for use in generation of a recognition moiety comprises at least one extracellular polymorphic epitope. In some embodiments, the target is the product of a gene that is located on chromosome 1, chromosome 2, chromosome 3, chromosome 4, chromosome 5, chromosome 6, chromosome 7, chromosome 8, chromosome 9, chromosome 10, chromosome 11, chromosome 12, chromosome 13, chromosome 14, chromosome 15, chromosome 16, chromosome 17, chromosome 18, chromosome 19, chromosome 20, chromosome 21, chromosome 22, or chromosome X.

[00188] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 1. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324,

KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[00189] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 1. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[00190] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 2. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

[00191] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 2. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

[00192] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 3. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2,

NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[00193] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 3. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[00194] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 4. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[00195] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 4. In some embodiments, the

recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[00196] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 5. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[00197] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 5. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13,

PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[00198] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 6. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

[00199] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 6. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1,

OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

[00200] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 7. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[00201] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 7. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[00202] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 8. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55,

SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[00203] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 8. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[00204] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 9. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[00205] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 9. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8,

OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[00206] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 10. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[00207] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 10. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[00208] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 11. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1,

NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[00209] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 11. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2,

OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[00210] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 12. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[00211] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 12. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119,

TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[00212] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 13. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[00213] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 13. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[00214] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 14. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[00215] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 14. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[00216] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 15. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[00217] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 15. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[00218] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 16. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[00219] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 16. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one

extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[00220] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 17. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[00221] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 17. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[00222] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 18. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[00223] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 18. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[00224] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 19. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[00225] In some embodiments, the the recognition moiety for use in the the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 19. In some embodiments, the recognition moiety for use in the the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[00226] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 20. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[00227] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 20. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the

group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[00228] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 21. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[00229] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 21. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[00230] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 22. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[00231] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome 22. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the

group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[00232] In some embodiments, the the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome X. In some embodiments, the recognition moiety for use in the aCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[00233] In some embodiments, the the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from chromosome X. In some embodiments, the recognition moiety for use in the iCAR or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[00234] The sequences encoding the variable regions of these antibodies can easily be cloned from the relevant hybridoma and used for constructing genes encoding scFvs against any desired target, including for example, scFvs against specific HLA Class-I allelic epitope variants, and which would be suitable for incorporation into a CAR construct using tools widely available as disclosed e.g., in Molecular Cloning: A Laboratory Manual (Fourth Edition) Green and Sambrook, Cold Spring Harbor Laboratory Press; Antibodies: A Laboratory Manual (Second Edition), Edited by Edward A. Greenfield, 2012 CSH laboratory press; Using Antibodies, A laboratory manual by Ed Harlow and David Lane, 1999 CSH laboratory press.

[00235] The present invention provides a database comprising DNA sequences of polymorphic variants lost in tumor cells due to LOH, and that encode cell-surface products, wherein the variation at the DNA sequence results in a variation at the amino acid sequence in an extracellular domain of the encoded protein. The information was retrieved from several databases open to the general public, such as TCGA, available on

the public National Institute of Health TCGA data portal (https://gdc.cancer.gov/), which provides, *inter alia,* data that can be used to infer relative copy number of the gene in a variety of tumor types and the cbio portal for TCGA data at http://www.cbioportal.org (Cerami et al., 2012, Gao et al., 2013); the Exome Aggregation Consortium (ExAC) database (exac.broadinstitute.org, Lek et al., 2016), providing, *inter alia*, allele frequencies of SNP variants in various populations; the Genotype-Tissue Expression (GTEX) database v6p (dbGaP Accession phs000424.v6.p1) (https://gtexportal.org/home, Consortium GT. Human genomics, 2015) which includes tissue expression data for genes; and databases providing structural information of proteins, such as the Human Protein Atlas (Uhlen et al., 2015); the Cell Surface Protein Atlas (Bausch-Fluck et al., 2015), a mass-spectrometry based database of N-glycosylated cell-surface proteins, and the UniProt database (www.uniprot.org/downloads).

[00236] The present invention further provides a method for genome-wide identification of genes that encode expressed cell-surface proteins that undergo LOH. The identified genes must meet the following criteria: 1) The gene encodes a transmembrane protein – therefore having a portion expressed on the cell surface to allow the iCAR or pCAR binding; 2) The gene has at least two expressed alleles (in at least one ethnic population checked); 3) The allelic variation found for that gene causes an amino acid change relative to the reference sequence in an extracellular region of the protein; 4) The gene is located in a chromosomal region which undergoes LOH in cancer; 5) The gene is expressed in a tissue-of-origin of a tumor type in which the corresponding region was found to undergo LOH.

[00237] In principle genes as described above, suitable to encode targets for iCAR or pCAR binding may be identified by any method known in the art, and not only by database mining. For example, the concept of LOH is not new and LOH information for specific genes, chromosomes, or genomic/chromosomal regions in specific tumors has already been published in the literature and candidate genes can therefore be derived from the available publications. Alternatively, such information can be found by whole genome hybridizations with chromosomal markers such as microsatellite probes (Medintz et al., 2000, Genome Res. 2000 Aug; 10(8): 1211–1218) or by any other suitable method (Ramos and Amorim, 2015, J. Bras. Patol. Med. Lab. 51(3):198-196).

[00238] Similarly, information regarding allelic variants is publicly available in various databases, and can also be easily obtained for a personalized case by genomic sequencing of a suspected region. Also, information regarding protein structure and expression pattern is publicly available and easily accessible as described above.

[00239] Accordingly, as information regarding the various criteria for many genes and SNPs is publicly available and the techniques for retrieving it are generally known, the main novelty of the application is using LOH as a criterion for choosing a target for iCAR or pCAR recognition, and the concept of personalizing treatment based on a specific allele lost in a specific patient.

[00240] As a non-limiting example, it was found according to the present invention that HLA genes, including non-classical HLA-I and HLA-II genes (e.g., HLA-A, HLA-B HLA-C, HLA-E, HLA-F, HLA-G, HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR HLA-K andor HLA-L) LOH, at varying frequencies, is a relatively frequent event in many tumor types (see **Figs. 10A-C**), which would make these genes good candidates to be used as targets for iCAR/pCAR recognition for the purpose of the present invention.

[00241] The recognition of the aCAR target on normal cells in any healthy essential tissue in the absence of the pCAR or iCAR target would be detrimental and is strictly forbidden. In this respect, the concept of pCAR-aCAR or iCAR-aCAR pairs, as proposed here, constitutes a fail-safe activation switch, as: i) cells not expressing the selected gene (in case the aCAR and the pCAR or iCAR target different products of the same gene) will not be targeted due to absence of the aCAR target antigen; ii) normal cells expressing this same gene will co-express both alleles and will not be targeted owing to the dominance of the pCAR or iCAR; iii) in case the pCAR or iCAR targets the product of a polymorphic housekeeping gene, all cells in the body will be protected; and iv) only tumor cells which express the aCAR target but not the pCAR or iCAR one will be attacked. In some embodiments, the recognition of the aCAR target on normal cells in any healthy essential tissue in the absence of the pCAR or iCAR target would be detrimental. In some embodiments, cells not expressing the selected gene (in case the aCAR and the pCAR or iCAR target different products of the same gene) will not be targeted due to absence of the aCAR target antigen. In some embodiments, normal cells expressing this same gene will co-express both alleles and will not be targeted

owing to the dominance of the pCAR or iCAR. In some embodiments, when the pCAR or iCAR targets the product of a polymorphic housekeeping gene, all cells in the body will be protected. In some embodiments, only tumor cells which express the aCAR target but not the pCAR or iCAR one will be attacked. In some embodiments, cells that express both the aCAR/iCAR pair targets or both aCAR/pCAR pair tarets will be protected.

[00242] As emphasized above, according to the invention there must be permanent dominance of the inhibitory signal over the activating signal. It is therefore necessaryl to ensure that no aCAR gene is expressed in a given killer cell, at any time, in the absence of its iCAR partner. This may be implemented through the tandem assembly of these iCAR-aCAR gene pairs as single-chain products or via a suitable bi-cistronic modality based, for example, on an internal ribosome entry site or on one of several viral self-cleaving 2A peptides. As suggested by the vast bulk of data reported on bi-cistronic expression, the iCAR gene will always be positioned upstream of its aCAR partner to guarantee favorable stoichiometry. Another option would be engeeniring the killer cells to express both aCAR and iCAR or pCAR by transfecting or transducing the killer cell with two independent constructs, each construct coding for either aCAR or iCAR/pCAR. Of course, this is not an issue when using a pCAR-aCAR gene pair. In some embodiments, the inhibitory signal is dominant over the activating signal. In some embodimetns, the aCAR and iCAR or pCAR are expressed simultaneously in the same cell.

[00243] Another attractive option for assuring iCAR dominance is detaching the aCAR recognition moiety from its activating/costimulatory portion so that both entities can only be assembled into one functional receptor in the presence of a heterodimerizing small molecule. The ability to tightly control the operative state of such split receptors by precise timing, dosage and location was recently demonstrated in the context of antitumor CARs (Wu et al., 2015).

[00244] In addition, the expected dominance is also likely to be intrinsic to the particular composition of the iCAR signaling elements incorporated into the intracellular portion in the selected iCAR design that should ‘compete’ with the signaling strength of the chosen aCAR platform. This capacity will also be influenced by the relative affinities of the two recognition moieties for their respective target

epitopes (which was dealt with above) and the overall avidities of their interactions. Concerning the latter, the proposed strategy secures both a favorable iCAR/aCAR stoichiometry and a balanced distribution of their respective target epitopes on normal cells. Again, this is not an issue when using a pCAR-aCAR gene pair.

[00245] To further assure safety, other conventional means currently implemented in the field of CAR and TCR immunotherapy can be employed, such as the use of suicide genes or the use of mRNA electroporation for transient expression.

[00246] While LOH often leaves the cells with only one allele of a given gene, it is frequently accompanied by duplication of the remaining chromosome, or chromosome part, resulting in 'copy number neutral'-LOH (Lo et al., 2008; O'Keefe et al., 2010; Sathirapongsasuti et al., 2011). Under these circumstances, the emergence of epitope-loss variants requires two independent events and is thus less likely. Expressing several pCAR-aCAR or iCAR-aCAR pairs in different fractions of the gene-modified cells will prevent the appearance of mutational escapees even in 'copy number loss' LOH cases, in which only a single copy of the target allele has been retained. Yet, as single-copy genes may become essential, their functional loss would be far less likely.

[00247] In view of the above, in one aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding an inhibitory chimeric antigen receptor (iCAR) capable of preventing or attenuating undesired activation of an effector immune cell, wherein the iCAR comprises an extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope absent from mammalian tumor cells due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue, or on vital organs the aCAR is expressed in; and an intracellular domain comprising at least one signal transduction element that inhibits an effector immune cell.

[00248] In some embodiments, the polymorphic cell surface epitope is part of an antigen encoded by a tumor suppressor gene or a gene genetically linked to a tumor suppressor gene, since such genes are likely to be lost due to LOH in tumors. Additionally, the polymorphic cell surface epitope may be part of an antigen encoded by a gene normally residing on a chromosome or chromosomal arm that often undergo LOH in cancer cells such as, but not limited to, chromosomal arms 3p, 6p, 9p, 10q, 17p,

17q, or 18q, or chromosome 19. These epitopes can readily be identified in the relevant databases as described herein.

[00249] In some embodiments, the polymorphic cell surface epitope is of a housekeeping gene product, such as the unclassified AP2S1, CD81, GPAA1, LGALS9, MGAT2, MGAT4B, VAMP3; the cell adhesion proteins CTNNA1 NM_001903, CTNNB1, CTNNBIP1 NM_020248, CTNNBL1 NM_030877, CTNND1 NM_001085458 delta catenin; the channels and transporters ABCB10 NM_012089, ABCB7 NM_004299, ABCD3 NM_002857, ABCE1 NM_002939, ABCF1 NM_001090, ABCF2 NM_005692, ABCF3 NM_018358, CALM1[1][7] Calmodulin grasps calcium ions, MFSD11 NM_024311 similar to MSFD10 aka TETRAN or tetracycline transporter-like protein[1], MFSD12 NM_174983, MFSD3 NM_138431, MFSD5 NM_032889, SLC15A4 NM_145648, SLC20A1 NM_005415, SLC25A11[1] mitochondrial oxoglutarate/malate carrier, SLC25A26 NM_173471, SLC25A28 NM_031212, SLC25A3 NM_002635, SLC25A32 NM_030780, SLC25A38 NM_017875, SLC25A39 NM_016016, SLC25A44 NM_014655, SLC25A46 NM_138773, SLC25A5 NM_001152, SLC27A4 NM_005094, SLC30A1 NM_021194, SLC30A5 NM_022902, SLC30A9 NM_006345, SLC35A2 NM_005660, SLC35A4 NM_080670, SLC35B1 NM_005827, SLC35B2 NM_178148, SLC35C2 NM_015945, SLC35E1 NM_024881, SLC35E3 NM_018656, SLC35F5 NM_025181, SLC38A2 NM_018976, SLC39A1 NM_014437, SLC39A3 NM_144564, SLC39A7 NM_006979, SLC41A3 NM_017836, SLC46A3 NM_181785, SLC48A1 NM_017842, the receptors ACVR1 NM_001105 similar to ACVRL1 TGF Beta receptor family Rendu-Osler-Weber syndrome, ACVR1B NM_004302,CD23[1] FCER2 low affinity IgE receptor (lectin); and the HLA/immunoglobulin/cell recognition group BAT1 aka DDX39B which is involved in RNA splicing, BSG Basigin Immunoglobulin Superfamily, extracelluar metalloproteinase, MIF macrophage migration inhibitory factor, and/or TAPBP [Wikipedia]. In some embodiments, the housekeeping gene is an HLA type I, a G-protein-coupled receptor (GPCR), an ion channel or a receptor tyrosine kinase, preferably an HLA-A, HLA-B, HLA-C. In some embodiments, the housekeeping gene is HLA-A. In some embodiments, the housekeeping gene is HLA-B. In some embodiments, the housekeeping gene is HLA-C.

[00250] Any relevant technology may be used to engineer a recognition moiety that confers to the aCARs and pCAR or iCARs specific binding to their targets. In some

embodiments, the extracellular domain comprises (i) an antibody, derivative or fragment thereof, such as a humanized antibody; a human antibody; a functional fragment of an antibody; a single-domain antibody, such as a Nanobody; a recombinant antibody; and a single chain variable fragment (ScFv); (ii) an antibody mimetic, such as an affibody molecule; an affilin; an affimer; an affitin; an alphabody; an anticalin; an avimer; a DARPin; a fynomer; a Kunitz domain peptide; and a monobody; or (iii) an aptamer. Preferably, the extracellular domain comprises an ScFv.

[00251] In some embodiments, the aCAR comprising an extracellular domain that specifically binds to a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope. In some embodiments, the aCAR extracellular domain binds to an epitope that is a tumor-associated antigen epitope. In some embodiments, the aCAR extracellular domain binds to an epitope that is a tumor-associated antigen is shared at least by cells of related tumor and normal tissue, and an intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell. In some embodiments, the aCAR used to treat the cancer is directed against or specifically binds to any membrane protein which is expressed on the tumor tissue as long as the iCAR target is expressed on every normal tissue in which the targeted aCAR protein is expressed. In some embodiments, the aCAR is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from but not limited to the following list of antigens: CD19, CD20, CD22, CD10, CD7, CD49f, CD56, CD74, CAIX Igκ, ROR1, ROR2, CD30, LewisY, CD33, CD34,CD38, CD123, CD28, CD44v6, CD44, CD41, CD133, CD138, NKG2D-L, CD139, BCMA, GD2,GD3, hTERT, FBP, EGP-2, EGP-40, FR-α, L1-CAM, ErbB2,3,4, EGFRvIII, VEGFR-2, IL-13Rα2, FAP, Mesothelin, c-MET, PSMA, CEA, kRas, MAGE-A1, MUC1MUC16, PDL1, PSCA, EpCAM, FSHR, AFP, AXL, CD80 CD89, CDH17,CLD18, GPC3, TEM8, TGFB1, NY-ESO-1, WT-1 and EGFR In some embodiments, the aCAR binds to CD19. In some embodiments, the aCAR directed against or specifically binds to, a non-polymorphic cell surface epitope of CD19.

[00252] In some embodiments, the iCAR is directed against or specifically binds to a single allelic variant of an antigen not including the ephrin receptors (e.g., EPHA 7) and claudins. In some embodiments, the iCAR is directed against or specifically binds to an epitope encoded by a single allelic variant of an HLA gene (HLA-A gene, HLA-B gene or HLA-C gene.

iii. INTRACELLULAR DOMAINS: aCAR, iCAR and pCAR

[00253] The present invention also provides for intracellular domains as part of the aCAR, iCAR, and/or pCAR. In some embodiments, the intracellular domain comprises at least one signal transduction element. In some embodiments, the intracellular domain comprises at least one signal transduction element that inhibits an effector immune cell.

[00254] Generally, any relevant technology may be used to engineer a signal transduction element that confers to the aCARs and pCAR or iCARs the ability to induce a cellular function, including for example, the ability to inhibit an effector immune cell or to activate or co-stimulate an effector immune cell.

[00255] In some embodiments, the at least one signal transduction element is capable of inhibiting an effector immune cell. In some embodiments, the at least one signal transduction element capable of inhibiting an effector immune cell is homologous to a signal transduction element of an immune checkpoint protein. In some embodiments, the immune checkpoint protein is selected from the group consisting of PD1, CTLA4, BTLA, 2B4, CD160, CEACAM (including for example, CEACAM1), KIRs (including for example KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, LIR1, LIR2, LIR3, LIR5, LIR8 and CD94), NKG2A; LAG3; TIM3; V-domain Ig suppressor of T cell activation (VISTA); STimulator of INterferon Genes (STING); immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing proteins, T cell immunoglobulin and ITIM domain (TIGIT), and adenosine receptor (e.g. A2aR). In some embodiments, the immune checkpoint protein is a negative immune regulator. In some embodiments, the negative immune regulatorr is selected from the group consisting of 2B4, LAG-3 and BTLA-4.

[00256] In some embodiments, the signal transduction element is capbale of activating or co-stimulating an effector immune cell. In some embodiments, the signal transduction element is an activating domain. In some embodiments, the signal transduction element is a co-stimulatory domain. In some embodiments, the signal transduction element that activates or co-stimulates an effector immune cell is homologous to an immunoreceptor tyrosine-based activation motif (ITAM), an activating killer cell immunoglobulin-like receptor, or an adaptor molecule, and/or a co-stimulatory signal transduction element. In some embodiments, the signal transduction

element that activates or co-stimulates an effector immune cell is homologous to an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the ITAM is from a protein including but not limited to CD3ζ or FcRγ chains. In some embodiments, the signal transduction element that activates or co-stimulates an effector immune cell is homologous to an an activating killer cell immunoglobulin-like receptor (KIR). In some embodiments, the KIR includes, for example, but is not limited to KIR2DS and KIR3DS. In some embodiments, the signal transduction element that activates or co-stimulates an effector immune cell is homologous to an adaptor molecule. In some embodiments, the adaptor molecule includes, for example, but is not limited to DAP12. In some embodiments, the signal transduction element that activates or co-stimulates an effector immune cell is homologous to a co-stimulatory signal transduction element. In some embodiments, the co-stimulatory signal transduction element is from a protein including but not limited to CD27, CD28, ICOS, CD137 (4-1BB), CD134 (OX40), and/or GITR. In some emndoiments, the aCAR comprise a signal transduction element.

[00257] In some embodiments, the extracellular domain is fused through a flexible hinge and transmembrane canonic motif to said intracellular domain.

[00258] In some embodiments, the use of a pCAR allows for uncoupling for uncoupling the activating moiety of the aCAR (FcRγ/CD3-ζ) from the recognition unit and the co-stimulatory element (*e.g.*, CD28, 4-1BB). In some embodiments, these elements are genetically placed on two different polypeptide products. In some embodiments, recoupling of these elements, which is mandatory for the aCAR function, will only take place by the addition of a heterodimerizing drug which can bridge the respective binding sites incorporated onto each of the polypeptides separately.

[00259] Instead of an activating domain (such as FcRy or CD3-ζ), an iCAR possesses a signaling domain derived from an inhibitory receptor which can antagonize T cell activation. In some embodiments, the iCAR possesses a signaling domain derived from an inhibitory receptor which can antagonize T cell activation. In some embodimetns, the iCAR signaling domain is derived from an inhibitory receptor, including for example but not limited to, a CTLA-4, a PD-1 or an NK inhibitory receptor.

iv. CAR-T VECTOR CONSTRUCTION (aCAR; iCAR; pCAR)

[00260] In some embodiments, the aCAR is encoded by a first nucleic acid vector and the iCAR or pCAR is encoded by a second nucleic acid vector. In some embodiments, the aCAR is encoded by a first nucleic acid vector and the iCAR or pCAR is encoded by a second nucleic acid vector. In some embodiments, the aCAR is encoded by a first nucleic acid vector and the iCAR or pCAR is encoded by a second nucleic acid vector. In some embodiments, the the nucleotide sequence encoding for the iCAR or pCAR is on a second vector.

[00261] In some embodiments, the present invention provides a vector comprising a nucleic acid molecule of the invention as defined in any one of the above embodiments, and at least one control element, such as a promoter, operably linked to the nucleic acid molecule.

[00262] In some embodiments, the vector is a lentiviral (LV) vector. In some embodiments, the LV vector is a commercially available LV vector. In some embodiments, the LV vector includes but is not limited to pLVX-Puro, pLVX-IRES-Puro/Neo/Hygro, pLVx-EF1a-IRES (TAKARA), and/or pcLV-EF1a (Sirion). In some embodiments, the LV vector is pLVX-Puro. In some embodiments, the LV vector is pLVX-IRES-Puro/Neo/Hygro. In some embodiments, the LV vector is pLVx-EF1a-IRES (TAKARA). In some embodiments, the LV vector is pcLV-EF1a (Sirion).

[00263] In some embodiments, the vector comprises an EF1 promoter. In some embodiments, the vector comprises a CMV promoter. In some embodiments, the vector comprises an PGK promoter. In some embodiments, the vector comprises a CD8 hinge. In some embodiments, the vector comprises a CD28 TM and 41BB costimulatory domain.

[00264] In some embodiments, the vector further comprises a nucleic acid molecule comprising a nucleotide sequence encoding an aCAR comprising an extracellular domain specifically binding a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared at least by cells of related tumor and normal tissue, and an intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell.

[00265] In some embodiments, the extracellular domain of the aCAR encoded by the nucleic acid comprised in the vector specifically binds to a non-polymorphic cell surface epitope of an antigen and the extracellular domain of the iCAR specifically binds a single allelic variant of a polymorphic cell surface epitope of a different antigen than that to which the extracellular domain of said aCAR binds.

[00266] In some embodiments, the extracellular domain of the iCAR encoded by the nucleic acid comprised in the vector, is directed against or specifically binds to a single allelic variant of HLA genes, including for example, HLA-A gene, HLA-B gene or HLA-C gene; or against a single allelic variant of a gene listed **Table 8.**

[00267] In some embodiments, the extracellular domain of the aCAR encoded by the nucleic acid comprised in the vector, is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as CD19. In some embodiments, the aCAR target is any target with an extracellular domain.

[00268] In some embodiments, the extracellular domain of the iCAR encoded by the nucleic acid comprised in the vector, is directed against or specifically binds to a single allelic variant of HLA genes, including for example, HLA-A gene, HLA-B gene or HLA-C gene or against a single allelic variant of a gene listed **Table 8**; and the extracellular domain of the aCAR encoded by the nucleic acid comprised in the vector, is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as CD19. In some embodiments, the aCAR target is any target with an extracellular domain.

[00269] In some embodiments, the at least one signal transduction element of the aCAR that activates or co-stimulates an effector immune cell is homologous to an immunoreceptor tyrosine-based activation motif (ITAM) of for example CD3ζ or FcRγ chains; a transmembrane domain of an activating killer cell immunoglobulin-like receptor (KIR) comprising a positively charged amino acid residue, or a positively charged side chain or an activating KIR transmembrane domain of e.g., KIR2DS and KIR3DS, or an adaptor molecule such as DAP12; or a co-stimulatory signal transduction element of for example CD27, CD28, ICOS, CD137 (4-1BB) or CD134 (OX40).

[00270] In some embodiments, the iCAR or pCAR is expressed by a first vector and the aCAR is expressed by a second vector. In some embodiments, the iCAR or pCAR and the aCAR are both expressed by the same vector.

[00271] In some embodiments, the nucleotide sequence of the vector comprises an internal ribosome entry site (IRES) between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR. In general, the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR can be in any sequential order, but in particular embodiments, the nucleotide sequence encoding for the aCAR is downstream of the nucleotide sequence encoding for the iCAR.

[00272] In some embodiments, the nucleotide sequences encoding for the aCAR iand the iCAR are encoded on a single vector. In some embodiments, the vector comprises an internal ribosome entry site (IRES) between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR. In some embodiments, the nucleotide sequence encoding for the aCAR is downstream of the nucleotide sequence encoding for the iCAR. In some embodiments, the nucleotide sequence comprises a viral self-cleaving 2A peptide located between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR. In some embodiments, the nucleotide sequence of the vector comprises a viral self-cleaving 2A peptide between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR. In some embodiments, the viral self-cleaving 2A peptide includes but is not limited to T2A from *Thosea asigna* virus (TaV), F2A from Foot-and-mouth disease virus (FMDV), E2A from *Equine rhinitis A* virus (ERAV) and/or P2A from *Porcine teschovirus-1* (PTV1). In some embodiments, the viral self-cleaving 2A peptide is T2A from *Thosea asigna* virus (TaV). In some embodiments, the viral self-cleaving 2A peptide is F2A from Foot-and-mouth disease virus (FMDV). In some embodiments, the viral self-cleaving 2A peptide is E2A from *Equine rhinitis A* virus (ERAV). In some embodiments, the viral self-cleaving 2A peptide is P2A from *Porcine teschovirus-1* (PTV1).

[00273] In some embodiments, the vector comprises a nucleotide sequence encoding the constitutive aCAR linked via a flexible linker to said iCAR.

[00274] The immune cells may be transfected with the appropriate nucleic acid molecule described herein by e.g., RNA transfection or by incorporation in a plasmid fit for replication and/or transcription in a eukaryotic cell or a viral vector. In some embodiments, the vector is selected from a retroviral or lentiviral vector.

[00275] Combinations of retroviral vector and an appropriate packaging line can also be used, where the capsid proteins will be functional for infecting human cells. Several amphotropic virus-producing cell lines are known, including PA12 (Miller, *et al.* (1985) *Mol. Cell. BioI.* 5:431-437); PA317 (Miller, *et al.* (1986) *Mol. Cell. Bioi.* 6:2895-2902); and CRIP (Danos, *et ai.* (1988) *Proc. Nati. Acad. Sci.* USA 85:6460-6464). Alternatively, non-amphotropic particles can be used, such as, particles pseudotyped with VSVG, RD 114 or GAL V envelope. Cells can further be transduced by direct co-culture with producer cells, e.g., by the method of Bregni, *et ai.* (1992) *Blood* 80: 1418-1422, or culturing with viral supernatant alone or concentrated vector stocks, e.g., by the method of Xu, *et ai.* (1994) *Exp. Hemat.* 22:223-230; and Hughes, *et ai.* (1992) *J Clin. Invest.* 89: 1817.

[00276] In another aspect, the present invention provides a method of preparing an inhibitory chimeric antigen receptor (iCAR) capable of preventing or attenuating undesired activation of an effector immune cell, according to the present invention as defined above, the method comprising: (i) retrieving a list of human genomic variants of protein-encoding genes from at least one database of known variants; (ii) filtering the list of variants retrieved in (i) by: (a) selecting variants resulting in an amino acid sequence variation in the protein encoded by the respective gene as compared with its corresponding reference allele, (b) selecting variants of genes wherein the amino acid sequence variation is in an extracellular domain of the encoded protein, (c) selecting variants of genes that undergo loss of heterozygosity (LOH) at least in one tumor, and (d) selecting variants of genes that are expressed at least in a tissue of origin of the at least one tumor in which they undergo LOH according to (c), thereby obtaining a list of variants having an amino acid sequence variation in an extracellular domain in the protein encoded by the respective gene lost in the at least one tumor due to LOH and expressed at least in a tissue of origin of the at least one tumor; (iii) defining a sequence region comprising at least one single variant from the list obtained in (ii), sub-cloning and expressing the sequence region comprising the at least one single variant and a sequence region comprising the corresponding reference allele thereby obtaining the

respective epitope peptides; (iv) selecting an iCAR binding domain, which specifically binds either to the epitope peptide encoded by the cloned sequence region, or to the epitope peptide encoded by the corresponding reference allele, obtained in (iii); and (vii) preparing iCARs as defined herein above, each comprising an iCAR binding domain as defined in (iv).

[00277] In some embodiments, the candidate variants of genes that are selected undergo LOH in at least 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% in a certain tumor type.

[00278] In some embodiments, the minor allele frequency for each variant selected equals or exceeds 1, 2, 3, 4 or 5% in at least one population.

[00279] In another aspect, the present invention is directed to a combination of two or more nucleic acid molecules, each one comprising a nucleotide sequence encoding a different member of a controlled effector immune cell activating system, said nucleic acid molecules being part of or forming a single continues nucleic acid molecule, or comprising two or more separate nucleic acid molecules, wherein the controlled effector immune activating system directs effector immune cells to kill tumor cells that have lost one or more chromosomes or fractions thereof due to Loss of Heterozygosity (LOH) and spares cells of related normal tissue, and wherein (a) the first member comprises an activating chimeric antigen receptor (aCAR) polypeptide comprising a first extracellular domain that specifically binds to a non-polymorphic cell surface epitope of an antigen or to a single allelic variant of a different polymorphic cell surface epitope and said non-polymorphic or polymorphic cell surface epitope is a tumor-associated antigen or is shared by cells of related abnormal and normal mammalian tissue; and (b) the second member comprises a regulatory polypeptide comprising a second extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope not expressed by an abnormal mammalian tissue due to LOH but present on all cells of related mammalian normal tissue.

[00280] In some embodiments, the first member is selected from: (a) a constitutive aCAR further comprising an intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell; and (b) a conditional aCAR further comprising an intracellular domain comprising a first member of a binding site for a heterodimerizing small molecule and optionally at least

one co-stimulatory signal transduction element, but lacking an activating signal transduction element; and the second member is: (c) an inhibiting chimeric antigen receptor (iCAR) further comprising an intracellular domain comprising at least one signal transduction element that inhibits an effector immune cell; or (d) a protective chimeric antigen receptor (pCAR) further comprising an extracellular regulatory region comprising a substrate for a sheddase; a transmembrane canonic motif comprising a substrate for an intramembrane-cleaving protease; and an intracellular domain, said intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell and a second member of a binding site for a heterodimerizing small molecule.

[00281] In some embodiments (i) the extracellular domain of the iCAR or pCAR specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen, which is a different antigen than that to which the extracellular domain of the aCAR binds; (ii) the extracellular domain of said pCAR or iCAR specifically binds a single allelic variant of a different polymorphic cell surface epitope of the same antigen to which the extracellular domain of said aCAR binds; or (iii) the extracellular domain of said pCAR or iCAR specifically binds a different single allelic variant of the same polymorphic cell surface epitope to which the extracellular domain of said aCAR binds.

[00282] In some pCAR embodiments, the substrate for a sheddase is a substrate for a disintegrin and metalloproteinase (ADAM) or a beta-secretase 1 (BACE1). In some embodiments, the substrate forms part of the extracellular domain and comprises Lin 12/Notch repeats and an ADAM protease cleavage site.

[00283] It is generally accepted that there is no consistent sequence motif predicting ADAM cleavage, but Caescu et al. (Caescu et al., 2009) disclose in Table 3 a large number of ADAM10 and/or ADAM17 substrate sequences, which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for ADAM in the pCAR of the present invention. In some embodiments, the ADAM substrate sequences are those of amyloid precursor protein, BTC, CD23, Collagen, DII-1, Ebola glycoprotein, E-cadherin, EGF, Epiregulin, Fas Ligand, growth hormone receptor, HB-EGF, type II interleukin-1 receptor, IL-6 receptor, L-selectin, N-cadherin, Notch, p55 TNF receptor, p75 TNF receptor, Pmel17, Prion protein, receptor-type protein tyrosine phosphatase Z, TGF-α, TNF or TR (Caescu et al., 2009).

[00284] It may be advantageous to use an ADAM10 cleavage sequence in the pCAR of the present invention because ADAM 10 is constitutively present at comparably high levels on *e.g.*, lymphocytes. In contrast to ADAM10, the close relative TACE/ADAM17 is detected at only low levels on unstimulated cells. ADAM17 surface expression on T cell blasts is rapidly induced by stimulation (Ebsen et al., 2013).

[00285] Hemming et al. (Hemming et al., 2009) report that no consistent sequence motif predicting BACE1 cleavage has been identified in substrates versus non-substrates, but discloses in Table 1 a large number of BACE1 substrates having BAC1 cleavage sequences, which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for BACE1 in the pCAR of the present invention.

[00286] In some pCAR embodiments, the substrate for an intramembrane-cleaving protease is a substrate for an SP2, a γ –secretase, a signal peptide peptidase (spp), a spp-like protease or a rhomboid protease.

[00287] Rawson et al. (Rawson, 2013) disclose that SP2 substrates have at least one type 2 membrane-spanning helix and include a helix-destabilizing motif, such as an Asp-Pro motif in a SP2 substrate. This paper discloses in Table 1 a number of SP2 substrates having SP2-cleavage sequences, which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for SP2 in the pCAR of the present invention.

[00288] Haapasalo and Kovacs (Haapasalo and Kovacs, 2011) teach that amyloid-β protein precursor (AβPP) is a substrate for presenilin (PS)-dependent γ-secretase (PS/γ-secretase), and that at least 90 additional proteins have been found to undergo similar proteolysis by this enzyme complex. γ-secretase substrates have some common features: most substrate proteins are type-I transmembrane proteins; the PS/γ-secretase-mediated γ-like cleavage (corresponding to the &- cleavage in AβPP, which releases AICD) takes place at or near the boundary of the transmembrane and cytoplasmic domains. The &-like cleavage site flanks a stretch of hydrophobic amino acid sequence rich in lysine and/or arginine residues. It appears that PS/γ-secretase cleavage is not dependent on a specific amino acid target sequence at or adjacent to the cleavage site, but rather perhaps on the conformational state of the transmembrane domain.

Haapasalo and Kovacs disclose in Table 1 a list of γ-secretase substrates, the cleavage sequences of which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for γ-secretases in the pCAR of the present invention.

[00289] Voss et al. (Voss et al., 2013) teach that so far no consensus cleavage site based on primary sequence elements within the substrate has been described for GxGD aspartyl proteases (spps). Transmembrane domains of membrane proteins preferentially adopt an α-helical confirmation in which their peptide bonds are hardly accessible to proteases. In order to make transmembrane domains susceptible for intramembrane proteolysis it was therefore postulated that their α-helical content needs to be reduced by helix destabilizing amino acids. Consistent with this hypothesis, various signal peptides have been shown to contain helix destabilizing amino acids within their h-region which critically influence their proteolytic processing by SPP. In addition, polar residues within the h-region of signal peptides may influence cleavage by SPP, as for instance serine and cysteine residues within the signal peptide of various HCV strains are critical for SPP cleavage. Whether these polar residues also simply affect the helical content of the signal peptides or the hydroxyl or sulfhydryl group in particular is required to trigger cleavage by SPP is not yet fully understood. Similarly, cleavage of the Bri2 transmembrane domain by SPPL2b is significantly increased when the α-helical content of the Bri2 transmembrane domainis reduced. Interestingly, only one amino acid residue out of four residues with a putative helix destabilizing potency significantly reduced the α-helical content of the Bri2 transmembrane domainin a phospholipid-based environment. This suggests that destabilization of an α-helical transmembrane domain is not simply caused by certain amino acid residues but that rather context and position of these amino acids determine their helix destabilizing potential and thus the accessibility of transmembrane domains to intramembrane proteolysis by SPP/SPPLs. Voss et al. further disclose in Table 1 a list of spp and spp-like substrates, the cleavage sequences of which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for spp in the pCAR of the present invention.

[00290] Bergbold et al. (Bergbold and Lemberg, 2013) teach that for rhomboid proteases, two different models for substrate recognition have been suggested. In the first model, the conformational flexibility of the substrate peptide backbone combined

with immersion of the membrane in the vicinity of the rhomboid active site is sufficient to provide specificity. For the well-characterized Drosophila substrate Spitz, a glycine–alanine motif has been shown to serve as a helix break that allows unfolding of the transmembrane domain into the rhomboid active site. The second model suggests that rhomboid proteases primarily recognize a specific sequence surrounding the cleavage site, and that transmembrane helix-destabilizing residues are a secondary feature required for some substrates only. The specific sequence has not yet been identified. Bergbold et al. disclose in Table 3 a list of rhomboid protease substrates, the cleavage sequences of which are hereby incorporated by reference as if fully disclosed herein, and which may serve as a substrate for rhomboid proteases in the pCAR of the present invention.

[00291] In view of the above, since in most cases no consensus motif has yet been established for the intramembrane-cleaving proteases, and since assays for identifying intramembrane-cleaving protease substrates are well known in the art as described in literature cited herein above, the pCAR may comprise an amino acid sequence identified as such and may further comprise transmembrane helix-destabilizing residues.

[00292] In some embodiments, the substrate forms part of the transmembrane canonic motif and is homologous to/derived from a transmembrane domain of Notch, ErbB4, E-cadherin, N-cadherin, ephrin-B2, amyloid precursor protein or CD44.

[00293] In some embodiments, the comprises a nucleotide sequence encoding an extracellular domain and an intracellular domain of said conditional aCAR as separate proteins, wherein each domain is independently fused to a transmembrane canonic motif and comprises a different member of a binding site for a heterodimerizing small molecule.

[00294] In some embodiments, the each one of the first and second member of the binding site for a heterodimerizing small molecule is derived from a protein selected from: (i) Tacrolimus (FK506) binding protein (FKBP) and FKBP; (ii) FKBP and calcineurin catalytic subunit A (CnA); (iii) FKBP and cyclophilin; (iv) FKBP and FKBP-rapamycin associated protein (FRB); (v) gyrase B (GyrB) and GyrB; (vi) dihydrofolate reductase (DHFR) and DHFR; (vii) DmrB homodimerization domain (DmrB) and DmrB; (viii) a PYL protein (a.k.a. abscisic acid receptor and as RCAR)

and ABI; and (ix) GAI *Arabidopsis thaliana* protein (a.k.a Gibberellic Acid Insensitive and DELLA protein GAI; GAI) and GID1 *Arabidopsis thaliana* protein (also known as Gibberellin receptor GID1; GID1).

v. CONSTRUCTION OF EFFECTOR CELLS

[00295] In still another aspect, the present invention provides a method for preparing a safe effector immune cell comprising: (i) transfecting a TCR-engineered effector immune cell directed to a tumor-associated antigen with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR or pCAR as defined herein above or transducing the cells with a vector or (ii) transfecting a naïve effector immune cell with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR or pCAR as defined herein above and a nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein above; or transducing an effector immune cell with a vector as defined herein above.

[00296] In some embodiments, the immune cell for use in engineering includes but is not limited to a T-cell, a natural killer cell, or a cytokine-induced killer cell. In some embodiments, the immune cell for use in engineering includes but is not limited to a Jurkat T-cell, a Jurkat-NFAT T-cell, and/or a peripheral blood mononuclear cell (PBMC).

[00297] In yet another aspect, the present invention provides a safe effector immune cell obtained by the method of the present invention as described above. The safe effector immune cell may be a redirected T cell expressing an exogenous T cell receptor (TCR) and an iCAR or pCAR, wherein the exogenous TCR is directed to a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared at least by cells of related tumor and normal tissue, and the iCAR or pCAR is as defined above; or the safe effector immune cell is a redirected effector immune cell such as a natural killer cell or a T cell expressing an iCAR or pCAR and an aCAR as defined above.

[00298] In some embodiments, the safe effector immune cell, expresses on its surface an aCAR comprising an extracellular domain that specifically binds to a non-

polymorphic cell surface epitope of an antigen and an iCAR or pCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of a different antigen to which the extracellular domain of said aCAR binds. In some embodiments, the extracellular domain of the iCAR or pCAR specifically binds a single allelic variant of a different polymorphic cell surface epitope are of the same antigen to which the extracellular domain of said aCAR binds; or the extracellular domain of the iCAR or pCAR specifically binds a different single allelic variant of the same polymorphic cell surface epitope area to which the extracellular domain of said aCAR binds.

[00299] In some embodiments, the extracellular domain of the aCAR expressed on the cell surface specifically binds to a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as CD19. In some embodiments, the target is any target with an extracellular domain.

[00300] In some embodiments, the extracellular domain of the iCAR and or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of an HLA-A, HLA-B, HLA-C, HLA-G, HLA-E, HLA-F, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA_DQ, or HLA-DR gene or against a single allelic variant of a gene listed **Table 8**.

[00301] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of an HLA-A gene, HLA-B gene or HLA-C gene or against a single allelic variant of a gene listed **Table 8**; and the extracellular domain of the aCAR expressed on the cell surface is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as, for example, but not limited to, CD19. In some embodiments, the aCAR target is any target with an extracellular domain.

[00302] In some embodiments, the aCAR and the iCAR are present on the cell surface as separate proteins.

[00303] In some embodiments, the expression level on the cell surface of the nucleotide sequence encoding the iCAR is greater than or equal to the expression level of the nucleotide sequence encoding the aCAR.

[00304] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of an at least one extracellular polymorphic epitope.

[00305] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[00306] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

[00307] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[00308] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[00309] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[00310] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

[00311] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[00312] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[00313] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCA1, AQP7, ASTN2,

C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[00314] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[00315] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[00316] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[00317] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[00318] In some embodiments, the recognition moiety for use in the aCAR, iCAR and/or pCAR provides specifity to at least one extracellular polymorphic epitope in a gene product from a gene selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[00319] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[00320] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[00321] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[00322] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[00323] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4,

LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[00324] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[00325] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[00326] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[00327] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of a gene selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[00328] In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a single allelic variant of HLA-A2. In some embodiments, the extracellular domain of the iCAR and/or pCAR expressed on the cell surface is directed against or specifically binds to a

single allelic variant of CD20. In some embdoiments, the iCAR will be directed toward HLA-A2. In some embodiments, the iCAR will be directed toward CD20. In some embodiments, the aCAR with be directed toward CD19. In some embodiments, the iCAR/aCAR set will be HLA-A2 and CD19 respectively. In some embodiments, the iCAR/aCAR set will include CD20 and CD19 respectively.

vi. PREPARATION OF TARGET CELLS

[00329] In some embodiments, the target cells are prepared and tested in an *in vitro* system. In some embodiments, an in vitro recombinant system will be established for testing the functionality of the iCAR and/or pCAR constructs in inhibiting the activity of the aCAR towards the off-target cells. In some embodiments, target cells expressing the aCAR epitope, iCAR epitope or both will be produced. In some embodiments, target cells expressing the aCAR epitope, pCAR epitope or both will be produced. In some embodiments, the recombinant cells expressing the aCAR epitope will represent the on-target 'on-tumor' cells while the cells expressing both aCAR and iCAR epitopes would represent the on target 'off-tumor' healthy cells.

[00330] In some embodiments, the iCAR/aCAR set will be HLA-A2 and CD19 respectively, recombinant cells expressing HLA-A2, CD19 or both will be produced by transfecting cell line (*e.g.,* Hela, Hela-Luciferase or Raji) with expression vector coding for these genes. For detection of recombinant CD19 and HLA-A2 expression, both genes will be fused to a protein tag (*e.g.,* HA or Flag or Myc etc). In some embodiments, the iCAR/aCAR target set will be CD20/CD19 and the recombinant cells will express CD19, CD20 or both.

[00331] In some embodiments, the expression vector comprising the iCAR/aCAR target set is transfected into a cell. In some embodiments, the expression vector is transfected into a cell to produce the target and off-tumor effects.

[00332] In some embodiments, the expression vector codes for a gene selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B,

FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B, LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PTGFRN, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

[00333] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2AD2A.

[00334] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

[00335] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

[00336] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

[00337] In some embodimetns, the expression vector codes for a gene selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

[00338] In some embodimetns, the expression vector codes for a gene selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14,

OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

[00339] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3, DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

[00340] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

[00341] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

[00342] In some embodimetns, the expression vector codes for a gene selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1,

OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12, SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

[00343] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

[00344] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

[00345] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

[00346] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11,

NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

[00347] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1, NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

[00348] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

[00349] In some embodimetns, the expression vector codes for a gene selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

[00350] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8,

SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

[00351] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7, GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

[00352] In some embodimetns, the expression vector codes for a gene selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

[00353] In some embodimetns, the expression vector codes for a gene selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

[00354] In some embodimetns, the expression vector codes for a gene selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

[00355] In some embodiments, the safe effector immune cells used for treating cancer as defined above express on their surface an aCAR comprising an extracellular domain that specifically binds to a tumor-associated antigen or a cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen expressed at least in a tissue of origin of the tumor, such as any of those listed above, which is a different antigen than that to which the extracellular domain of said aCAR binds. In some embodiments, the iCAR is expressed in the same tissue as the aCAR is expressed in. In some embodiments, the aCAR and iCAR are different alleles of the same gene. In some embodiments, the aCAR and iCAR are different proteins, and hence are different alleles.

[00356]

A. IN VITRO ASSAYS

[00357] In some embodiments, the iCAR and/or pCAR will be tested for activity in effects, including effectiveness and ability to inhibit, using a variety of assays. In some embodiments, the inhibitory effect of the iCAR and/or pCAR will be tested *in-vitro* and/or *in-vivo*. In some embodiments, the inhibitory effect of the iCAR and/or pCAR will be tested *in-vitro*. In some embodiments, the inhibitory effect of the iCAR and/or pCAR will be tested *in-vivo*. In some embodiments, the *in vitro* assays measure cytokine secretion and/or cytotoxicity effects. In some embodiments, the *in vivo* assays will evaluate the iCAR and/or pCAR inhibition and protection to on-target off tumor xenografts. In some embodiments, the the *in vivo* assays will evaluate the iCAR and/or pCAR inhibition and protection to on-target off tumor tissue and/or viral organs.

i. Luciferase Cytotoxicity Assay

[00358] In some embodimetns, the iCAR and/or pCAR are evaluated using a luciferase cytotoxitiy assay. Generally, for a luciferase cytotoxic assay, recombinant target cells (which can be referred to as "T") are engineered to express firefly luciferase. In some embodiments, commercial Hela-Luc cells can be transfected with DNA coding for the target proteins. The *in vitro* luciferase assay can be performed according to the Bright-Glo Luciferase assay (commercially available from Promega or BPS Biosciences or other commercial vendors). Transduced effector (E) T cells (which have been transduced with both iCAR or pCAR and aCAR or aCAR or mock CAR) can be incubated for 24-48 hrs with recombinant target cells expressing HLA-A2, CD19 or both CD19 and HLA-A2, or CD20, or both CD20 and CD19 to be tested in different effector to target ratios. In some embodiments, the iCAR/aCAR or pCAR/aCAR pair comprises any of aCAR, pCAR and/or iCAR with the components as described above. In some embodiments, the iCAR/aCAR pair comprises an HLA-A2 targeted iCAR and a CD19 targeted aCAR. In some embodiments, the iCAR/aCAR pair comprises a CD20 targeted iCAR and a CD19 targeted aCAR. Cell killing will be quantified indirectly by estimating the number of live cells with the Bright-Glo Luciferase system.

[00359] In some embodiments, the 'off-tumor' cytotoxicity can be optimized by sorting transduced T cell populations according to iCAR/aCAR expression level or by selecting sub population of recombinant target cells according to their target expression, including for example, expression of the gene product encoding for at least one

extracellular polymorphic epitope. In some embodiments, the aCAR, iCAR, and/or pCAR target is any target with an extracellular domain. In some embodiments, the sorting is based on CD19 or HLA-A2 expression level.

[00360] In some embodiments, the iCAR and/or pCAR is examined to determine whether the iCAR transduced T cells can discriminate between the 'on-tumor' cells (e.g., tumor cells) and 'off-tumor' cells (e.g., non-tumor cells) *in vitro*. Generally, this is tested by examining the killing effect of transduced T cells incubated with a mix of 'on-tumor' and 'off-tumor' cells at a ratio of 1:1. In some embodiments, the ratio is 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, or 1:8. The on tumor recombinant cells can be distinguished from the 'off-tumor' recombinant cells by luciferase expression in embodiments where only one cell population will be engineered to express the luciferase gene at a time). Killing can be quantified after 24-48 hrs of co-incubation using the Bright-Glo Luciferase assay (Promega).

[00361] In some embodiments, the iCAR/aCAR and/or pCAR/aCAR transduced T cells exhibit about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, and/or about 95% less off-tumor cell killing as compared to T cells transduced with aCAR but not transduced with the iCAR and/or pCAR. In some embodiments, the iCAR/aCAR and/or pCAR/aCAR transduced T cells exhibit about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, or about 10-fold less off-tumor cell killing as compared to T cells transduced with aCAR but not transduced with the iCAR and/or pCAR.

ii. Caspase 3

[00362] In some embodiments, caspase 3-detection assays are employed to examine the iCAR and/or pCAR to determine the level of apoptis of the 'on-tumor' cells (e.g., tumor cells) and 'off-tumor' cells (e.g., non-tumor cells) *in vitro*. In some embodiments, caspase_3-detection of cytotoxic lymphocyte (CTL) induced apoptosis by an antibody to activated cleaved caspase 3 is examined.

[00363] Generally, one of the pathways by which CTLs kill target cells is by inducing apoptosis through the Fas ligand. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family. Typically, sequential activation of caspases plays a significant role in the execution-phase of cell apoptosis and as such, cleavage of pro-caspase 3 to caspase 3 results in conformational change and expression of catalytic

activity. The cleaved activated form of caspase 3 can be recognized specifically by a monoclonal antibody.

[00364] In some embodiments, transduced T cells can be incubated with either 'on-tumor' (e.g., mimicking tumor) and 'off-tumor' cells (e.g., mimicking non-tumor) recombinant cells. In some embodiments, the 'on-tumor' (e.g., tumor) and 'off-tumor' cells (e.g., non-tumor) recombinant cells have been previously labeled with CFSE ((5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester)) or other cell tracer dye (e.g., CellTrace Violet). In some embodiments, co-incubation of target cells with effector cells occurs for about 1 hour to 6 about hours, about 2 hours to about 5 hours, or about 2 to about 4 hrs. In some embodiments, target cell apoptosis is quantified by flow cytometry. Cells can be permeabilized and fixed by an inside staining kit (Miltenyi or BD bioscience) and stained with an antibody for activated caspase 3 (BD bioscience).

[00365] In some embodiments, the iCAR/aCAR and/or pCAR/aCAR transduced T cells induce about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, and/or about 95% less off-tumor cell apoptosis as compared to T cells transduced with aCAR but not transduced with the iCAR and/or pCAR. In some embodiments, the aCAR/iCAR and/or aCAR/pCAR transduced T cells induce about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, or about 10-fold less off-tumor cell apoptosis as compared to T cells transduced with aCAR but not transduced with the iCAR and/or pCAR.

iii. Time-lapse microscopy

Time lapse micros CTL-

[00366] Time lapse microscopy of the iCAR and/or pCAR transduced T cells can be employed in order to discern target binding. In some embodiments, target cells will be labeled with a reporter gene (for example but not limited to a fluorescent proten such as mCherry). In some embodiments, transduced T cells are incubated with either 'on-tumor' or 'off-tumor' cells for up to 5 days. In some embodiments, time lapse microscopy can be used to visualize killing. In some embodiments, flow cytometry analysis using viable cell number staining and CountBright beads (Invitrogen) for determining target cell number at end-point time will be conducted.

[00367] In some embodiments, in order to determine if the aCAR/iCAR or aCAR/pCAR transduced T cells can discern targets *in vitro*, each recombinant target

cells ('on-tumor' or 'off-tumor') is labeled with a different reporter protein (for example GFP and mCherry). In some embodiments, any report protein pair would work, so long as the reporter pair contains two reporters which are easily distinguishable. In some embodiments, transduced T cells (Effector cells) will be co-incubated with the recombinant cells (target cells) at a 1:1 ratio of E/T. In some embodiments, the ration of effector to target (E/T) includes but is not limited to 16:1, 12:1, 10:1, 8:1, 6:1, 4:1, 2:1, or 1:1. In some embodimetns, the cell fate is then examined by microscopy imaging.

iv. Cytokine release

[00368] Cytkine release can be examined in order to determine T cells activation. In some embodiments, iCAR/aCAR and/or pCAR/aCAR transduced T cells are incubated with the recombinant target cells and cytokine production for one or more cytokines is quantified, for example, either by measuring cytokine secretion in cell culture supernatant according to BioLegend's ELISA MAXTM Deluxe Set kit or by flow cytometry analysis of the percentage of T cells producing cytokines. For the flow cytometry analysis, a Golgi stop is generally employed to prevent the secretion of the cytokines. In some embodiments, following a 6 hour and 18 hour to 24 hour incubation of the transduced T cells with target cells, T cells will be permeabilized and fixed by an inside staining kit (Miltenyi) and stained with antibodies for the T cell markers (CD3 and CD8) and for one or more cytokines. In some embodiments, the cytokines include but are not limited to IL-2, INFγ, and/or TNFα.

v. CD107a staining

[00369] Staining for CD107a can also be examined in order to determine cytolytic activity of the transduced T cells. Generally, degranulating of T cells can be identified by the surface expression of CD107a, a lysosomal associated membrane protein (LAMP-1), and surface expression of LAMP-1 has been shown to correlate with CD8 T cell cytotoxicity. Furthter, this molecule is located on the luminal side of lysosomes. Typically, upon activation, CD107a is transferred to the cell membrane surface of activated lymphocytes. Moreover, CD107a is expressed on the cell surface transiently and is rapidly re-internalized via the endocytic pathway. Therefore, while not being bound by theory, CD107a detection is maximized by antibody staining during cell

stimulation and by the addition of monensin (for example, to prevent acidification and subsequent degradation of endocytosed CD107a antibody complexes).

[00370] In some embodiments, the aCAR/iCAR and/or aCAR/pCAR transduced transduced T cells are incubated with the target cells for about 6 ours to about 24 hrs and CD107a expression on the CD8 T cells is examined. In some embodiments, the target cells expresso only one target protein recognized by aCAR (as in tumor cells) or target cells expressing both target proteins recognized by aCAR and iCAR (as in normal cells). In some embodiments, the iCAR and/or pCAR transduced transduced T cells are incubated with the target cells for about 6 ours to about 24 hrs in the presence of monensin and CD107a expression on the CD8 T cells is followed by flow cytometry using conjugated antibodies against the T cell surface markers (for example, CD3 and CD8) and a conjugated antibody for CD107a.

vi. Quantitation of Secreted Cytokines by ELISA

[00371] In some embodiments, following co-cultivation of transduced T-cells (Jurkat, or primary T- cells) expressing iCAR or aCAR or both aCAR and iCAR with modified target cells, expressing iCAR or aCAR or both aCAR and iCAR antigens on their cell surface, conditioned medium will be collected, and cytokine's concentration will be measured by cytokine ELISA. In some embodiments, the cytokine is selected from the group consisting of IL-2, INFγ and/or TNFα. In some embodiments, the cytokine is selected from the group consisting of IL-2. In some embodiments, the cytokine is selected from the group consisting of INFγ. In some embodiments, the cytokine is selected from the group consisting of TNFα. In some embodiments, a decrease of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% is demonstrated with dual CAR (aCAR/iCAR) transduced cells.

vii. Cytokines Secretion Measured by Cytometric Bead Array (CBA) Assay

[00372] Cytometric Bead Array (CBA) is used to measure a variety of soluble and intracellular proteins, including cytokines, chemokines and growth factors. In some embodiments, T-cells (primary T-cells or Jurkat cells) transduced with aCAR or both aCAR and iCAR constructs (Effector cells) are stimulated with modified target cells expressing both iCAR and aCAR or aCAR or iCAR target antigens on their cell surface.

In some embodiments, the effector to target ratio ranges from 20:1 up to 1:1. In some embodiments, the effector to target ratio ranges from 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1,2:1, or 1:1. In some embodimens, following several hours of co-incubation the effector cells produce and secrete cytokines which indicate their effector state. In some embodiments, the supernatant of the reaction is collected, and secreted IL-2 was measured and quantified by multiplex CBA assay.

[00373] In some embodiments, a decrease of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% is demonstrated with dual CAR (aCAR/iCAR) transduced cells were co-incubated with target cells expressing both target antigens as compared to IL-2 secretion resulted from co-incubation of the same effector cells with target cells expressing only one target. In some embodiments, a decrease of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% in IL-2 secretion was demonstrated when dual CAR (aCAR/iCAR) transduced cells were co-incubated with target cells expressing both target antigens as compared to IL-2 secretion resulted from co-incubation of the same effector cells with target cells expressing only one target. In some embodiments, a decrease of 86%. In some embodiments, the aCAR is a CD19 aCAR. In some embodimetns, the iCAR is an HLA-A2 iCAR. In some embodiments, the iCAR is a CD20 iCAR. In some embodiments, the aCAR/iCAR pair is CD19 aCAR and HLA-A2 iCAR. In some embodiments, the aCAR/iCAR pair is CD19 aCAR and a CD20 iCAR

viii. T-cell degranulation assay as measured by CD107a staining

[00374] In some embodiments, degranulating of T cells can be identified by the surface expression of CD107a, a lysosomal associated membrane protein (LAMP-1). In some embodiments, surface expression of LAMP-1 has been shown to correlate with CD8 T cell cytotoxicity. In some embodiments, granulation (CD107a) is a marker for killing potential.

B. IN VIVO ASSAYS

[00375] In some embodiments, the iCAR/aCAR and/or iCAR/pCAR pairs are tested for effectiveness *in vivo*. In some embodiments, NOD/SCID/γc- or similar mice are inoculated intravenously with tumor cells. In some embodiments, the tumor cells are CD19 positive NALM 6 (ATCC, human B-ALL cell line) cells that are engineered to express firefly luciferase. In some embodiments, for establishment of and/or differentiation between 'on-target' cells and 'off-tumor' cells, NALM 6 can be engineered to express the iCAR and/or pCAR epitope thereby representing the healthy cells. In some embodiments, the iCAR and/or pCAR epitope comprises at least one extracellular polymorphic epitope. In some embodiments, the iCAR and/or pCAR epitope is from HLA-A2 or CD20. Other cells that could be employed in these assays include but are not limited to Raji or any other recombinant cell lines. In some embodiemnts, such assays can be in a PDX (patient derived xenograft) model.

[00376] For the assay, mice will be divided into study groups; one group will be injected with the NALM 6 cells while the other will be injected with the NALM-6 expressing the iCAR epitope. Several days later, mice will be infused intravenously with T cells transduced with aCAR, aCAR/iCAR and a control group of untransduced T cells or no T cells. Mice will be sacrificed and tumor burden will be quantified according to total flux.

[00377] According to one embodiment of the assay, in order to test whether the T cells expressing the iCAR and/or pCAR construct could discriminate between the target cells and off target cells *in vivo* within the same organism, mice are injected with a 1:1 mixture of the 'on-tumor'/'off-tumor NALM-6 cells, followed by injection of transduced T cells expressing either the aCAR alone or both aCAR and iCAR. With this embodiment, upon sacrifice of the mice the presence of the 'on-tumor' and 'off-tumor cells in the spleen and bone marrow will be analyzed by flow cytometry for the two markers, CD19 and the iCAR epitope.

i. In Vivo CTL assay in human xenograft mouse models

[00378] In some embodiments, to test whether T-cells expressing both aCAR and iCAR constructs discriminate between the target cells and 'off-target' cells within the

same organism and effectively kill the target cells while sparing the 'off-target' cells will be assessed by an in-vivo CTL assay.

[00379] In some embodiments, transduced T-cells with iCAR or aCAR or both iCAR and aCAR will be injected i.v. to naïve NOD/SCID/γc- or similar mice and up to several hours later, target cells expressing iCAR, aCAR or both will be injected. In some embodiments, these targets will be labeled with either CFSE / CPDE or similar cell trace dye in different concentrations (high, medium and low) which will allow further discrimination between them. In some embodiments, percentage of specific killing will be calculated, as described in Example 5.

ii. Tumor growth kinetics in human xenograft mouse models

[00380] In some embodiments, the tumor cells express either the iCAR target, aCAR target or both. In some embodiments, an aCAR tumor cell line could be the CD19 positive NALM 6 (ATCC, human BALL cell line). In some embodiments, tumor cells that express both the aCAR and iCAR (i.e. 'off-tumor' cells) are NALM 6 engineered to express the iCAR epitope (for example HLA- A2) thereby representing the healthy cells. In some embodiments, NALM 6 and NAlM 6-HLA-A2 can also be engineered to express a reporter gene (e.g. firefly luciferase), for easy detection.

[00381] In some embodiments, monitoring will be conducted by measuring tumor volume by mechanical means (caliper) and also by using in-vivo imaging systems (IVIS). In some embodiments, tumor burden can be quantified, and infiltrating T-cell populations can be analyzed by FACS.

iii. Toxicity and Tumor Growth Kinetics in Transgenic Mouse Models

[00382] In some embodiments, transgenic mice that express the human aCAR and iCAR targets will also be used to determine the efficacy of the transduced T-cells. In some embodiments, system will allow us to monitor efficacy and toxicity issues.

C. IN VIVO USES: TREATMENT, BIOMARKERS

[00383] In yet another aspect, the present invention provides a method of selecting a personalized biomarker for a subject having a tumor characterized by LOH, the method comprising (i) obtaining a tumor biopsy from the subject; (ii) obtaining a sample of normal tissue from the subject, e.g., PBMCs; and (iii) identifying a single allelic variant

of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, thereby identifying a personalized biomarker for the subject.

[00384] In some embodiments, the biomarker is used to customize a treatment of the subject, so the method further comprises the steps of treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient an effector immune cell as defined above, wherein the iCAR is directed to the single allelic variant identified in (iii). In some embodiments, the present invention provides a method of selecting a personalized biomarker for a subject having a tumor characterized by LOH, the method comprising (i) obtaining a tumor biopsy from the subject; (ii) obtaining a sample of normal tissue from the subject, e.g. PBMCs; (iii) identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, based on the LOH candidate score, wherein an allelic variant is identified as a personalized biomarker for the subject.

[00385] In a further aspect, the present invention provides a method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient an effector immune cell as defined above, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

[00386] In a similar aspect, the present invention provides a method of reducing tumor burden in a subject having a tumor characterized by LOH, comprising administering to the patient an effector immune cell as defined above, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient or at least on vital tissues the aCAR is expressed in.

[00387] In another similar aspect, the present invention provides a method of increasing survival of a subject having a tumor characterized by LOH, comprising administering to the patient an effector immune cell as defined above, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope

absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

[00388] In still a further aspect, the present invention is directed to a safe effector immune cell as defined above for use in treating, reducing tumor burden in, or increasing survival of, a patient having a tumor characterized by LOH, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

[00389] In yet a further aspect, the present invention is directed to a method for treating cancer in a patient having a tumor characterized by LOH comprising: (i) identifying or receiving information identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, (ii) identifying or receiving information identifying a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared by cells at least of related tumor and normal tissue in said cancer patient; (iii) selecting or receiving at least one nucleic acid molecule defining an iCAR as defined herein above and at least one nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein above, or at least one vector as defined herein above, wherein the iCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (i) and the aCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (ii); (iv) preparing or receiving at least one population of safe redirected effector immune cells by transfecting effector immune cells with the nucleic acid molecules of (iii) or transducing effector immune cells with the vectors of (iii); and (v) administering to said cancer patient at least one population of safe redirected immune effector cells of (iv).

[00390] In a similar aspect, the present invention provides at least one population of safe redirected immune effector cells for treating cancer in a patient having a tumor characterized by LOH, wherein the safe redirected immune cells are obtained by (i) identifying or receiving information identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue, (ii) identifying or receiving information

identifying a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared by cells at least of related tumor and normal tissue in said cancer patient; (iii) selecting or receiving at least one nucleic acid molecule defining an iCAR as defined herein above and at least one nucleic acid molecule comprising a nucleotide sequence encoding an aCAR as defined herein above, or at least one vector as defined herein above, wherein the iCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (i) and the aCAR comprises an extracellular domain that specifically binds to a cell surface epitope of (ii); (iv) preparing or receiving at least one population of safe redirected effector immune cells by transfecting effector immune cells with the nucleic acid molecules of (iii) or transducing effector immune cells with the vectors of (iii).

[00391] In some embodiments referring to any one of the above embodiments directed to treatment of cancer or safe immune effector cells for use in treatment of cancer, (i) the extracellular domain of the iCAR specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen, which is a different antigen than that to which the extracellular domain of the aCAR binds; (ii) the extracellular domain of said iCAR specifically binds a single allelic variant of a different polymorphic cell surface epitope of the same antigen to which the extracellular domain of said aCAR binds; or (iii) the extracellular domain of said iCAR specifically binds a different single allelic variant of the same polymorphic cell surface epitope to which the extracellular domain of said aCAR binds.

[00392] In some embodiments, the treating results in reduced on-target, off-tumor reactivity, as compared with a treatment comprising administering to the cancer patient at least one population of immune effector cells expressing an aCAR of (iii) but lacking and iCAR of (iii).

[00393] In some embodiments, the safe effector immune cells used for treating cancer as defined above express on their surface an aCAR comprising an extracellular domain that specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen

expressed at least in a tissue of origin of the tumor or of a housekeeping protein, which is a different antigen than that to which the extracellular domain of said aCAR binds.

[00394] In some embodiments, the safe effector immune cells used for treating cancer as defined above express on their surface an aCAR comprising an extracellular domain that specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen expressed at least in a tissue of origin of the tumor or of a housekeeping protein, such as an HLA genes (including for example, HLA-A, HLA-B, HLA-C, HLA-G, HLA-E, HLA-F, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA-DQ, or HLA-DR) which is a different antigen than that to which the extracellular domain of said aCAR binds.

[00395] In some embodiments, the safe effector immune cells used for treating cancer as defined above express on their surface an aCAR comprising an extracellular domain that specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen expressed at least in a tissue of origin of the tumor, such as an HLA-A, which is a different antigen than that to which the extracellular domain of said aCAR binds.

[00396] In some embodiments, more than one population of immune effector cells are administered, and the different populations express different pairs of aCARs and iCARs having specific binding to cell surface epitopes of different gene products.

[00397] In some embodiments, the safe effector immune cells used in the method of treating cancer are selected from T cells, natural killer cells or cytokine-induced killer cells. In some embodiments, the safe effector immune cell is autologous or universal (allogeneic) effector cells. In some embodiments, the iCAR used in any one of the methods of treating cancer defined above is directed to all tissues of the patient on which the target-antigen of the aCAR is present, wherein the target antigen of the aCAR is a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope is present, and said epitope is a tumor-associated antigen or is shared at least by cells of related tumor and normal tissue.

[00398] In some embodiments, the cancer is selected from Acute Myeloid Leukemia [LAML], Adrenocortical carcinoma [ACC], Bladder Urothelial Carcinoma [BLCA], Brain Lower Grade Glioma [LGG], Breast invasive carcinoma [BRCA], Cervical squamous cell carcinoma and endocervical adenocarcinoma [CESC], Cholangiocarcinoma [CHOL], Colon adenocarcinoma [COAD], Esophageal carcinoma [ESCA], Glioblastoma multiforme [GBM], Head and Neck squamous cell carcinoma [HNSC], Kidney Chromophobe [KICH], Kidney renal clear cell carcinoma [KIRC], Kidney renal papillary cell carcinoma [KIRP], Liver hepatocellular carcinoma [LIHC], Lung adenocarcinoma [LUAD], Lung squamous cell carcinoma [LUSC], Lymphoid Neoplasm Diffuse Large B-cell Lymphoma [DLBC], Mesothelioma [MESO], Ovarian serous cystadenocarcinoma [OV], Pancreatic adenocarcinoma [PAAD], Pheochromocytoma and Paraganglioma [PCPG], Prostate adenocarcinoma [PRAD], Rectum adenocarcinoma [READ], Sarcoma [SARC], Skin Cutaneous Melanoma [SKCM], Stomach adenocarcinoma [STAD], Testicular Germ Cell Tumors [TGCT], Thymoma [THYM], Thyroid carcinoma [THCA], Uterine Carcinosarcoma [UCS], Uterine Corpus Endometrial Carcinoma [UCEC], Uveal Melanoma [UVM].

[00399] In some embodiments, the iCAR and/or pCAR for use in the treatment of cancer is any iCAR and/or pCAR described herein. In some embodiments, the iCAR and/or pCAR used to treat the cancer, such as any one of the cancer types recited above, is directed against or specifically binds to a single allelic variant of an HLA genes (including for example, HLA-A, HLA-B, HLA-C, HLA-G, HLA-E, HLA-F, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA-DQ, or HLA-DR, HLA-B gene or HLA-C gene or against a single allelic variant of a gene listed **Table 8** In some embodiments, the iCAR used to treat the cancer, such as any one of the cancer types recited above, is directed against or specifically binds to a single allelic variant of an HLA-A gene, HLA-B gene or HLA-C gene or against a single allelic variant of a gene listed **Table 8**; and the aCAR used to treat the cancer, such as any one of the cancer types recited above, is directed against or specifically binds to, a non-polymorphic cell surface epitope selected from the antigens listed in **Table 1**, such as CD19.

[00400] For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives

such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

[00401] Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[00402] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00403] The compositions may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen free water, before use.

[00404] The compositions may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[00405] For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and

cartridges of, e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00406] For purposes of clarity, and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values recited herein, should be interpreted as being preceded in all instances by the term "about." Accordingly, the numerical parameters recited in the present specification are approximations that may vary depending on the desired outcome. For example, each numerical parameter may be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00407] The term "about" as used herein means that values of 10% or less above or below the indicated values are also included.

EXEMPLARY EMBODIMENTS

[00408] In some embodiments, the methods of the present invention provide for the following exemplary embodiments.

1. A nucleic acid molecule comprising a nucleotide sequence encoding an inhibitory chimeric antigen receptor (iCAR) or protective chimeric antigen receptor (pCAR) capable of preventing or attenuating undesired activation of an effector immune cell, wherein the iCAR or pCAR comprises an extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope absent from mammalian tumor cells due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue; and an intracellular domain comprising at least one signal transduction element that inhibits an effector immune cell.

2. The nucleic acid molecule of claim 1, wherein the polymorphic cell surface epitope is of a housekeeping gene product, such as an HLA gene, a G-protein-coupled receptor (GPCR), an ion channel or a receptor tyrosine kinase, preferably an HLA-A, HLA-B or HLA-C; or a polymorphic cell surface epitope of a gene selected from Table 8.

3. The nucleic acid molecule claim 1, wherein said extracellular domain comprises (i) an antibody, derivative or fragment thereof, such as a humanized antibody; a human

antibody; a functional fragment of an antibody; a single-domain antibody, such as a Nanobody; a recombinant antibody; and a single chain variable fragment (ScFv); (ii) an antibody mimetic, such as an affibody molecule; an affilin; an affimer; an affitin; an alphabody; an anticalin; an avimer; a DARPin; a fynomer; a Kunitz domain peptide; and a monobody; or (iii) an aptamer.

4. The nucleic acid molecule of claim 1, wherein said mammalian tissue is human tissue and said related mammalian normal tissue is normal tissue from which the tumor developed.

5. The nucleic acid molecule of claim 1, wherein said effector immune cell is a T cell, a natural killer cell or a cytokine-induced killer cell.

6. The nucleic acid molecule of claim 1, wherein said at least one signal transduction element capable of inhibiting an effector immune cell is homologous to a signal transduction element of an immune checkpoint protein.

7. The nucleic acid molecule of claim 6, wherein said immune checkpoint protein is selected from the group consisting of PD1; CTLA4; BTLA; 2B4; CD160; CEACAM, such as CEACAM1; KIRs, such as KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, LIR1, LIR2, LIR3, LIR5, LIR8 and CD94– NKG2A; LAG3; TIM3; V-domain Ig suppressor of T cell activation (VISTA); STimulator of INterferon Genes (STING); immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing proteins, T cell immunoglobulin and ITIM domain (TIGIT), and adenosine receptor (e.g. A2aR).

8. The nucleic acid molecule of claim 1, wherein said extracellular domain is fused through a flexible hinge and transmembrane canonic motif to said intracellular domain.

9. A vector comprising a nucleic acid molecule of any one of claims 1 to 8 and at least one control element, such as a promoter, operably linked to the nucleic acid molecule.

10. The vector of claim 9, further comprising a nucleic acid molecule comprising a nucleotide sequence encoding an aCAR comprising an extracellular domain specifically binding a non-polymorphic cell surface epitope of an antigen or a single allelic variant of a polymorphic cell surface epitope, wherein said epitope is a tumor-associated antigen or is shared at least by cells of related tumor and normal tissue, and an intracellular domain

comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell.

11. The vector of claim 10, wherein the extracellular domain of the aCAR specifically binds to a non-polymorphic cell surface epitope of an antigen and the extracellular domain of the iCAR specifically binds a single allelic variant of a polymorphic cell surface epitope of a different antigen than that to which the extracellular domain of said aCAR binds.

12. The vector of claim 10 or 11, wherein the extracellular domain of the aCAR specifically binds to a non-polymorphic cell surface epitope selected from the antigens listed in Table 1, such as CD19.

13. The vector of claim 10, wherein said at least one signal transduction element that activates or co-stimulates an effector immune cell is homologous to an immunoreceptor tyrosine-based activation motif (ITAM) of for example CD3ζ or FcRγ chains; an activating killer cell immunoglobulin-like receptor (KIR), such as KIR2DS and KIR3DS, or an adaptor molecule such as DAP12; or a co-stimulatory signal transduction element of for example CD27, CD28, ICOS, CD137 (4-1BB) or CD134 (OX40).

14. The vector of claim 10, wherein the nucleotide sequence comprises an internal ribosome entry site (IRES) between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR

15. The vector of claim 14, wherein the nucleotide sequence encoding for the aCAR is downstream of the nucleotide sequence encoding for the iCAR.

16. The vector of claim 10, wherein the nucleotide sequence comprises a viral self-cleaving 2A peptide between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR.

17. The vector of claim 16, wherein the viral self-cleaving 2A peptide is selected from the group consisting of T2A from Thosea asigna virus (TaV), F2A from Foot-and-mouth disease virus (FMDV), E2A from Equine rhinitis A virus (ERAV) and P2A from Porcine teschovirus-1 (PTV1).

18. The vector of claim 10, comprising a nucleotide sequence encoding said constitutive aCAR linked via a flexible linker to said iCAR.

19. A method of preparing an inhibitory chimeric antigen receptor (iCAR) capable of preventing or attenuating undesired activation of an effector immune cell, as defined in claims 1 to 8, the method comprising:

(i) retrieving a list of human genomic variants of protein-encoding genes from at least one database of known variants;

(ii) filtering the list of variants retrieved in (i) by:

(a) selecting variants resulting in an amino acid sequence variation in the protein encoded by the respective gene as compared with its corresponding reference allele,

(b) selecting variants of genes wherein the amino acid sequence variation is in an extracellular domain of the encoded protein,

(c) selecting variants of genes that undergo loss of heterozygosity (LOH) at least in one tumor, and

(d) selecting variants of genes that are expressed at least in a tissue of origin of the at least one tumor in which they undergo LOH according to (c), thereby obtaining a list of variants having an amino acid sequence variation in an extracellular domain in the protein encoded by the respective gene lost in the at least one tumor due to LOH and expressed at least in a tissue of origin of the at least one tumor;

(iii) defining a sequence region comprising at least one single variant from the list obtained in (ii), sub-cloning and expressing the sequence region comprising the at least one single variant and a sequence region comprising the corresponding reference allele thereby obtaining the respective epitope peptides;

(iv) selecting an iCAR binding domain, which specifically binds either to the epitope peptide encoded by the cloned sequence region, or to the epitope peptide encoded by the corresponding reference allele, obtained in (iii); and

(vii) preparing iCARs as defined in any one of claims 1 to 8, each comprising an iCAR binding domain as defined in (iv).

20. The method of claim 19, wherein the minor allele frequency for each variant equals or exceeds 1, 2, 3, 4 or 5%.

21. A method for preparing a safe effector immune cell comprising: (i) transfecting a TCR-engineered effector immune cell directed to a tumor-associated antigen with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR of any one of claims 1 to 8 or transducing the cells with a vector of claim 9; or (ii) transfecting a naïve effector immune cell with a nucleic acid molecule comprising a nucleotide sequence encoding an iCAR of any one of claims 1 to 8 and a nucleic acid molecule comprising a nucleotide sequence encoding an aCAR defined in any one of claims 10 to 13; or transducing an effector immune cell with a vector of any one of claims 10 to 18.

22. A safe effector immune cell obtained by the method of claim 21.

23. The safe effector immune cell of claim 22, expressing on its surface an aCAR comprising an extracellular domain that specifically binds to a non-polymorphic cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of a different antigen to which the extracellular domain of said aCAR binds.

24. The safe effector immune cell of claim 22 or 23, wherein the extracellular domain of the aCAR specifically binds to a non-polymorphic cell surface epitope selected from the antigens listed in Table 1, such as CD19.

25. The safe effector immune cell of claim 22, wherein the aCAR and the iCAR are present on the cell surface as separate proteins.

26. The safe effector immune cell of claim 22, wherein the expression level of said nucleotide sequence encoding the iCAR is greater than or equal to the expression level of the nucleotide sequence encoding the aCAR.

27. A method of selecting a personalized biomarker for a subject having a tumor characterized by LOH, the method comprising

(i) obtaining a tumor biopsy from the subject;

(ii) obtaining a sample of normal tissue from the subject, e.g. PBMCs;

(iii) identifying a single allelic variant of a polymorphic cell surface epitope that is not expressed by cells of the tumor due to LOH, but that is expressed by the cells of the normal tissue,

thereby identifying a personalized biomarker for the subject.

28. A method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient an effector immune cell of claim 22, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

29. A safe effector immune cell of claim 22 for use in treating patient having a tumor characterized by LOH, wherein the iCAR is directed to a single allelic variant encoding a polymorphic cell surface epitope absent from cells of the tumor due to loss of heterozygosity (LOH) but present at least on all cells of related mammalian normal tissue of the patient.

30. The safe effector immune cell for the use of claim 29, wherein the treating results in reduced on-target, off-tumor reactivity, as compared with a treatment comprising administering to the cancer patient at least one population of immune effector cells expressing an aCAR of (iii) but lacking and iCAR of (iii).

31. The safe effector immune cell for the use of claim 29, expressing on its surface an aCAR comprising an extracellular domain that specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope of an antigen and an iCAR comprising an extracellular domain that specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen expressed at least in a tissue of origin of the tumor or of a housekeeping protein, such as an HLA-A, which is a different antigen than that to which the extracellular domain of said aCAR binds.

32. The safe effector immune cell for the use of claim 28, which is an autologous or a universal (allogeneic) effector cell.

33. The safe effector immune cell for the use of any one of claims 28 to 32, selected from a T cell, natural killer cell or cytokine-induced killer cell.

34. A combination of two or more nucleic acid molecules, each one comprising a nucleotide sequence encoding a different member of a controlled effector immune cell activating system, said nucleic acid molecules forming a single continues nucleic acid molecule or comprising two or more separate nucleic acid molecules, wherein the controlled effector immune activating system directs effector immune cells to kill tumor cells that have lost one or more chromosomes or fractions thereof due to Loss of Heterozygosity (LOH) and spares cells of related normal tissue, and wherein

(a) the first member comprises an activating chimeric antigen receptor (aCAR) polypeptide comprising a first extracellular domain that specifically binds to a non-polymorphic cell surface epitope of an antigen or to a single allelic variant of a different polymorphic cell surface epitope and said non-polymorphic or polymorphic cell surface epitope is a tumor-associated antigen or is shared by cells of related abnormal and normal mammalian tissue; and

(b) the second member comprises a regulatory polypeptide comprising a second extracellular domain that specifically binds to a single allelic variant of a polymorphic cell surface epitope not expressed by an abnormal mammalian tissue due to LOH but present on all cells of related mammalian normal tissue.

35. The combination of claim 34, wherein the first member is selected from:

(a) a constitutive aCAR further comprising an intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell; and

(b) a conditional aCAR further comprising an intracellular domain comprising a first member of a binding site for a heterodimerizing small molecule and optionally at least one co-stimulatory signal transduction element, but lacking an activating signal transduction element; and the second member is:

(c) an inhibiting chimeric antigen receptor (iCAR) further comprising an intracellular domain comprising at least one signal transduction element that inhibits an effector immune cell; or

(d) a protective chimeric antigen receptor (pCAR) further comprising an extracellular regulatory region comprising a substrate for a sheddase; a transmembrane canonic motif comprising a substrate for an intramembrane-cleaving protease; and an intracellular domain, said intracellular domain comprising at least one signal transduction element that activates and/or co-stimulates an effector immune cell and a second member of a binding site for a heterodimerizing small molecule.

36. The combination of claim 34 or 35, wherein:

(i) the extracellular domain of the iCAR or pCAR specifically binds a single allelic variant of a polymorphic cell surface epitope of an antigen, which is a different antigen than that to which the extracellular domain of the aCAR binds

(ii) the extracellular domain of said pCAR or iCAR specifically binds a single allelic variant of a different polymorphic cell surface epitope of the same antigen to which the extracellular domain of said aCAR binds; or

(iii) the extracellular domain of said pCAR or iCAR specifically binds a different single allelic variant of the same polymorphic cell surface epitope to which the extracellular domain of said aCAR binds.

37. The combination of claim 34, wherein said substrate for a sheddase is a substrate for a disintegrin and metalloproteinase (ADAM) or a beta-secretase 1 (BACE1).

38. The combination of claim 37, wherein said substrate forms part of the extracellular domain and comprises Lin 12/Notch repeats and an ADAM protease cleavage site.

39. The combination of claim 34, wherein said substrate for an intramembrane-cleaving protease is a substrate for an SP2, a y–secretase, a signal peptide peptidase (spp), a spp-like protease or a rhomboid protease.

40. The combination of claim 39, wherein said substrate forms part of the transmembrane canonic motif and is homologous to/derived from a transmembrane domain of Notch, ErbB4, E-cadherin, N-cadherin, ephrin-B2, amyloid precursor protein or CD44.

41. The combination of claim 34, comprising a nucleotide sequence encoding an extracellular domain and an intracellular domain of said conditional aCAR as separate proteins, wherein each domain is independently fused to a transmembrane canonic motif and comprises a different member of a binding site for a heterodimerizing small molecule.

42. The combination of claim 34, wherein each one of said first and second member of said binding site for a heterodimerizing small molecule is derived from a protein selected from:

(i) Tacrolimus (FK506) binding protein (FKBP) and FKBP;

(ii) FKBP and calcineurin catalytic subunit A (CnA);

(iii) FKBP and cyclophilin;

(iv) FKBP and FKBP-rapamycin associated protein (FRB);

(v) gyrase B (GyrB) and GyrB;

(vi) dihydrofolate reductase (DHFR) and DHFR;

(vii) DmrB homodimerization domain (DmrB) and DmrB;

(viii) a PYL protein (a.k.a. abscisic acid receptor and as RCAR) and ABI;

(ix) GAI *Arabidopsis thaliana* protein (a.k.a Gibberellic Acid Insensitive and DELLA protein GAI; GAI) and GID1 *Arabidopsis thaliana* protein (also known as Gibberellin receptor GID1; GID1).

LENGTHY TABLES

[00409] The patent application contains a lengthy table section. Copies of the tables are submitted concurrently herewith on CD-ROM [MPCA1]

EXAMPLES

[00410] With regard to the examples, the following terminology is employed.

[00411] When the term chromosome is employed, this generally refers to the chromosome the SNP lies on. For the SNP analysis, position refers to the genomic position of the SNP (assembly GRCh37.p13). The snp_id when used refers to the dbSNP rs ID, where one exists.

[00412] The term "ref" refers to the reference nucleotide allele. The term "alt" refers to the alternative nucleotide allele.

[00413] The term "quality" refers to the quality score from Exome Aggregation Consortium (ExAC). The term "filter_status" refers to filter information from ExAC.

[00414] The term "allele_frequency" refers to the global allele frequency from ExAC. The term "max_allele_frequency" refers to the global allele frequency of most common alternative allele (generally, this is only relevant when the SNP has more than two alternative alleles at the same site, and this can often mean sequencing errors anyway).

[00415] The term "het_allele_count" refers to the number of participants in ExAC who were heterozygotes. The term "AFR_AF" refers to minor allele frequency from African genomes. The term "AMR_AF" refers to minor allele frequency in Latino genomes. The term "EAS_AF" refers to minor allele frequency in East Asian genomes. The term "FIN_AF" refers to minor allele frequency in Finnish genomes. The term "NFE_AF" refers to minor allele frequency in Non-Finnish-European genomes. The term "OTH_AF" refers to minor allele frequency in Other genomes. The term "SAS_AF" refers to minor allele frequency in South Asian genomes.

[00416] The term "max_AF" refers to maximum minor allele frequency amongst the populations categorized in ExAC (0.5 is maximum allowable allele frequency).

[00417] The term "gene" refers to the HUGO symbol of the gene in which the SNP falls.

[00418] The term "hgnc_ID" refers to the HUGO Gene Nomenclature Committee numeric ID of the gene in which the SNP falls.

[00419] The term "consequence" refers to the impact of the SNP on the translated protein product. Can be one of several, including: missense_variant, frameshift_variant, inframe_deletion, stop_gained.

[00420] The term "protein_consequence" reports the amino acid substitution and the location thereof on the reference protein transcript (e.g. p.Arg482Gln).

[00421] The term "aa_affected" refers to the numeric location of the affected amino acid on the consensus protein transcript.

[00422] The term "allele_1" refers to the amino acid encoded by the reference allele.

[00423] The term "allele_2" refers to the amino acid encoded by the alternative allele.

[00424] The term "sift_score" refers to the score and interpretation of the predicted functional effect of the amino acid substitution by the SIFT algorithm. Uses version sift5.2.2. Scores range from 0-1. A low score means than an amino acid substitution is more likely to be tolerated.

[00425] The term "polyphen_score" refers to the score and interpretation of the predicted functional effect of the amino acid substitution by the polyphen algorithm.

Uses PolyPhen (v2.2.2). Scores range from 0-1. A low score means than an amino acid substitution is more likely to be deleterious.

[00426] The term "polyphen_numeric" refers to the extracted numeric only score from the polyphen algorithm.

[00427] The term "protein_domains_affected" refers to the predicted protein domains based on the following algorithms: Gene3D, hmmpanther, Prosite.

[00428] The term "BLOSUM_score" refers to the score for the amino acid substitution based on the BLOSUM62 matrix from https://www.ncbi.nlm.nih.gov/IEB/ToolBox/C_DOC/lxr/source/data/BLOSUM62. A negative score indicates an amino acid substitution that has occurred less frequently over time in evolution (more likely to affect protein function).

[00429] The term "allele_1_one_letter" refers to the one letter amino acid code of the reference amino acid allele.

[00430] The term "allele_2_one_letter" refers to the one letter amino acid code of the alternative amino acid allele.

[00431] The term "mono_allelic_expression" refers to whether or not the gene that the SNP falls in undergoes mono-allelic expression in humans. The database established by Savova et al. was used for this annotation[7]. A 1 in this column indicates that the gene displays mono-allelic expression. A 0 in this column indicates that the gene did not display mono-allelic expression in the Savova et al. database. An NA in this column means that the gene was not annotated in the Savova et al. paper.

[00432] The term "extracellular" refers to whether or not the SNP falls in an extracellular domain of the affected protein. A 1 in this column indicates that the SNP is in an extracellular domain and a 0 indicates that it is not. Uniprot was used for annotation of protein domains.

[00433] The term "Pdb_id" refers to the protein databank ID of the affected protein if it exists. In the case where many protein databank entries exist for one protein, only the first ID is included.

[00434] The term "aa_context_21aa_allele_1" refers to A 21 amino acid window surrounding the SNP amino acid on the consensus protein sequence. The sequence consists of the 10 amino acids from the preceding part of the consensus protein

sequence. A check was made to ensure that the reference amino acid matched the consensus protein sequence at the affected position. If these two amino acids were not the same, then the entry reads "discrepancy with uniprot fasta based on consensus isoform".

[00435] The term "aa_context_21aa_allele_2: The same amino acid window as above, but inserting amino acid allele 2 into the middle.

[00436] The term "gtex_mean: Average gene expression across tissues (in RPKM). This consists of the mean value of the median RPKM values across tissues from GTEX. For example, if the values for a given gene were Lung (median)= 3, Breast (median) = 2, Pancreas (median) = 5, then the value reported in this entry would be 3.33.

[00437] The term "gtex_min: The lowest gene expression for a tissue across all tissues. This value is derived from the list of the median values of gene expression across all tissues. For example, if the values for a given gene were Lung (median)= 3, Breast (median) = 2, Pancreas (median) = 5, then the value reported in this entry would be 2.

[00438] The term "gtex_max: The highest gene expression for a tissue across all tissues. This value is derived from the list of the median values of gene expression across all tissues. For example, if the values for a given gene were Lung (median)= 3, Breast (median) = 2, Pancreas (median) = 5, then the value reported in this entry would be 5.

[00439] The term "gtex_std_dev: The standard deviation of gene expression values across tissues for a given gene. For example, if the values for a given gene were Lung (median)= 3, Breast (median) = 2, Pancreas (median) = 5, then the value reported in this entry would be 1.5.

[00440] The term "cell_surface_protein_atlas: A binary marker for whether or not the protein was annotated as a membrane protein in the cell surface protein atlas (wlab.ethz.ch/cspa/). A 1 indicates that the gene was annotated as a membrane protein in this database.

[00441] The term "human_protein_atlas_membrane_proteins: A binary marker for whether or not the protein was annotated as a membrane protein in the human protein atlas (https://www.proteinatlas.org/). A 1 indicates that the gene was annotated as a membrane protein in this database.

[00442] The term "subcellular_map_proteome_membrane_proteins: A binary marker for whether or not the protein was annotated as a membrane protein in the subcellular map of the proteome (http://science.sciencemag.org/content/early/2017/05/10/science.aal3321/). A 1 indicates that the gene was annotated as a membrane protein in this database.

[00443] The term "n_membrane_databases_w_gene: The total number of databases with the gene annotated as a gene that is expressed on the cell membrane. Maximum=3, minimum=0.

[00444] The term "membrane_protein_call: A textual interpretation of the number of membrane databases that the included the gene. If the gene was included in one database, then the call is a "low-confidence" membrane protein. If the gene was included in two databases, then the call is a "medium-confidence" membrane protein. If the gene was included in three databases, then the call is a "high-confidence" membrane protein.

[00445] The term "ratio_gtex_std_dev_to_mean: The ratio of the standard deviation of gene expression across tissues over the mean gene expression across tissues. For example, if the values for a given gene were Lung (median)= 3, Breast (median) = 2, Pancreas (median) = 5, then the value reported in this entry would be 1.5/3.33= 0.45. This is meant to be a measure of the uniformity of expression across tissues. A low value indicates that the gene is uniformly expressed. A high value suggests that the gene tends to be expressed in some tissues and not others.

[00446] The term "universally_expressed: A binary marker of whether a gene seems to be universally expressed. A gene is said to be universally expressed if the gtex_mean is > 10, the gtex_min. The term "> 1, and ratio_gtex_std_dev_to_mean < 1. A 1 in this column indicates that the gene in question met these criteria.

[00447] The term "disease: the TCGA barcode for the disease analyzed for LOH data in this row of the spreadsheet.

[00448] The term "mean_expression_in_tissue: The mean gene expression in the tissue analyzed. Several tissue categorizations may map onto a single TCGA tumor type. The mapping from tissues in GTEX to TCGA tumor types is given in the file "tcga_disease_tissue_lookup.txt". A representative sample is given below:

tcga_disease	gtex_tissues
acc	Adrenal.Gland
blca	Bladder
brca	Breast...Mammary.Tissue
cesc	Cervix...Endocervix,Cervix...Ectocervix

[00449] The term "mean_expression_in_other_tissues: The mean gene expression in all other tissues except for the tissue analyzed. For example, if the gene being analyzed was PSMA (a prostate specific gene), then this value would be very low when the tumor type analyzed was PRAD (prostate adenocarcinoma).

[00450] The term "cohens_d: The Cohen's *d* measure of the separation of the expression in the tissue analyzed vs all other tissues. This is meant to be a measure of how much this gene is uniquely expressed in the tissue analyzed. A high Cohen's *d* would suggest that this gene is uniquely expressed in the tissue analyzed and therefore might be a good aCAR target.

[00451] The term "proportion_w_LOH_relative: The proportion of tumors in the tumor type analyzed that display evidence of LOH. The threshold for calling a genomic segment suggesting LOH was -0.1 (in relative copy number units). The relative copy number of a segment was the log of the copy number signal in the tumor divided by the copy number signal in the matched normal. These data were obtained from the cbio portal and the technique was validated in part 1.

[00452] The term "CI_95_low_relative: The lower boundary of the 95% confidence interval on the proportion of tumors undergoing LOH at this locus. The prop.test function in R was used for this calculation. This function calculates a binomial confidence interval with Yates' continuity correction.

[00453] The term "CI_95_high_relative: The upper boundary of the 95% confidence interval on the proportion of tumors undergoing LOH at this locus. The prop.test function in R was used for this calculation. This function calculates a binomial confidence interval with Yates' continuity correction.

[00454] The term "mutsig_hits_on_chr: The genes on the same chromosome as the SNP that pass statistical significance (q-value < 0.25) for being drivers in cancer. The Mutsig 2.0 algorithm was used. The format is "Gene symbol, q=q-value; Gene symbol 2, …"

[00455] The term "tsg_on_chr_mutated_in_disease: A binary indicator variable for whether or not one of the genes passing statistically significance from mutsig is a tumor suppressor gene. The list of tumor suppressor genes used for this annotation was the list from the table published by Vogelstein et al[9]. A 1 in this column indicates that the gene is annotated as a tumor suppressor gene.

[00456] The term "hallmark_tsg_on_chr_mutated_in_disease: A binary indicator variable for whether any of the genes identified as significantly mutated in the tumor type analyzed and on the same chromosome as the SNP are "hallmark" tumor suppressor genes. "Hallmark" tumor suppressor genes are a small list of very-well validated tumor suppressor genes that are more likely to be mutated early in tumor development. These genes were: TP53, PTEN, APC, MLL3, MLL2, VHL, CDKN2A, and RB1. A 1 in this column indicates that one of these hallmark TSGs exists on the same chromosome as the SNP in question and is significantly mutated in the tumor type analyzed.

[00457] The term "gistic_deletion_n_peaks: The number of GISTIC peaks on the chromosome on which the SNP falls. A higher number suggests (loosely) that there are more selective forces driving loss of genetic material on this chromosome.

[00458] The term "gistic_deletion_best_q_value: The lowest GISTIC q-value for genomic loss on the chromosome on which the SNP falls. A very low q-value suggests that there is a significant selective pressure to lose genomic material somewhere on the chromosome.

[00459] The term "proportion_of_patients_eligible: The estimated proportion of patients who would have i) germline heterozygosity of the SNP and ii) LOH of the SNP in tumor. The estimate of the proportion of patients with germline heterozygosity of the SNP assumes Hardy-Weinberg equilibrium, using the equation proportion heterozygote = 2pq. Where p is the global allelic fraction of the SNP and q = 1-p.

[00460] The term "proportion_of_patients_eligible_max_ethnicity_targeted: The estimated proportion of patients who would have i) germline heterozygosity of the SNP and ii) LOH of the SNP in tumor. The estimate of the proportion of patients with germline heterozygosity of the SNP assumes Hardy-Weinberg equilibrium, using the equation proportion heterozygote = 2pq. Where p is the maximum population-restricted

allelic fraction of the SNP and q = 1-p. For example, in some cases the population used might be African and in some cases it might be South Asian.

[00461] The term "cumulative_score: A score that quantifies the degree to which a SNP is a good candidate for an iCAR target. Scores range from 0 to theoretical 1. For more information on the calculation of this score, please see the section titled "Cumulative score to rank candidate SNPs."

EXAMPLE 1. ASSESSMENT OF RATE OF LOH OF HLA GENES ACROSS CANCERS

Introduction:

[00462] A therapeutic strategy is proposed to address vulnerabilities incurred by genomic loss in cancer cells. The proposed strategy uses a combination of activating-CAR T-cells (aCAR) and inhibitory-CAR T-cells (iCAR) to more safely target tumors that have lost genomic segments encoding cell-membrane proteins heterozygous for the maternal and paternal alleles (*i.e.*, with polymorphic protein coding changes).

[00463] iCARs can decrease off-tumor toxicity of CAR-T therapy without decreasing anti-tumor efficacy if the target of the iCAR is expressed only by non-tumor tissues. One such scenario in which iCAR targets are expressed only by non-tumor cells occurs when the iCAR antigen is encoded by a portion of the genome that has been deleted in tumor cells. One gene family that is highly polymorphic and known to be expressed on all cells is HLA.

[00464] The HLA proteins are nearly universally expressed by mammalian cells to allow for the presentation of non-self antigens to cells of the immune system. HLA genes also tend to be quantitatively highly expressed, making them more amenable to therapeutic targeting. The RNA expression of the HLA genes is higher than 99.3 percent of other protein coding genes in the genome (Figure 4). The mean tissue expression of HLA genes and their genomic locations is included in Table 3 as well as the lengthy table provided herewith on CD-ROM.

[00465] The goal of this section is to identify cancer types in which the HLA gene undergoes frequent deletion. Secondary analyses include attempts to identify drivers of genomic loss at the HLA locus.

[00466] We executed a detailed plan for identifying cancers with selective pressures that drove frequent copy-loss of HLA genes (Figure 5).

Frequency of HLA loss across tumor types using ABSOLUTE data:

[00467] We used copy number profiles from the TCGA that had been processed by the ABSOLUTE algorithm to assess ground-truth estimates of the rate of allelic loss of HLA-A. Publicly available ABSOLUTE segmented copy-number data were downloaded from (https://www.synapse.org/#!Synapse:syn1710464.2)[1]. The ABSOLUTE algorithm outputs the integer copy level of each allelic segment within a single cancer genome. In the case of loss of a single copy of chromosome 6 (harboring the HLA locus), then the allelic copy numbers would be: 1 for the retained segment and 0 for the segment that was lost. In the case of copy-neutral loss of heterozygosity, then the retained segment would have copy number 2 and the lost segment would have copy number 0. Publically available copy number data processed by ABSOLUTE were available for 12 tumor types (Table 4). Lung squamous cell carcinoma (LUSC) had the highest frequency of HLA-A LOH compared to the other tumor types (Figure 6). Uterine/endometrial cancers (UCEC) had the lowest frequency of HLA-A LOH of all the evaluable tumors (AML samples were not included due to ABSOLUTE data not being available). Of 588 deletions of the HLA-A gene, none had an intragenic breakpoint (Figure 7). Most deletions of HLA-A genes encompassed large portions of the chromosome (Figure 8). While ABSOLUTE copy number data were not available for AML samples, manual inspection of the relative copy number data in these samples revealed no deletions (Figure 11).

Validation of Relative Copy Number Data Compared to ABSOLUTE Copy Number Data:

[00468] We sought to obtain the frequency of LOH of as many tumor types as were publicly available. However, these data had not been processed by ABSOLUTE and the raw data to process by ABSOLUTE are not publicly available. Instead, we used relative copy number data on 32 tumor types from TCGA (Figure 13). These data were downloaded from cbioportal (cbioportal.org/data_sets.jsp). The relative copy number data were obtained from Affymetrix SNP 6.0 arrays of tumor samples.

[00469] In order to determine whether accurate estimates of LOH could be obtained from relative copy number data, we computed the rate of LOH with relative data for the

tumors that had already had LOH data from ABSOLUTE. These data consisted of a segmented copy number file. Each segment is assigned a relative copy-ratio. The copy ratio is defined as the log of the ratio of density of signal in tumor compared to the matched normal (in Affymetrix arrays). The normalization to a matched control (usually from peripheral blood) helps to remove any germline copy-number variants from mistakenly being interpreted as somatic. A segment is said to have undergone genomic loss if the relative copy number of that genomic segment is below a given threshold. For example, if the relative copy number of segment 321 is -0.4 and the threshold for copy-loss is -0.3, then segment 321 is said to have undergone copy-loss and because we lack direct allelic information, it is said to have undergone LOH as well.

[00470] We first attempted to determine the optimal copy number cutoff for labeling relative copy number segments as having undergone LOH. The concordance of ABSOLUTE and relative copy number estimates of LOH was highest with a cutoff of -0.1 for relative copy number (Table 5 and Figure 9). This threshold also happens to be the threshold used by the TCGA copy number group to define copy-loss in the TCGA Tumorscape portal (http://portals.broadinstitute.org/tcga/home). The correlation between the fraction of individuals with HLA-A LOH in relative data vs ABOSLUTE data was 0.55. This reasonably high correlation enabled us to move forward with the analysis of all tumor types with relative copy number data available.

Fraction of patients with HLA-LOH across 32 tumor types using relative copy number data

[00471] The portion of patients that had LOH of HLA-A was computed for all 32 tumors available from TCGA (Figure 10A; COAD and READ were analyzed together). The tumor with the highest rate of HLA-A LOH was kidney chromophobe cancer. The tumor with the lowest rate of HLA-A LOH was uveal melanoma (Table 6). To ensure that the rate of LOH we had derived in these analyses was robust to small perturbation of genomic position, we analyzed the rate of LOH of the upstream and downstream genes of HLA-A to see if their rate of HLA-LOH was similar to HLA-A. As expected, the rate of LOH of the upstream and downstream genes, HLA-G and ZNRD1 respectively was exactly the same as for HLA-A. (Figure 3 A-C). These data demonstrate that the HLA-A LOH calls are robust to small deviations in genomic position. Next, we sought to determine whether the other HLA genes (A, B, C) had

similar rates of LOH compared to HLA-A. These genes all fall within a 1.3Mb region on chromosome 6p. In genomic distance, this is a small region. We repeated the HLA-A analysis on HLA-B and HLA-C. The pattern of LOH was nearly identical between all three HLA genes across the 32 tumors analyzed (Figure 10A-C).

Addition of Selection Pressure to HLA-A LOH rates

[00472] Intratumoral genomic heterogeneity is a recently appreciated feature of nearly all human cancers analyzed to date[2, 3]. Therapies targeted to genetic alterations only present in a fraction of tumor cells may only affect the tumor cells harboring said alterations. An iCAR strategy that targets antigens not present on tumor cells may protect some tumor cells from aCAR attack if the antigen is not clonally deleted. We therefore sought to identify tumors in which HLA genes were likely to undergo clonal LOH. LOH that occurs early in evolution is likely to be driven by selective forces in tumor initiation and/or maintenance. We therefore looked for tumor suppressors on chromosome 6 (harboring HLA locus) in three ways. First, we looked for genes that were significantly mutated on chromosome 6 in each of the tumor types assessed[4]. The spreadsheet reports the genes with significant mutation on chromosome 6 under the "chr6_mutsig_sig_genes" column.

[00473] Second, we looked for regions of significantly deleted genes, signifying likely deleted tumor suppressors. We used the results of GISTIC2.0 run on these data. The spreadsheet reports the number of GISTIC deletion peaks on chromosome 6 ($q<0.25$) and the lowest q-value of these deletion peaks. Generally, the more GISTIC deletion peaks and the lower the q-value, the stronger the selection pressure. However, it is also possible to have the scenario where one very strong GISTIC peak predominates and the number of peaks would be small, but the significance of the driver is for certain. In general, the lowest q-value should be the strongest correlate of tumor suppressor driver presence on a given chromosome.

[00474] Third, we overlapped the set of genes that were significantly mutated in each tumor with a list of known tumor suppressor genes to determine if any of the mutated genes was likely to drive loss of chromosome 6[5]. We were able to identify two tumor types with possible mutational drivers. In adrenocortical carcinoma, the DAXX gene was significantly mutated ($q=.0571$) and in Diffuse Large B Cell Lymphoma, the TNFAIP3 gene was significantly mutated ($q=0.00278$). DAXX encodes a histone

chaperone, mutations of which are associated with longer telomeres in adrenocortical carcinoma[6]. TNFAIP3 encodes a negative regulator of NF-kappaB signaling. Mutations of this gene occurring in DLBCL have been shown to therefore increase NF-kappaB signaling[7].

Table 3. Genomic loci analyzed for LOH. Genomic coordinates are in the hg19 human genome assembly.

Gene	Protein	Chromosome	Start Position	End Position	RNA Expression (RPKM)
HLA-A	HLA-A	6	29941260	29945884	226.6
HLA-B	HLA-B	6	31353872	31357188	422.4
HLA-C	HLA-C	6	31268749	31272130	193.4

Table 4. Tumor types with ABSOLUTE data

Disease Name	TCGA Abbreviation	Number of Samples	Number of Samples Finished ABSOLUTE
Bladder urothelial carcinoma	BLCA	138	90
Breast invasive carcinoma	BRCA	880	750
Colon adenocarcinoma	COAD	422	349
Glioblastoma multiforme	GBM	580	485
Head and Neck squamous cell carcinoma	HNSC	310	270
Kidney renal clear cell carcinoma	KIRC	497	373
Acute Myeloid Leukemia	LAML	200	0
Lung adenocarcinoma	LUAD	357	292
Lung squamous cell carcinoma	LUSC	344	261
Ovarian serous cystadenocarcinoma	OV	567	457
Rectum adenocarcinoma	READ	164	147

Uterine corpus endometrial carcinoma	UCEC	498	378

Table 5. Correlation (Pearson) of LOH rate by relative copy number data vs ABSOLUTE copy number data. Correlation peaks for a threshold value of -0.1.

Deletion threshold	Correlation (r^2)
0	0.01
-0.05	0.49
-0.1	0.55
-0.15	0.53
-0.2	0.46
-0.25	0.44
-0.3	0.21
-0.35	0.10
-0.4	0.07
-0.45	0.09
-0.5	0.08

Table 6. Numbers and rates of LOH for all 32 cancers in the TCGA dataset.

TCGA Abbreviation	Total samples (n)	Number with LOH (n)	Fraction with LOH
KICH	66	57	0.863636364
ACC	90	46	0.511111111
PAAD	184	51	0.277173913
KIRP	288	73	0.253472222
LUSC	501	124	0.24750499
SARC	257	63	0.245136187
ESCA	184	45	0.244565217
KIRC	528	98	0.185606061
BLCA	408	73	0.178921569
OV	579	96	0.165803109
THYM	123	20	0.162601626
HNSC	522	81	0.155172414
CESC	295	45	0.152542373
STAD	441	66	0.149659864
BRCA	1080	159	0.147222222
DLBC	48	7	0.145833333
LUAD	516	65	0.125968992
COADREAD	616	77	0.125
GBM	577	72	0.124783362
TGCT	150	18	0.12
CHOL	36	4	0.111111111
MESO	87	9	0.103448276
UCS	56	5	0.089285714
UCEC	539	31	0.057513915
LGG	513	24	0.046783626

PRAD	492	19	0.038617886
SKCM	104	4	0.038461538
LIHC	370	14	0.037837838
PCPG	162	3	0.018518519
THCA	499	9	0.018036072
UVM	80	0	0
LAML	0	0	NA

[00475] Based on the above, we concluded that HLA region LOH is a common event in many tumors, however and the percentage of LOH varies between tumor types. Therefore, HLA genes are good candidates for iCAR targets.

References for Example 1:

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EXAMPLE 2. GENOME-WIDE IDENTIFICATION OF GERMLINE ALLELES THAT ENCODE EXPRESSED CELL-SURFACE PROTEINS THAT UNDERGO LOSS OF HETEROZYGOSITY

Introduction:

[00476] Inhibitory-CAR-T cells can decrease off-tumor toxicity of CAR-T therapy without decreasing anti-tumor efficacy if the target of the iCAR is expressed only by non-tumor tissues. One such scenario in which iCAR targets will be expressed only by non-tumor cells is where the iCAR antigen is encoded by a portion of the genome that has been deleted in tumor cells. The goal of this section of the workflow is to identify such alleles.

Allele identification:

[00477] We used the Exome Aggregation Consortium (ExAC) database as an input to the analysis (exac.broadinstitute.org). The ExAC database is a compilation of exomes from various population-level sequencing studies totaling 60,706 exomes[1]. ExAC contains information about each variant including the number of counts of the reference allele compared to the alternative allele (allelic frequency). The allelic frequency information is extended to subpopulations within the database as detailed in Table 7.

Table 7. Subpopulations within the ExAC database. Note: Not all positions in genome have sufficient coverage in exome such that all individuals in this table are represented. Source: http://exac.broadinstitute.org/faq.

Population ancestry	Population Abbreviation	Number of Individuals
African	AFR	5,203
Latino	AMR	5,789
East Asian	EAS	4,327
Finnish	FIN	3,307
Non-Finnish European	NFE	33,370
South Asian	SAS	8,256
Other	OTH	454

[00478] The following filters were applied to variants from the ExAC database: i) the variant must affect the amino acid composition of the encoded protein ii) the variant must have a minor allele frequency of greater than 0.05 (5%) in at least one of the populations in Table 6. The analysis corrected for scenarios where the minor allele had an allele fraction greater than 0.5 (50%). If more than three alleles at a site were observed, then the most prevalent substitution was used (these sites are often sites of sequencing error and should be interpreted with caution).

[00479] A SNP was counted as having an impact on the composition of the protein if any of the SNP produced any of the following variant classes: 'missense_variant', 'inframe_deletion', 'start_lost','stop_gained', 'inframe_insertion', 'stop_retained_variant', 'frameshift_variant', 'stop_lost', 'coding_sequence_variant', 'protein_altering_variant'.

The analysis started with 9,362,319 variants and 29,904 variants passed these two filters. These variants fell in 10,302 genes. All alleles matching these two filters were included in the analysis.

Identification of expressed genes:

[00480] We used the Genotype-Tissue Expression (GTEX) database v6p (dbGaP Accession phs000424.v6.p1) for the identification of genes that are expressed in various tissue types (https://gtexportal.org/home/)[2]. The GTEX database consists of RNA-sequencing of 8,555 human samples from diverse healthy tissue types. Several annotations were obtained from this database. First, we determined the average expression of each gene across all tissues. The mean expression for each gene was calculated by taking the per-tissue median expression data and computing the mean of these values across tissues. These data were obtained from the file GTEx_Analysis_v6p_RNA-seq_RNA-SeQCv1.1.8_gene_median_rpkm.gct from available at https://gtexportal.org/home/datasets.

[00481] The mean expression of each gene corresponding to each tumor type was also included. To obtain these data we created a mapping of tumor types to corresponding normal tissues. For example, the pancreatic cancer TCGA data would be annotated with pancreas tissue from GTEX. In some cases, the mapping was more approximate. For example, the glioblastomas expression data were mapped from all tissues annotated as brain in GTEX. A table with these mappings (titled tcga_disease_tissue_lookup.txt) is attached Several measures were computed to assess the homogeneity or overexpression

of each gene in each tissue/tumor type. For each tumor type, a cohen's-D score was computed to establish possible over-expression of the gene. Genes overexpressed in particular tissues, are likely to be good aCAR targets. Conversely, we measured the standard deviation of gene expression across tissues and compared this to the mean expression across all tissues. When this ratio was low, the gene is evenly expressed across all tissues. Genes with even expression across all tissues are likely to be better iCAR targets.

[00482] A gene was called "universally expressed" if it met the following criteria: (i) the mean express across tissues was greater than 10 RPKM. (ii) The tissues with the least expression had an RPKM greater than 1. (iii) The ratio of the standard deviation in median RPKM across tissues compared to the mean RPKM was less than 1. Only 1,092 genes were annotated as universally expressed.

[00483] Candidates were selected only based on the UniProt annotation. For transmembrane proteins, there is usually clear prediction for segments of the protein that are extracellular.

[00484] **Table 8** presents a list of 1167 good candidate genes identified by the above method having extracellular polymorphic epitopes sorted according to chromosome location.

Annotation of alleles

Impact of allele on protein function:

[00485] For an iCAR to effectively recognize only cancer cells that have lost one allele of a membrane protein, the protein's structure out to be sufficiently different based on which allele is encoded. Several measures were taken to quantify the effect of each SNP on the resulting protein. First, the reported SNP variant class (e.g. missense, nonsense) was reported in the column 'consequence'. The effect on the consensus protein translation was included in the 'protein_consequence' (e.g., p.Arg482Gln) column. The SIFT algorithm attempts to predict whether a protein variant will have an effect on the protein structure, and therefore function[6]. The score can range from 0 (deleterious) to 1 (benign). SIFT scores (version sift5.2.2) were included for every SNP for which a score was available. Scores are not available for frameshift mutations for example. PolyPhen

(v2.2.2) was also used to make prediction on the possibility that a variant may affect protein structure and function. The Polyphen algorithm reports scores in the opposite manner of SIFT, with a score of 0 corresponding to benign and a score of 1 corresponding to deleterious.

[00486] One classic measure of an amino acids substitution probability of inducing a structural change is to use the BLOSUM62 substitution matrix. We downloaded the BLOSUM62 matrix from https://www.ncbi.nlm.nih.gov/IEB/ToolBox/C_DOC/lxr/source/data/BLOSUM62. Each SNP was annotated with the BLOSUM62 score corresponding to its substitution.

Classification of allele as falling in the extracellular portion of the protein:

[00487] For an iCAR to recognize an allele, the allele must fall on the extracellular portion of the protein. For each SNP, we extracted the position of the amino acid affected in the consensus translation and compared this to domains annotated as extracellular from the Uniprot database. The Uniprot database was downloaded from www.uniprot.org/downloads. Many false negatives are possible due to a lack of characterization of the domains of all proteins. A total of 3288 SNPs in 1167 genes were annotated as extracellular (Table 8).

Annotations of Peptide Context of SNP:

[00488] The peptide context of the alleles analyzed will likely matter when trying to generate antibodies that recognize these sequences. We include for reference the 10 amino acids preceding and flanking the amino acid encoded by the SNP (21 amino acid sequence total). The uniprot database was used for the consensus amino acid sequence. We annotate any conflicts where the uniprot database sequence did not match the amino acid encoded by either SNP at the predicted position, so as not to include any false sequences. These 21 amino acid sequences could be useful as input to B-cell epitope prediction programs such as Bepipred.

Cancer-specific annotations:

Proportion of tumors undergoing LOH

[00489] Finding patients whose tumors could benefit from the proposed therapy would require an iCAR target would be a SNP that undergoes loss of heterozygosity (LOH) in a large fraction of tumors. Segments copy number files were downloaded from the cbio cancer genomics portal http://www.cbioportal.org/ [8]. As an example, the proportion of

uveal melanoma tumors undergoing LOH for all SNPs is shown in Figure 12.

Potential driver alterations on chromosomes harboring candidate SNPs

[00490] One possible mechanism of resistance genomically targeted therapy is if one of the intended genomic alterations in only present in a fraction of the cancer cells. One mechanism to attempt to identify targets likely to be present in the earliest stages of tumor development is to identify driver events for each tumor. The most frequent mechanism of tumor suppressor gene inactivation is mutation and subsequent LOH of the non-mutated chromosome. We attempted to find driver genes, particularly tumor suppressor genes (TSGs) likely to undergo this process in each tumor type. We used the results of MUTSIG 2.0 run on all tumors in this analysis to identify genes significantly mutated in each tumor type. We annotated whether or not one of the genes that was significantly mutated was included in a list of "hallmark" tumor suppressor genes including TP53, PTEN, APC, MLL3, MLL2, VHL, CDKN2A, RB1. Finally, the list of driver genes, TSG, and "hallmark" TSGs were annotated onto a SNP if they fell on the same chromosome as the SNP.

[00491] While mutations in driver genes that subsequently undergo LOH is one mechanism that may mark events likely to occur early in tumor evolution, focal deletion of genomic segments containing a tumor suppressor gene is another. We used the GISTIC algorithm to identify regions of DNA that undergo genomic deletion at a rate higher than average. The GISTIC algorithm identifies "peaks" of statistical significance along chromosome arms that suggest a negative selective pressure on these regions. For each SNP, we recorded the number of deletion peaks on the chromosome that the SNP fell on. We also recorded the lowest q-value of any of these peaks. A lower q-value suggests stronger selective pressure.

Cumulative score to rank candidate SNPs:

[00492] In an effort to provide a continuous "score" for the candidate SNPs, we combined several different metrics that should be associated with better SNP candidates. The score consists of the product of the percentile rank of each of the following:

1. proportion of tumors with LOH at that SNP (higher is better) 2. prevalence of the allele (higher is better) 3. ratio of the standard deviation of expression values across

tissues to the median (lower is better, more consistent) 4. whether or not there is a tumor suppressor gene on the chromosome (having one is better than not having one)

[00493] To illustrate, we will calculate the score for a theoretical SNP. If only 32% of the SNPs had a tumor suppressor gene on the chromosome, then the percentile rank for having one would be 0.68. If the allele had a minor allele fraction of 0.49 (where 0.5 is the highest possible), then the percentile rank would be 0.99. If the rate of LOH was 0.10, and 75% of SNPs had more LOH than that, then the percentile rank would be 0.25. If the ratio of standard deviation of expression values across tissues to the median for the gene harboring this SNP was 1.3 and that is better than 90% of other genes, then the percentile rank is 0.9. The total score for this SNP would then be 0.68*0.99*0.25*0.9=0.15.

[00494] Any SNP with a score greater than 0.4 was considered "top-hit".

Table 8. Exemplary iCAR Targets

Chr. No.	Gene
1	ABCA4
1	ADAM30
1	ASTN1
1	C1orf101
1	CACNA1S
1	CATSPER4
1	CD101
1	CD164L2
1	CD1A
1	CD1C
1	CD244
1	CD34
1	CELSR2
1	CHRNB2
1	CLCA2
1	CLSTN1
1	CR1
1	CR2
1	CRB1
1	CSF3R
1	CSMD2
1	ECE1
1	ELTD1

1 EMC1
1 EPHA10
1 EPHA2
1 ERMAP
1 FCAMR
1 FCER1A
1 FCGR1B
1 FCGR2A
1 FCGR2B
1 FCGR3A
1 FCRL1
1 FCRL3
1 FCRL4
1 FCRL5
1 FCRL6
1 GJB4
1 GPA33
1 GPR157
1 GPR37L1
1 GPR88
1 HCRTR1
1 IGSF3
1 IGSF9
1 IL22RA1
1 ITGA10
1 KIAA1324
1 KIAA2013
1 LDLRAD2
1 LEPR
1 LRIG2
1 LRP8
1 LRRC52
1 LRRC8B
1 LRRN2
1 LY9
1 MR1
1 MUC1
1 MXRA8
1 NCSTN
1 NFASC
1 NOTCH2
1 NPR1
1 NTRK1
1 OPN3
1 OR10J1
1 OR10J4

1 OR10K1
1 OR10R2
1 OR10T2
1 OR10X1
1 OR11L1
1 OR14A16
1 OR14I1
1 OR14K1
1 OR2AK2
1 OR2C3
1 OR2G2
1 OR2G3
1 OR2L2
1 OR2M7
1 OR2T1
1 OR2T12
1 OR2T27
1 OR2T29
1 OR2T3
1 OR2T33
1 OR2T34
1 OR2T35
1 OR2T4
1 OR2T5
1 OR2T6
1 OR2T7
1 OR2T8
1 OR2W3
1 OR6F1
1 OR6K2
1 OR6K3
1 OR6K6
1 OR6N1
1 OR6P1
1 OR6Y1
1 PEAR1
1 PIGR
1 PLXNA2
1 PTCH2
1 PTCHD2
1 PTGFRN
1 PTPRC
1 PTPRF
1 PVRL4
1 RXFP4
1 S1PR1

1	SCNN1D
1	SDC3
1	SELE
1	SELL
1	SELP
1	SEMA4A
1	SEMA6C
1	SLAMF7
1	SLAMF9
1	SLC2A7
1	SLC5A9
1	TACSTD2
1	TAS1R2
1	TIE1
1	TLR5
1	TMEM81
1	TNFRSF14
1	TNFRSF1B
1	TRABD2B
1	USH2A
1	VCAM1
1	ZP4
2	ABCG5
2	ALK
2	ASPRV1
2	ATRAID
2	CD207
2	CHRNG
2	CLEC4F
2	CNTNAP5
2	CRIM1
2	CXCR1
2	DNER
2	DPP10
2	EDAR
2	EPCAM
2	GPR113
2	GPR148
2	GPR35
2	GPR39
2	IL1RL1
2	ITGA4
2	ITGA6
2	ITGAV
2	LCT
2	LHCGR

2 LRP1B
2 LRP2
2 LY75
2 MARCO
2 MERTK
2 NRP2
2 OR6B2
2 PLA2R1
2 PLB1
2 PROKR1
2 PROM2
2 SCN7A
2 SDC1
2 TGOLN2
2 THSD7B
2 TMEFF2
2 TMEM178A
2 TPO
2 TRABD2A
3 ACKR2
3 ALCAM
3 ANO10
3 ATP13A4
3 CACNA1D
3 CACNA2D2
3 CACNA2D3
3 CASR
3 CCRL2
3 CD200
3 CD200R1
3 CD86
3 CD96
3 CDCP1
3 CDHR4
3 CELSR3
3 CHL1
3 CLDN11
3 CLDN18
3 CLSTN2
3 CSPG5
3 CX3CR1
3 CXCR6
3 DCBLD2
3 DRD3
3 EPHB3
3 GABRR3

3 GP5
3 GPR128
3 GPR15
3 GPR27
3 GRM2
3 GRM7
3 HEG1
3 HTR3C
3 HTR3D
3 HTR3E
3 IGSF11
3 IL17RC
3 IL17RD
3 IL17RE
3 IL5RA
3 IMPG2
3 ITGA9
3 ITGB5
3 KCNMB3
3 LRIG1
3 LRRC15
3 LRRN1
3 MST1R
3 NAALADL2
3 NRROS
3 OR5AC1
3 OR5H1
3 OR5H14
3 OR5H15
3 OR5H6
3 OR5K2
3 OR5K3
3 OR5K4
3 PLXNB1
3 PLXND1
3 PRRT3
3 PTPRG
3 ROBO2
3 RYK
3 SEMA5B
3 SIDT1
3 SLC22A14
3 SLC33A1
3 SLC4A7
3 SLITRK3
3 STAB1

3	SUSD5
3	TFRC
3	TLR9
3	TMEM44
3	TMPRSS7
3	TNFSF10
3	UPK1B
3	VIPR1
3	ZPLD1
4	ANTXR2
4	BTC
4	CNGA1
4	CORIN
4	EGF
4	EMCN
4	ENPEP
4	EPHA5
4	ERVMER34-1
4	EVC2
4	FAT1
4	FAT4
4	FGFRL1
4	FRAS1
4	GPR125
4	GRID2
4	GYPA
4	GYPB
4	KDR
4	KIAA0922
4	KLB
4	MFSD8
4	PARM1
4	PDGFRA
4	RNF150
4	TENM3
4	TLR1
4	TLR10
4	TLR6
4	TMEM156
4	TMPRSS11A
4	TMPRSS11B
4	TMPRSS11E
4	TMPRSS11F
4	UNC5C
5	ADAM19
5	ADRB2

5 BTNL3
5 BTNL8
5 BTNL9
5 C5orf15
5 CATSPER3
5 CD180
5 CDH12
5 CDHR2
5 COL23A1
5 CSF1R
5 F2RL2
5 FAM174A
5 FAT2
5 FGFR4
5 FLT4
5 GABRA6
5 GABRG2
5 GPR151
5 GPR98
5 GRM6
5 HAVCR1
5 HAVCR2
5 IL31RA
5 IL6ST
5 IL7R
5 ITGA1
5 ITGA2
5 KCNMB1
5 LIFR
5 LNPEP
5 MEGF10
5 NIPAL4
5 OR2V1
5 OR2Y1
5 OSMR
5 PCDH1
5 PCDH12
5 PCDHA1
5 PCDHA2
5 PCDHA4
5 PCDHA8
5 PCDHA9
5 PCDHB10
5 PCDHB11
5 PCDHB13
5 PCDHB14

5 PCDHB15
5 PCDHB16
5 PCDHB2
5 PCDHB3
5 PCDHB4
5 PCDHB5
5 PCDHB6
5 PCDHGA1
5 PCDHGA4
5 PDGFRB
5 PRLR
5 SEMA5A
5 SEMA6A
5 SGCD
5 SLC1A3
5 SLC22A4
5 SLC22A5
5 SLC36A3
5 SLC6A18
5 SLC6A19
5 SLCO6A1
5 SV2C
5 TENM2
5 TIMD4
5 UGT3A1
6 BAI3
6 BTN1A1
6 BTN2A1
6 BTN2A2
6 BTN3A2
6 BTNL2
6 CD83
6 DCBLD1
6 DLL1
6 DPCR1
6 ENPP1
6 ENPP3
6 ENPP4
6 EPHA7
6 GABBR1
6 GABRR1
6 GCNT6
6 GFRAL
6 GJB7
6 GLP1R
6 GPR110

6 GPR111
6 GPR116
6 GPR126
6 GPR63
6 GPRC6A
6 HFE
6 HLA-A
6 HLA-B
6 HLA-C
6 HLA-DPA1
6 HLA-DPB1
6 HLA-DQA1
6 HLA-DQA2
6 HLA-DQB1
6 HLA-DQB2
6 HLA-DRB1
6 HLA-DRB5
6 HLA-E
6 HLA-F
6 HLA-G
6 IL20RA
6 ITPR3
6 KIAA0319
6 LMBRD1
6 LRFN2
6 LRP11
6 MAS1L
6 MEP1A
6 MICA
6 MICB
6 MUC21
6 MUC22
6 NCR2
6 NOTCH4
6 OPRM1
6 OR10C1
6 OR12D2
6 OR12D3
6 OR14J1
6 OR2B2
6 OR2B6
6 OR2J1
6 OR2W1
6 OR5V1
6 PKHD1
6 PTCRA

6 RAET1E
6 RAET1G
6 ROS1
6 SDIM1
6 SLC22A1
6 SLC44A4
6 TAAR2
6 TREM1
6 TREML1
6 TREML2
7 AQP1
7 CD36
7 CDHR3
7 CNTNAP2
7 DPP6
7 EGFR
7 EPHA1
7 EPHB6
7 ERVW-1
7 GHRHR
7 GJC3
7 GPNMB
7 GRM8
7 HYAL4
7 KIAA1324L
7 LRRN3
7 MET
7 MUC12
7 MUC17
7 NPC1L1
7 NPSR1
7 OR2A12
7 OR2A14
7 OR2A2
7 OR2A25
7 OR2A42
7 OR2A7
7 OR2AE1
7 OR2F2
7 OR6V1
7 PILRA
7 PKD1L1
7 PLXNA4
7 PODXL
7 PTPRN2
7 PTPRZ1

7 RAMP3
7 SLC29A4
7 SMO
7 TAS2R16
7 TAS2R4
7 TAS2R40
7 TFR2
7 THSD7A
7 TMEM213
7 TTYH3
7 ZAN
7 ZP3
8 ADAM18
8 ADAM28
8 ADAM32
8 ADAM7
8 ADAM9
8 CDH17
8 CHRNA2
8 CSMD1
8 CSMD3
8 DCSTAMP
8 FZD6
8 GPR124
8 NRG1
8 OR4F21
8 PKHD1L1
8 PRSS55
8 SCARA3
8 SCARA5
8 SDC2
8 SLC10A5
8 SLC39A14
8 SLC39A4
8 SLCO5A1
8 TNFRSF10A
8 TNFRSF10B
9 ABCA1
9 AQP7
9 C9orf135
9 CA9
9 CD72
9 CNTNAP3
9 CNTNAP3B
9 ENTPD8
9 GPR144

9	GRIN3A
9	IZUMO3
9	KIAA1161
9	MAMDC4
9	MEGF9
9	MUSK
9	NOTCH1
9	OR13C2
9	OR13C3
9	OR13C5
9	OR13C8
9	OR13C9
9	OR13D1
9	OR13F1
9	OR1B1
9	OR1J2
9	OR1K1
9	OR1L1
9	OR1L3
9	OR1L6
9	OR1L8
9	OR1N1
9	OR1N2
9	OR1Q1
9	OR2S2
9	PCSK5
9	PLGRKT
9	PTPRD
9	ROR2
9	SEMA4D
9	SLC31A1
9	TEK
9	TLR4
9	TMEM2
9	VLDLR
10	ABCC2
10	ADAM8
10	ADRB1
10	ANTXRL
10	ATRNL1
10	C10orf54
10	CDH23
10	CDHR1
10	CNNM2
10	COL13A1
10	COL17A1

10 ENTPD1
10 FGFR2
10 FZD8
10 GPR158
10 GRID1
10 IL15RA
10 IL2RA
10 ITGA8
10 ITGB1
10 MRC1
10 NPFFR1
10 NRP1
10 OPN4
10 PCDH15
10 PKD2L1
10 PLXDC2
10 PRLHR
10 RGR
10 SLC29A3
10 SLC39A12
10 TACR2
10 TCTN3
10 TSPAN15
10 UNC5B
10 VSTM4
11 AMICA1
11 ANO3
11 APLP2
11 C11orf24
11 CCKBR
11 CD248
11 CD44
11 CD5
11 CD6
11 CDON
11 CLMP
11 CRTAM
11 DCHS1
11 DSCAML1
11 FAT3
11 FOLH1
11 GDPD4
11 GDPD5
11 GRIK4
11 HEPHL1
11 HTR3B

11 IFITM10
11 IL10RA
11 KIRREL3
11 LGR4
11 LRP4
11 LRP5
11 LRRC32
11 MCAM
11 MFRP
11 MPEG1
11 MRGPRE
11 MRGPRF
11 MRGPRG
11 MRGPRX2
11 MRGPRX3
11 MRGPRX4
11 MS4A4A
11 MTNR1B
11 MUC15
11 NAALAD2
11 NAALADL1
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11 NRXN2
11 OR10A2
11 OR10A5
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11 OR10D3
11 OR10G4
11 OR10G7
11 OR10G8
11 OR10G9
11 OR10Q1
11 OR10S1
11 OR1S1
11 OR2AG1
11 OR2AG2
11 OR2D2
11 OR4A15
11 OR4A47
11 OR4A5
11 OR4A8P
11 OR4C11
11 OR4C13
11 OR4C15
11 OR4C16
11 OR4C3

11 OR4C46
11 OR4C5
11 OR4D6
11 OR4D9
11 OR4S2
11 OR4X1
11 OR51E1
11 OR51L1
11 OR52A1
11 OR52E1
11 OR52E2
11 OR52E4
11 OR52E6
11 OR52I1
11 OR52I2
11 OR52J3
11 OR52L1
11 OR52N1
11 OR52N2
11 OR52N4
11 OR52W1
11 OR56B1
11 OR56B4
11 OR5A1
11 OR5A2
11 OR5AK2
11 OR5AR1
11 OR5B17
11 OR5B3
11 OR5D14
11 OR5D16
11 OR5D18
11 OR5F1
11 OR5I1
11 OR5L2
11 OR5M11
11 OR5M3
11 OR5P2
11 OR5R1
11 OR5T2
11 OR5T3
11 OR5W2
11 OR6A2
11 OR6T1
11 OR6X1
11 OR8A1

11	OR8B12
11	OR8B2
11	OR8B3
11	OR8B4
11	OR8D1
11	OR8D2
11	OR8H1
11	OR8H2
11	OR8H3
11	OR8I2
11	OR8J1
11	OR8J2
11	OR8J3
11	OR8K1
11	OR8K3
11	OR8K5
11	OR8U1
11	OR9G1
11	OR9G4
11	OR9Q2
11	P2RX3
11	PTPRJ
11	ROBO3
11	SIGIRR
11	SLC22A10
11	SLC3A2
11	SLC5A12
11	SLCO2B1
11	SORL1
11	ST14
11	SYT8
11	TENM4
11	TMEM123
11	TMPRSS4
11	TMPRSS5
11	TRPM5
11	TSPAN18
11	ZP1
12	ANO4
12	AVPR1A
12	CACNA2D4
12	CD163
12	CD163L1
12	CD27
12	CD4
12	CLEC12A

12 CLEC2A
12 CLEC4C
12 CLEC7A
12 CLECL1
12 CLSTN3
12 GPR133
12 GPRC5D
12 ITGA7
12 ITGB7
12 KLRB1
12 KLRC2
12 KLRC3
12 KLRC4
12 KLRF1
12 KLRF2
12 LRP1
12 LRP6
12 MANSC1
12 MANSC4
12 OLR1
12 OR10AD1
12 OR10P1
12 OR2AP1
12 OR6C1
12 OR6C2
12 OR6C3
12 OR6C4
12 OR6C6
12 OR6C74
12 OR6C76
12 OR8S1
12 OR9K2
12 ORAI1
12 P2RX4
12 P2RX7
12 PTPRB
12 PTPRQ
12 SCNN1A
12 SELPLG
12 SLC38A4
12 SLC5A8
12 SLC6A15
12 SLC8B1
12 SLCO1B1
12 SLCO1B7
12 SSPN

12	STAB2
12	TAS2R10
12	TAS2R13
12	TAS2R20
12	TAS2R30
12	TAS2R31
12	TAS2R42
12	TAS2R43
12	TAS2R46
12	TAS2R7
12	TMEM119
12	TMEM132B
12	TMEM132C
12	TMEM132D
12	TMPRSS12
12	TNFRSF1A
12	TSPAN8
12	VSIG10
13	ATP4B
13	ATP7B
13	FLT3
13	FREM2
13	KL
13	PCDH8
13	SGCG
13	SHISA2
13	SLC15A1
13	SLITRK6
13	TNFRSF19
14	ADAM21
14	BDKRB2
14	C14orf37
14	CLEC14A
14	DLK1
14	FLRT2
14	GPR135
14	GPR137C
14	JAG2
14	LTB4R2
14	MMP14
14	OR11G2
14	OR11H12
14	OR11H6
14	OR4K1
14	OR4K15
14	OR4K5

14	OR4L1
14	OR4N2
14	OR4N5
14	OR4Q2
14	SLC24A4
14	SYNDIG1L
15	ANPEP
15	CD276
15	CHRNA7
15	CHRNB4
15	CSPG4
15	DUOX1
15	DUOX2
15	FAM174B
15	GLDN
15	IGDCC4
15	ITGA11
15	LCTL
15	LTK
15	LYSMD4
15	MEGF11
15	NRG4
15	OCA2
15	OR4F4
15	OR4M2
15	OR4N4
15	PRTG
15	RHCG
15	SCAMP5
15	SEMA4B
15	SEMA6D
15	SLC24A1
15	SLC28A1
15	TRPM1
15	TYRO3
16	ATP2C2
16	CACNA1H
16	CD19
16	CDH11
16	CDH16
16	CDH3
16	CDH5
16	CNGB1
16	CNTNAP4
16	GDPD3
16	GPR56

16 GPR97
16 IL4R
16 ITFG3
16 ITGAL
16 ITGAM
16 ITGAX
16 KCNG4
16 MMP15
16 MSLNL
16 NOMO1
16 NOMO3
16 OR2C1
16 PKD1
16 PKD1L2
16 SCNN1B
16 SEZ6L2
16 SLC22A31
16 SLC5A11
16 SLC7A6
16 SPN
16 TMC5
16 TMC7
16 TMEM204
16 TMEM219
16 TMEM8A
17 ABCC3
17 ACE
17 AOC3
17 ASGR2
17 C17orf80
17 CD300A
17 CD300C
17 CD300E
17 CD300LG
17 CHRNB1
17 CLEC10A
17 CNTNAP1
17 CPD
17 CXCL16
17 FAM171A2
17 GCGR
17 GLP2R
17 GP1BA
17 GPR142
17 GUCY2D
17 ITGA2B

17 ITGA3
17 ITGAE
17 ITGB3
17 KCNJ12
17 LRRC37A
17 LRRC37A2
17 LRRC37A3
17 LRRC37B
17 MRC2
17 NGFR
17 OR1A2
17 OR1D2
17 OR1G1
17 OR3A1
17 OR3A2
17 OR4D1
17 OR4D2
17 RNF43
17 SCN4A
17 SDK2
17 SECTM1
17 SEZ6
17 SLC26A11
17 SPACA3
17 TMEM102
17 TMEM132E
17 TNFSF12
17 TRPV3
17 TTYH2
17 TUSC5
18 APCDD1
18 CDH19
18 CDH20
18 CDH7
18 COLEC12
18 DCC
18 DSC1
18 DSG1
18 DSG3
18 DYNAP
18 MEP1B
18 PTPRM
18 SIGLEC15
18 TNFRSF11A
19 ABCA7
19 ACPT

19 BCAM
19 C19orf38
19 C19orf59
19 C5AR1
19 CATSPERD
19 CATSPERG
19 CD320
19 CD33
19 CD97
19 CEACAM1
19 CEACAM19
19 CEACAM21
19 CEACAM3
19 CEACAM4
19 CLEC4M
19 DLL3
19 EMR1
19 EMR2
19 EMR3
19 ERVV-1
19 ERVV-2
19 FAM187B
19 FCAR
19 FFAR3
19 FPR1
19 GFY
19 GP6
19 GPR42
19 GRIN3B
19 ICAM3
19 IGFLR1
19 IL12RB1
19 IL27RA
19 KIR2DL1
19 KIR2DL3
19 KIR2DL4
19 KIR3DL1
19 KIR3DL2
19 KIR3DL3
19 KIRREL2
19 KISS1R
19 LAIR1
19 LDLR
19 LILRA1
19 LILRA2
19 LILRA4

19 LILRA6
19 LILRB1
19 LILRB2
19 LILRB3
19 LILRB4
19 LILRB5
19 LINGO3
19 LPHN1
19 LRP3
19 MADCAM1
19 MAG
19 MEGF8
19 MUC16
19 NCR1
19 NOTCH3
19 NPHS1
19 OR10H1
19 OR10H2
19 OR10H3
19 OR10H4
19 OR1I1
19 OR2Z1
19 OR7A10
19 OR7C1
19 OR7D4
19 OR7E24
19 OR7G1
19 OR7G2
19 OR7G3
19 PLVAP
19 PTGIR
19 PTPRH
19 PTPRS
19 PVR
19 SCN1B
19 SHISA7
19 SIGLEC10
19 SIGLEC11
19 SIGLEC12
19 SIGLEC5
19 SIGLEC6
19 SIGLEC8
19 SIGLEC9
19 SLC44A2
19 SLC5A5
19 SLC7A9

19	TARM1
19	TGFBR3L
19	TMC4
19	TMEM91
19	TMPRSS9
19	TNFSF14
19	TNFSF9
19	TRPM4
19	VN1R2
19	VSIG10L
19	VSTM2B
20	ABHD12
20	ADAM33
20	ADRA1D
20	APMAP
20	ATRN
20	CD40
20	CD93
20	CDH22
20	CDH26
20	CDH4
20	FLRT3
20	GCNT7
20	GGT7
20	JAG1
20	LRRN4
20	NPBWR2
20	OCSTAMP
20	PTPRA
20	PTPRT
20	SEL1L2
20	SIGLEC1
20	SIRPA
20	SIRPB1
20	SIRPG
20	SLC24A3
20	SLC2A10
20	SSTR4
20	THBD
21	CLDN8
21	DSCAM
21	ICOSLG
21	IFNAR1
21	IFNGR2
21	IGSF5
21	ITGB2

	21	KCNJ15
	21	NCAM2
	21	TMPRSS15
	21	TMPRSS2
	21	TMPRSS3
	21	TRPM2
	21	UMODL1
	22	CACNA1I
	22	CELSR1
	22	COMT
	22	CSF2RB
	22	GGT1
	22	GGT5
	22	IL2RB
	22	KREMEN1
	22	MCHR1
	22	OR11H1
	22	P2RX6
	22	PKDREJ
	22	PLXNB2
	22	SCARF2
	22	SEZ6L
	22	SSTR3
	22	SUSD2
	22	TMPRSS6
	22	TNFRSF13C
X		ATP6AP2
X		ATP7A
X		EDA2R
X		FMR1NB
X		GLRA4
X		GPR112
X		GUCY2F
X		HEPH
X		P2RY10
X		P2RY4
X		PLXNA3
X		PLXNB3
X		VSIG4
X		XG

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6. Ng PC, Henikoff S. Sift: Predicting amino acid changes that affect protein function. *Nucleic acids research*. 2003;31:3812-3814
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EXAMPLE 3. DNA SEQUENCING ANALYSIS FOR VERIFICATION OF HLA LOH IN KICH SAMPLES

Library Preparation and Sequencing

Purpose- based on the in-silico analysis, KICH cancer was chosen as the first tumor type for wet verification of the HLA LOH prediction. The aim was to identify the HLA genotype for each pateinet based on DNA derived from normal tissue, and then to analyse the HLA allotype in the cancer tisse in an attempt to identify loss of one of the HLA alleles.

[00495] For that matter, HLA allotype was determined for DNA derived from 6 frozen matche KICH samples (Normal and Cancer) RC-001-RC003, TNEABA1l, TNEABNWE, 2rDFRAUB, 2RDFRNQG, IOWT5AVJ, IOWT5N74. In addition, two DNA matched samples OG-001-OG-002 (Normal and Cancer) were also analysed. A DNA library was prepared sequence analysis was conducted in order to identify the sample's HLA typing. DNA was extracted from 6 frozen matched KICH samples (Normal and tumor) and a library was prepared as described below.

[00496] TruSight HLA Sequencing libraries were prepared using TruSight® HLA v2 Sequencing Panel (Illumina, San Diego, California, U.S.A.) at Genotypic Technology Pvt. Ltd., Bangalore, India.

[00497] Briefly, HLA Amplicons were generated using the primers provided in the TruSight HLA Sequencing Kit. Amplicons were confirmed on Agarose Gel followed by cleanup of the amplicons using Aample Purification Beads provided in the kit. Amplicons were normalized and fragmented by Tagmentation reaction. Post Tagmentation different amplicons of each individual sample were pooled and proceeded for enrichment PCR. Barcoding of the samples was done during enrichment PCR using Nextera XT Index Kit v2 (Illumina). Final PCR product was purified using Sample Purification Beads followed by quality control check of the libraries. Libraries were quantified by Qubit fluorometer (Thermo Fisher Scientific, MA, USA) and its fragment size distribution was analyzed on Agilent Bioanalyzer.

Illumina Adapter Sequences:
5' - AATGATACGGCGACCACCGAGATCTACAC [i5] TCGTCGGCAGCGTC
5' - CAAGCAGAAGACGGCATACGAGAT [i7] GTCTCGTGGGCTCGG
[i5, i7] – Unique dual index sequence to identify sample-specific sequencing data

[00498] The table below depict the HLA genotype of the above samples.

[00499] As seen below, we can infer the lost allele from the analysis, for example, patient #RC001 exhibits loss of HLA-A30 allele in the tumor samples and becomes hemizygout to HLA-32; patient #RC003 lost HLA-1 in the tumor sample and becomes hemizygout to HLA-30. The identified lost allele will determine the relevant iCAR for each patient. Cases where tumor samples were contaminated with normal cells, could exhibit clear HLA allele loss in this method.

Table 9: HLA genotype of the matched KICH samples

Sample_ID	HLA-A	HLA-B	HLA-C
OG_001_NAT_NORMAL	02:06:01:--	7:02:01	03:04:01:--
	24:02:01:--	15:01:01:--	07:02:01:--
OG_001_TUM_TUMOR	02:06:01:--	7:02:01	03:04:01:--
	24:02:01:--	15:01:01:--	07:02:01:--
OG_002_NAT_NORMAL	02:01:01:--	15:01:01:--	3:03:01
	24:02:01:--	55:01:01	X
OG_002_TUM_TUMOR	02:01:01:--	15:01:01:--	3:03:01
	24:02:01:--	55:01:01	X

RC_002_NAT_A_NORMAL	03:01:01:--	7:02:01	06:02:01:--
	68:02:01:--	58:02:01	7:18:00
RC_002_TUM_A_TUMOR	03:01:01:--	7:02:01	06:02:01:--
	68:02:01:--	58:02:01	7:18:00
RC_003_NAT_A_NORMAL	01:01:01:01	7:02:01	07:01:01:--
	30:04:01	49:01:01	07:02:01:--
RC_003_TUM_A_TUMOR	30:04:01	7:02:01	07:01:01:--
	X	49:01:01	07:02:01:--
RC_001_NAT_B_NORMAL	30:04:01	53:01:01	04:01:01:--
	32:01:01	58:02:01	06:02:01:--
RC_001_TUM_B_TUMOR	32:01:01	53:01:01	04:01:01:--
	X	58:02:01	06:02:01:--
2RDFRAUB_ Tumor	03:01:01:--	7:02:01	07:02:01:--
	32:01:01	38:01:01	12:03:01:--
SO_7534_SET3_2RDFRNQG_ Normal	03:01:01:--	7:02:01	07:02:01:--
	32:01:01	38:01:01	12:03:01:--
IOWT5AVJ _Tumor	34:02:01	15:03:01:--	02:10:01:--
	68:01:01:--	81:01:00	8:04:01
IOWT5N74 _Normal	34:02:01	15:03:01:--	02:10:01:--
	68:01:01:--	81:01:00	8:04:01
TNEAB1L_ Tumor	02:01:01:--	8:01:01	03:04:01:--
	03:01:01:--	40:01:02	07:01:01:--
TNEABNWE_ Normal	02:01:01:--	8:01:01	03:04:01:--
	03:01:01:--	40:01:02	07:01:01:--

x- no variant reads

Exome sequencing

In addition to the HLA sequencing, we also performed exome sequencing in order to confirm HLA-LOH and to identify additional LOH events across the genome

[00500] The Illumina paired end raw reads (150X2, HiSeq) were quality checked using FastQC. Illumina raw reads were processed by Trim Galore software for adapter clipping and low quality base trimming using parameters of minimum read length 50bp and minimum base quality 30. The processed reads were aligned to the reference human genome (hg19) using Bowtie2. Then aligned .bam files for each of the samples were processed to get the final PCR duplicate removed .bam files and alignment quality was checked using Qualimap.

[00501] Variants were identified using SAMtools and BCFtools. In this case, joint genotyping is done to identify variants in each pair of samples (each normal and tumor pair). Therefore, for each pair a merged .vcf is generated. Potential variants are identified from each of these merged .vcf files using read depth threshold >20 and mapping quality >30. From each pair of the filtered merged .vcf, sample-wise .vcf files were generated. The filtered variants were further annotated for genes, protein change and the impact of the variations using Variant Studio.

[00502] The below table describes the extent of chromosome loss for the above samples. RC001, RC002 and RC003 exhibit extensive chromosome loss including chromosome 6 which codes for HLA genes, hence, for these samples, HLA can be used as iCAR target, in addition to many other targets coded on chromosomes 1, 2, 3, 4 (for RC002), 5, 6, 8 (for RC003), 9 (RC001, RC002), 10 (RC001, RC003), 11 (RC003), 13 (RC001, RC003), 14 (RC002), 17 (RC001, RC003), 19 (RC001), 21 (RC001, RC003), 22(RC001, RC002).

Table 10 chromosome loss

Chr	RC001	RC002	RC003	OG001	OG002	2RD	IOW	TNE
1	++	++	++					
2	++	+	++			+		
3	++	++				+		
4		++						
5	++	++						
6	++	+	++				+	
7								
8			++					

9	++	++					++	
10	++		++					
11			++					
12							+	
13	++		++					
14		+				+		
15						+		
16								
17	++		++					
18								
19	++							
20								
21	++		++					
22	++	++					++	

\+ LOH (Chr loss) for about 50% of the cells
++ LOH (Chr loss) for almost 100% of the cells

[00503] For RC001, Fig. 14 depict the loss of a chromosomal region adjacent to the tumor suppressor protein TP53, coded on chromosome 17. Genes coded on chromosome 17 which were identified as iCAR targets can be used to treat patient RC001.

Abbreviations: ADP, adenosine diphosphate; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; APRIL, a proliferation-inducing ligand; BAFF, B cell activation factor of the TNF family; BCMA, B cell maturation antigen; BCR, B cell receptor; BM, bone marrow; CAIX, carbonic anhydrase IX; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukemia; CNS, central nervous system; CSPG4, chondroitin sulfate proteoglycan 4; DC, dendritic cell; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EGFRvIII, variant III of the EGFR; EphA2, erythropoietin-producing hepatocellular carcinoma A2; FAP, fibroblast activation protein; FR-α, folate receptor-alpha; GBM, glioblastoma multiforme; GPI, glycophosphatidylinositol; H&N, head and neck; HL, Hodgkin's lymphoma; Ig, immunoglobulin; L1-CAM, L1 cell adhesion molecule; MM, multiple myeloma; NB, neuroblastoma; NF-KB, nuclear factor-KB; NHL, non-Hodgkin's lymphoma; NK, natural killer; NKG2D-L, NKG2D ligand; PBMC, peripheral blood mononuclear cell; PC, plasma cell; PLL, prolymphocytic leukemia; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; RCC, renal cell carcinomas; ROR1, receptor tyrosine kinase-like orphan receptor 1; TCL, T cell leukemia/lymphoma; Th2, T helper 2; TNBC, triple-negative breast cancer; TNFR, tumor necrosis factor receptor; VEGFR-2, vascular endothelial growth factor-2.

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EXAMPLE 4. VERIFICATION OF LOH AT THE PROTEIN LEVEL

[00504] LOH can be detected at the protein level by differential staining of normal vs. tumor cell samples using allele specific antibodies. For example, verification of HLA-

LOH in cancer samples, can be done using commercial HLA antibodies specific to the patient's HLA allotype. Table 11 below details an example for available allele-specific antibodies, which can be used.

[00505] Samples will be subjected to immuno-histochemistry (IHC) staining as described in the IHC protocol below.

Table 11: Allele-specific anti-HLA antibodies

Antibody	Manufacturer
Anti-human HLA-A2 APC (BB7.2)	eBiosciences
Anti-human HLA-A2 PE-cy7 (BB7.2)	eBiosciences
Anti-human HLA-A3 FITC (GAP A3)	eBiosciences
Anti-human HLA-A3 PE (GAP A3)	eBiosciences
Mouse Anti-HLA Class 1 Antigen A25, A32 Antibody	US Biological
HLA Class 1 Antigen A30, A31	MyBioSource
mouse anti-human HLA-B7-PE (BB7.1)	Millipore
HLA-A2 antibody (BB7.2)	Novus
HLA B7 antibody (BB7.1)	Novus
Mouse anti-human HLA-B27-FITC (clone HLA.ABC.m3)	Millipore

IHC protocol

Frozen tissues samples –

[00506] Frozen tissues are often fixed in a formalin-based solution, and embedded in OCT (Optimal Cutting Temperature compound), that enables cryosectioning of the sample. Tissues in OCT are kept frozen at -80oC. Frozen blocks are removed from -80oC prior to sectioning, equilibrated in cryostat chamber, and cut to thin sections (often 5-15μm thick). Sections are mounted on a histological slide. Slides can be stored at -20oC to -80oC. Prior to

IHC staining, slides are thawed at room temperature (RT) for 10-20min.

Paraffin-embedded tissues –

[00507] Tissues are embedded in a Formaldehyde Fixative Solution. Prior to addition of the paraffin wax, tissues are dehydrated by gradual immersion in increasing concentrations of ethanol (70%, 90%, 100%) and xylene for specific times and durations at RT. Then, the tissues are embedded in paraffin wax.

[00508] The paraffin-embedded tissues are cut in a microtome to a 5-15µm thick sections, floated in a 56oC water bath, and mounted on a histological slide. Slides can be kept at RT.

[00509] Prior to IHC staining, paraffin-embedded sections require a rehydration step. REHYDRATION - sections are rehydrated by immersion in xylene (2 X 10min), followed by decreasing concentrations of ethanol – 100% X2, each for 10min 95% ethanol – 5min 70% ethanol – 5min 50% ethanol – 5min Rinsing in dH2O.

Immunofluorescence detection:

[00510] Protocol:

1. Rehydrate slides in wash buffer (PBSX1) for 10min. Drain the wash buffer.
2. Perform antigen retrieval – if needed (heat-induced antigen retrieval or enzymatic retrieval).
3. For intracellular antigens, perform permeabilization – incubate the slides in 0.1% triton X-100 in PBSX1 for 10min at RT.
4. BLOCKING - Block the tissue in blocking buffer for 30 min. at RT. Blocking buffer depends on the detection method, usually 5% animal serum in PBSX1, or 1% BSA in PBSX1
5. PRIMARY ANTIBODY - Dilute primary antibody in incubation buffer (*e.g.*,1% BSA, 1% donkey serum in PBS, other incubation buffers can also be used), according to antibody manufacturer instructions. Incubate the tissue in diluted primary antibody at 4oC overnight. The primary antibody may be a monoclonal anti-HLA-A, anti-HLA-B or anti-HLA-C allele-specific antibody as detailed above.
    - If a conjugated primary antibody is used, protect from light, and proceed to step 8.
    - As a negative control, incubate the tissue with incubation buffer only, with no primary antibody.
    - Also, perform isotype matched control of the monoclonal antibody used in the experiment.
    -
6. 6. WASH - wash slides in wash buffer – 3 X 5-15 min.
7. 7. SECONDARY ANTIBODY - Dilute secondary antibody in incubation buffer according to antibody manufacturer instructions. Incubate the tissue in diluted secondary antibody for 30-60 min at RT. Protect from light.
8. 8. WASH - wash slides in wash buffer – 3 X 5-15 min.
9. 9. DAPI staining – Dilute DAPI incubation buffer (~300nM - 3µM). Add 300µl of DAPI solution to each section. Incubate at RT for 5-10min.
10. 10. WASH – wash slide once with X1 PBS.
11. 11. Mount with an antifade mounting media.
12. 12. Keep slides protected from light.
13. 13. Visualize slides using a fluorescence microscope.

Chromogenic detection:

[00511] Protocol:

1. 1. Rehydrate slides in wash buffer (PBSX1) for 10min. Drain the wash buffer.
2. 2. Perform antigen retrieval – if needed- see above.
3. 3. For HRP reagents, block endogenous peroxidase activity with 3.0% hydrogen peroxide in methanol for at least 15min.
4. 4. Wash the sections by immersing them in dH2O for 5min.
5. 5. For intracellular antigens, perform permeabilization – incubate the slides in 0.1% triton X-100 in PBSX1 for 10min at RT.
6. 6. BLOCKING - Block the tissue in blocking buffer for 30 min. at RT. Blocking buffer depends on the detection method, usually 5% animal serum in PBSX1, or 1% BSA in PBSX1.
7. 7. PRIMARY ANTIBODY - Dilute primary antibody in incubation buffer (*e.g.,*1% BSA, 1% donkey serum in PBS, other incubation buffers can also be used), according to antibody manufacturer instructions. Incubate the tissue in diluted primary antibody at 4°C overnight
8. 8. WASH - wash slides in wash buffer – 3 X 5-15 min.
9. 9. SECONDARY ANTIBODY - Incubate the tissue in HRP-conjugated secondary antibody for 30-60 min at RT.
10. 10. WASH - wash slides in wash buffer 3 X 5-15min.
11. 11. Add ABC-HRP reagent according to manufacturer guidelines. Incubate at RT for 60min.
12. 12. Prepare DAB solution (or other chromogen) according to manufacturer guidelines, and apply to tissue sections. The chromogenic reaction turns the epitope sites brown (usually few seconds - 10 minutes). Proceed to the next step when the intensity of the signal is appropriate for imaging
13. 13. WASH - wash slides in wash buffer – 3 X 5-15 min.
14. 14. Wash slides in dH2O – 2 X 5-15min.
15. 15. Nuclei staining – add Hematoxylin solution. Incubate at RT for 5min.
16. 16. Dehydrate tissue sections - 95% ethanol – 2 X 2min. 100% ethanol – 2 X 2min. Xylene – 2 X 2min.
17. 17. Mount with an antifade mounting media
18. 18. Visualize slides using a bright-field illumination

EXAMPLE 5. CAR-T DESIGN AND CONSTRUCTION

[00512] The purpose of the study is to create a synthetic receptor which will inhibit the on-target 'off-tumor' effect of CAR-T therapy. To that extent a library of CAR constructs composed of activating and inhibitory CARs was established.

[00513] The first set of constructs included an inhibitory CAR directed at HLA type I sequence (HLA-A2) and an activating CAR directed at tumor antigen (CD19). The next set of constructs to be used for the sake of proof of concept, includes activating CAR

sequences directed at CD19 and an inhibitory CAR sequences directed at CD20. Additional constructs directed at target antigens identified by future bioinformatics analysis will be constructed. Target candidates will be prioritized according to set forth criteria (exemplary criteria include but are not limited to, target expression pattern, target expression level, antigenicity and more). A CD19 aCAR, CD20 iCAR, and HLA-A2 iCAR have been constructed, as described in Figures 15 and 21.

[00514] iCAR constructs were designed and synthesized using commercial DNA synthesis. The transmembrane and intracellular domains up to the first annotated extracellular domain of PD-1 (amino acid 145-288) was fused downstream to HLA-A2 scFv (DNA sequence coding for HLA-A2, was retrieved from hybridoma BB7.2, (ATCC cat #: HB-82), producing anti HLA-A2).

[00515] Similar constructs with CTLA4 (amino acids 161-223) or with other sequences derived from additional negative immune regulators (for example 2B4, LAG-3 and BTLA-4) will be designed and their signaling sequences will be fused downstream to the HLA-A2 scFv.

[00516] For iCAR detection and sorting, a reporter gene (*e.g.*, eGFP) was integrated downstream to the iCAR sequence via IRES sequences and followed by an antibiotic resistance gene (*i.e.*, hygromycin) separated by P2A sequence, as illustrated in Figure 15.

[00517] For the aCAR construct, CD19 scFV was fused to 2nd generation CAR construct composed of CD8 hinge sequence followed by CD28 transmembrane and 41BB co-stimulation 1 and CD3ζ. Additional aCAR constructs composed of other signaling or structural element will also be designed and constructed (e.g. CD28 hinge, CD28 signaling domain or both CD28 and 41BB signaling domains). For aCAR detection and sorting, RFP a reporter gene was integrated downstream to the aCAR sequence via IRES sequences followed by antibiotic resistance gene (Puromycin resistance) separated by P2A sequence (Figure 15).

[00518] Both aCAR and iCAR sequences were cloned into lentivirus transfer vector and then used for viral particle production using HEK-293T packaging cells.

EXAMPLE 6. PRODUCTION OF EFFECTOR CELLS

[00519] To study the effect of the iCAR constructs on modulating CD19 CAR activation, recombinant Jurkat effector cells were constructed as detailed in Table 12 below. Jurkat (ATCC TIB152), a CD4+ T-cell line and Jurkat-NFAT (a Jurkat cell-line purchased from BPS Biosciences, engineered to express a firefly luciferase protein, under the control of NFAT response elements) were transduced using retronectin-coated (Takara) lentiviral vector bound plates or in the presence of polybrene. Transduced cells were further subjected to antibiotic selection to yield the cell-lines described in Table 12. Following selection, the cells were subjected to flow cytometry analysis to verify the expression of the reporter protein coded on each construct.

Table 12 - recombinant effector cell-lines

Recombinant effector cell-line	Parental Cell	Construct 1 (aCAR-RFP)	Construct 2 (iCAR-GFP)
CD19 aCAR Jurkat	Jurkat	CD19 aCAR	-
CD19aCAR/HLA-A2 iCAR Jurkat	Jurkat	CD19 aCAR	HLA-A2 iCAR
HLA-A2 iCAR Jurkat	Jurkat	-	HLA-A2 iCAR
CD19aCAR/CD20 iCAR Jurkat	Jurkat	CD19 aCAR	CD20 iCAR
CD20 iCAR Jurkat	Jurkat	-	CD20 iCAR
CD19 aCAR Jurkat-NFAT	Jurkat-NFAT	CD19 aCAR	-
CD19aCAR/HLA-A2 iCAR Jurkat-NFAT	Jurkat-NFAT	CD19 aCAR	HLA-A2 iCAR
HLA-A2 iCAR Jurkat-NFAT	Jurkat-NFAT	-	HLA-A2 iCAR
CD19aCAR/CD20 iCAR Jurkat-NFAT	Jurkat-NFAT	CD19 aCAR	CD20 iCAR
CD20 iCAR Jurkat-NFAT	Jurkat-NFAT	-	CD20 iCAR

[00520] In addition, activated T-cells, derived from peripheral blood obtained from healthy donors will be transduced with viral particles coding for aCAR, iCAR or both, at different multiplicity of infection (MOI). FACS selection based on reporter gene expression will be used for sorting and selection of cell population expressing different level of aCAR, iCAR or both.

EXAMPLE 7. PREPARATION OF TARGET CELLS

[00521] An in-vitro recombinant system was established for testing the functionality of iCAR constructs in inhibiting the activity of the aCAR towards off-target cells. For this purpose, target cells expressing the aCAR epitope, iCAR epitope or both were produced. The recombinant cells expressing the aCAR epitope represent the 'on-target' 'on-tumor' cells, while the cells expressing both aCAR and iCAR epitopes represent the 'on-target' 'off-tumor' healthy cells.

[00522] As our first iCAR/aCAR set is based on HLA-A2 and CD19 respectively, recombinant cells expressing HLA-A2 or CD19 or both were produced, by transfecting cell line (*e.g.*, Hela, ATCC CRM-CCL-2,Hela-Luciferase- GenTarget SCO32-Bsd or Raji- ATCC CCL-86) with expression vectors coding for these genes.

[00523] For detection of recombinant HLA A-2 expression, Myc tag was inserted. For the second iCAR/aCAR set comprised of CD20 iCAR/CD19 aCAR, recombinant cells expressing CD20 or CD19 or both were constructed (target cells are detailed in Table 15).

Table 13 - Target cell-lines

Set#	Parental cell	Target protein 1	Target protein 2	Purpose	Modeling

1		Raji	CD19	None	A model for cancer cells expressing endogenous CD19	On-tumor
		Raji	CD19	HLA-A2	A model for normal cells expressing endogenous CD19; recombinant HLA-A2	Off-tumor
		Thp1	None	HLA_A2	A model for normal cells expressing endogenous HLA-A2 and negative to CD19	Negative control
2		Hela	HLA-A2	None	A model for normal cells expressing endogenous HLA-A2 and negative to CD19	Negative control
		Hela	HLA-A2	CD19	A model for normal cells expressing recombinant	Off-tumor

					CD19; HLA-A2	
	4	Hela	CD19	None	A model for cancer cells expressing recombinant CD19	On-tumor
		Hela	CD19	CD20	A model for normal cells expressing recombinant CD19; CD20	Off-tumor
		Hela	CD20	None	A model for normal cells expressing endogenous CD20 and negative to CD19	Negative control
3		Hela-Luciferase	HLA-A2	None	Negative control to be used in killing assay	Negative control
		Hela-	HLA-	CD19	A model for normal cells expressing recombinant CD19; HLA-A2 (killing	Off-tumor

		Luciferase	**A2**		**assay)**	
	5	**Hela-Luciferase**	**CD19**	**None**	**A model for cancer cells expressing recombinant CD19 (killing assay)**	**On-tumor**
		Hela-Luciferase	**CD19**	**CD20**	**A model for normal cells expressing recombinant CD19; CD20 (killing assay)**	**Off-tumor**
		Hela-Luciferase	**CD20**	**None**	**Negative control (killing assay)**	**Negative control**

Assays-

[00524] iCAR's inhibitory effect will be tested both in-vitro and in-vivo.

[00525] In the in-vitro assays, we will focus on measuring cytokine secretion and cytotoxicity effects, while in-vivo, we will evaluate the iCAR inhibition and protection to 'on-target off- tumor' xenografts. We will limit T-cells lacking iCAR from contaminating the results by sorting T-cells to be iCAR/aCAR double positive using reporter genes. As a negative control for iCAR blocking activity, we may use T-cells transduced with CAR lacking the scFv domain (i.e. mock transduction).

EXAMPLE 8. IN VITRO ASSAYS

Luciferase Cytotoxic T lymphocyte (CTL) Assay

[00526] Assay will be performed using Hela-Luc recombinant target cells described above, engineered to express firefly luciferase and one or two CAR target antigens. In-vitro luciferase assay will be performed according to the Bright-Glo Luciferase assay manufacture's protocol (Promega) and bioluminescence as a readout.

[00527] T-cells (transduced with both iCAR and pCAR or iCAR and aCAR or aCAR or mock CAR) will be incubated for 24-48 hrs. with the recombinant target cells expressing HLA-A2 or CD19 or both HLA-A2 and CD19 or CD20 or both CD20 and CD19 in different effector to target ratios. Cell killing will be quantified with the Bright-Glo Luciferase system.

[00528] The 'off-tumor' cytotoxicity is optimized by sorting transduced T-cells population according to iCAR/aCAR expression level or by selecting sub population of recombinant target cells according to their CD19, HLA-A2 or CD20 expression level. To test whether iCAR transduced T-cells can discriminate between the 'on- tumor' and 'off-tumor' cells in vitro, we will test the killing effect of transduced T-cells incubated with a mix of 'on-tumor' and 'off-tumor' cells at a ratio of 1:1 and more. The 'on-tumor' recombinant cells will be distinguished from the 'off-tumor' recombinant cells by Luciferase expression (only one cell population will be engineered to express the luciferase gene at a given time). Killing will be quantified after 24-48 hrs of co-incubation.

Caspase 3 Activity Assay- Detection of CTL Induced Apoptosis by an anti- activated Caspase 3 (CASP3).

[00529] One of the pathways by which cytotoxic T-cells kill target cells is by inducing apoptosis through the Fas ligand. Sequential activation of caspases plays a significant role in the execution-phase of cell apoptosis. Cleavage of pro-caspase 3 to caspase 3 results in conformational change and expression of catalytic activity. The cleaved activated form of caspase 3 can be specifically recognized by a monoclonal antibody.

[00530] Transduced T-cells will be co-cultured for 2-4 hrs with either 'on-tumor' or 'off-tumor' recombinant cells, previously labeled with CFSE or other cell tracer dye (e.g. CellTrace Violet). Following cell permeabilization and fixation by an inside staining kit (e.g. Miltenyi or BD bioscience) activated CASP3 will be detected by specific antibody staining (BD bioscience), and apoptotic target cells will be detected and quantified by flow cytometry.

Time Lapse Microscopy CTL

[00531] Transduced T-cells will be incubated with either 'on-tumor' or 'off-tumor' cells for up to 5 days. Time lapse microscopy will be used to visualize killing. Alternatively, flow cytometry analysis using viable cell number staining and CountBright beads (Invitrogen) for determining target cells number at end-point time will be conducted.

[00532] In order to demonstrate the effectiveness of aCAR/iCAR transduced T-cells in discerning targets in vitro, each recombinant target cells ('on-tumor' or 'off-tumor') is labeled with a different reporter protein (e.g. GFP and mCherry). Transduced T-cells (Effector cells) will be co-incubated with a mix of recombinant cells expressing one or two target antigens (Target cells) at different E/T ratios. Each cell-line's fate will be followed by microscopy imaging.

Cytokine Release

[00533] Upon T-cell activation, the cells secrete cytokines which can be quantified and used for evaluating T-cell activation and inhibition. Cytokines can be detected intracellularly by flow cytometry or by measurement of the secreted proteins in the medium by ELISA or Cytometric Bead Array (CBA).

Quantitation of Secreted Cytokines by ELISA

[00534] Following co-cultivation of transduced T-cells (Jurkat, or primary T- cells) expressing iCAR or aCAR or both aCA and iCAR with modified target cells, expressing iCAR or aCAR or both aCAR and iCAR antigens on their cell surface, conditioned medium will be collected, and cytokine's concentration will be measured

by cytokine ELISA (IL-2, INFγ and or TNFα) according to the manufacture instruction (e.g. BioLegened or similar), and by Cytometric Bead Array (Miltenyi or similar).

iCAR Specific Inhibition as Measured by IL-2 ELISA

[00535] Jurkat CD19 aCAR and Jurkat CD19 aCAR/HLA-A2 iCAR effector cells were co-cultured with Raji, Raji-HLA-A2 and Thp1 target cells and the corresponding supernatants were collected for IL-2 measurement by ELISA, as illustrated in Figure 16A. Incubation of Jurkat CD19-aCAR/HLA-A2-iCAR with Raji target cells ('tumor') expressing CD19 showed IL-2 secretion, however incubation of these effector cells with Raji-HLA-A2 target cells expressing both CD19 and HLA-A2 ('off-tumor') resulted in more than 80% inhibition of IL-2 secretion. Conversely, IL-2 secretion was not affected when CD19 aCAR Jurkat cells were incubated with Raji or Raji-HLA-A2 target cells (Figure 16B). This result, together with the NFAT activation assay described below points toward the potency of the iCAR construct to specifically protect normal cells expressing an antigen not expressed on tumor cells.

Quantitation of Cytokine Release by Flow Cytometry

[00536] Transduced T-cells (Jurkat, or primary T- cells) expressing iCAR or aCAR or both aCAR and iCAR co-cultured for 6-24 hrs. with recombinant target cells, expressing iCAR or aCAR or both aCAR and iCAR target antigens on their cell surface, will be subjected to Golgi transport blocker (e.g. Brefeldin A, monensin) to enable cytokine intracellular accumulation. T-cells will then be permed and fixed by an inside staining kit (e.g. Miltenyi) and stained with anti CD3 and CD8 and for IL-2 and or INFγ and or TNFα.

Cytokines Secretion Measured by Cytometric Bead Array (CBA) Assay

[00537] Cytometric Bead Array (CBA) is used to measure a variety of soluble and intracellular proteins, including cytokines, chemokines and growth factors.

[00538] T-cells (primary T-cells or Jurkat cells) transduced with aCAR or both aCAR and iCAR constructs (Effector cells) were stimulated with modified target cells expressing both iCAR and aCAR or aCAR or iCAR target antigens on their cell surface (Figure 17A). Following several hours of co-incubation the effector cells produce and

secrete cytokines which indicate their effector state. The supernatant of the reaction was collected, and secreted IL-2 was measured and quantified by multiplex CBA assay.

[00539] As shown in the Figure 17B, a specific inhibition of IL-2 secretion was demonstrated for aCAR/iCAR transduced Jurkat T-cells co-cultured with target cells expressing both target antigens. A decrease of 86% in IL-2 secretion was demonstrated when dual CAR (aCAR/iCAR) transduced cells were co-incubated with target cells expressing both target antigens as compared to IL-2 secretion resulted from co-incubation of the same effector cells with target cells expressing only one target.

NFAT activation assay

[00540] For determination of T-cell activation as measured by NFAT activation, Jurkat-NFAT cells were transduced with different combinations of aCAR and iCAR, as detailed in Table 12. Effector Jurkat-NFAT cell-lines, expressing CD19 aCAR, HLA-A2 iCAR or both, were cocultured with target cells expressing either CD19 (Raji cells-'on-target') both CD19 and HLA-A2 (Raji-HLA-A2 'off-tumor') or HLA-A2 (Thp1 'off tumor') as described in Table 13. As a positive control, effector cells were stimulated in the presence of PMA and Ionomycin, which trigger calcium release required for NFAT signaling. Following 16 hrs. incubation at 37°C, luciferase was quantified using BPS Biosciences kit "One step luciferase assay system" according to the manufacturer's instructions. As expected, Jurkat NFAT cell-line expressing the CD19-CAR construct were specifically activated in the presence of Raji cell-line expressing CD19, while, no activation was shown when these cells were co-cultured with Thp1 cell-line which does not express CD19 (Figure 18).

[00541] The inhibitory effect of HLA-A2 iCAR on CD19 aCAR induced NFAT activation can be seen in Figure 21. Jurkat-NFAT-cell line expressing both CD19 aCAR and HLA-A2 iCAR was specifically inhibited when co-incubated with Raji-HLA-A2, expressing CD19 and HLA-A2 as compared to the activation induced by Raji cells expressing CD19 only. In contrast, Jurkat-NFAT cell-line expressing only CD19-CAR was similarly activated by both Raji and Raji-A2 cell-lines. Under these conditions, the inhibition of NFAT activation was calculated as ~30% (Figure 19).

[00542] The effect of different E/T ratios was tested. Assay was repeated several times with E/T ratios of 10:1, 5:1, 1:1. The results given in Figure 20 indicate that an increased inhibitory effect can be obtained with a higher E/T ratio. The results are

presented as a ratio of the mean luminescence value from co-culture each effector cell-line with 'off-tumor' target cells to the mean value from coculture with 'on-target' presenting cells. As shown, Jurkat-NFAT-cell line expressing both CD19 aCAR and HLA-A2 iCAR was specifically inhibited when co-incubated with Raji-HLA-A2 expressing CD19 and HLA-A2 proteins, however, no inhibition was detected when this cell-line was co-cultured with Raji cell-line expressing CD19 only. On the contrary, Jurkat-NFAT cell line expressing CD19 aCAR, was equally activated regardless of the CD19 expressing target cell line it was co-cultured with (Raji or Raji-HLA-A2).

T-cell degranulation assay as measured by CD107a staining

[00543] Degranulating of T cells can be identified by the surface expression of CD107a, a lysosomal associated membrane protein (LAMP-1). Surface expression of LAMP-1 has been shown to correlate with CD8 T cell cytotoxicity. This molecule is located on the luminal side of lysosomes. Upon activation, CD107a is transferred to the cell membrane surface of activated lymphocytes. CD107a is expressed on the cell surface transiently and is rapidly re-internalized via the endocytic pathway. Therefore, CD107a detection is maximized by antibody staining during cell stimulation and by the addition of monensin (to prevent acidification and subsequent degradation of endocytosed CD107a antibody complexes).

[00544] The transduced T cells will be incubated with the target cells for 6-24 hrs in the presence of monensin and will follow CD107a expression on the CD8 T cells by flow cytometry using conjugated antibodies against the T cell surface markers (CD3,CD8) and a conjugated antibody for CD107a.

[00545] Granulation (CD107a) as a marker for the killing potential. The most critical function of cytolytic T cells is the ability to kill target cells. Cytotoxic CD8+ T lymphocytes mediate the killing of target cells via two major pathways: perforin-granzyme–mediated activation of apoptosis and fas–fas ligand–mediated induction of apoptosis. Induction of these pathways depends on the release of cytolytic granules from the responding CD8+ T cells. Degranulation is a prerequisite to perforin-granzyme–mediated killing and is required for immediate lytic function mediated by responding antigen-specific CD8+ T cells. Cytotoxicity does not require de novo synthesis of proteins by the effector CD8+ T cell; instead, pre-formed lytic granules located within the cytoplasm are released in a polarized fashion toward the target cell.

The lytic granules are membrane-bound secretory lysosomes that contain a dense core composed of various proteins, including perforin and granzymes. The granule core is surrounded by a lipid bilayer containing numerous lysosomal-associated membrane glycoproteins (LAMPs), including CD107a (LAMP-1), CD107b (LAMP-2), and CD63 (LAMP-3). During the process of degranulation, the lytic granule membrane merges with the plasma membrane of the activated CD8+ T cell and the contents of the granule are then released into the immunological synapse between the CD8+ T cell and the target cell. As a result of this process, the granular membrane, including CD107a, CD107b, and CD63 glycoproteins therein, is incorporated into the plasma membrane of the responding CD8+ T cell. High-level expression of CD107a and b on the cell surface of activated T cells requires degranulation, because degranulation inhibitors, such as colchicine, dramatically reduce cell-surface expression of CD107a and b. Importantly, these proteins are rarely found on the surface of resting T lymphocytes. Thus, labeling responding cells with antibodies to CD107a and b and measuring their expression by flow cytometry can directly identify degranulating CD8+ T cells (Betts and Koup, 2004).

Experimental settings:

[00546] PBMC's transduced with iCAR+aCAR / aCAR constructs (Effector cells) are stimulated with either PMA+Ionomycin (Positive Control) or modified target cells that express iCAR+aCAR/ aCAR/ iCAR antigens on their cell surface. During several hours of co-incubation, the effector cells degranulate, CD107a can be detected on the cell surface. This expression is transient and the CD107a is rapidly re-internalized via the endocytic pathway. Therefore, CD107a detection is maximized by antibody staining during cell stimulation and by the addition of monensin (to prevent acidification and subsequent degradation of endocytosed CD107a antibody complexes). BFA is required for optimal cytokine expression.

EXAMPLE 9. IN VIVO MODELS

In vivo CTL assay in human xenograft mouse models

[00547] To test whether T-cells expressing both aCAR and iCAR constructs could discriminate between the target cells and 'off-target' cells within the same organism and

effectively kill the target cells while sparing the 'off-target' cells will be assessed by an in-vivo CTL assay.

[00548] Transduced T-cells with iCAR or aCAR or both iCAR and aCAR will be injected i.v. to naïve NOD/SCID/γc- or similar mice. Several hours later, target cells expressing iCAR, aCAR or both will be injected. These targets will be labeled with either CFSE / CPDE or similar cell trace dye in different concentrations (high, medium and low) which will allow further discrimination between them. 18hrs following targets injection, mice will be sacrificed, spleens will be harvested, and the elimination of the specific target will be assessed by FACS. Percentage of specific killing will be calculated according to the formula below:

$$\left\{1-\left[\left(\frac{\%pop_{high}(day1)}{\%pop_{high}(day0)}\right)\div\left(\frac{\%pop_{medium}(day1)}{\%pop_{medium}(day0)}\right)\right]\right\}\times 100$$

Tumor growth kinetics in human xenograft mouse models

[00549] NOD/SCID/γc- or similar mice will be inoculated with tumor cells. Inoculation can be i.p / i.v. or s.c. The tumor cells will express either the iCAR target, aCAR target or both. An example for one possible aCAR tumor cell line could be the CD19 positive NALM 6 (ATCC, human BALL cell line). Example of tumor cells that express both the aCAR and iCAR (*i.e.*, 'off-tumor' cells), is the NALM 6 engineered to express the iCAR epitope (for example HLA- A2) thereby representing the healthy cells. NALM 6 and NALM 6-HLA-A2 can also be engineered to express a reporter gene (e.g. firefly luciferase), for easy detection. Mice will be divided into several study groups inoculated with all possible combinations of target cells. As an example, one group will be injected with the NALM 6 cells while the other will be injected with the NALM-6 expressing the iCAR epitope. Several days later, while the tumor has already been established, mice will be infused intravenously with T-cells transduced with aCAR, or aCAR/iCAR, or iCAR. In addition, control groups of untransduced T-cells, no T-cells or T-cells transduced without a signaling domain will also be included. Mice will be monitored until tumor reaches the experimental end point i.e. the maximal allowed tumor volume. Monitoring will be by measuring tumor volume by mechanical means (caliper) and also by using in-vivo imaging systems (IVIS). On the end point day, mice will be sacrificed, tumor burden will be quantified, and infiltrating T-cell populations will be analyzed by FACS. To test whether the T-cells expressing the iCAR

construct could discriminate between the target cells and 'off-target' cells within the same organism, we will inject mice with several possible mixtures in several ratios of the 'on-tumor'/'off-tumor NALM-6 cells, followed by injection of transduced T-cells expressing either the aCAR alone or both aCAR and iCAR. Upon sacrifice of the mice the presence of the 'on-tumor' and 'off-tumor cells in the spleen and bone marrow will be analyzed by flow cytometry for the two markers, CD19 and the iCAR epitope.

Toxicity and Tumor Growth Kinetics in Transgenic Mouse Models

[00550] Transgenic mice that express the human aCAR and iCAR targets will also be used to determine the efficacy of the transduced T-cells. Under these settings the mice have a fully functional immune system, and the potential toxicity of the iCAR/ aCAR transduced T-cells can be evaluated. The CAR construct will contain scFv that matches the human antigens, while the signaling domains will be modified to activate or inhibit murine T-cells. One example for such a model is the HHD-HLA-A2 mice that express only human HLA-A2 molecule while all other proteins are solely murine. The scFv of the CD19 aCAR will be directed in this case to the murine CD19 homolog. Human target cells lacking HLA molecules (e.g. LCL 721.221 cells or C1R-neoATCC® CRL-2369™ or similar) will be used. The targets will be modified to express the murine CD19. This system will allow monitoring of efficacy and toxicity issues.

mAbs Production

[00551] Several pairs of preserved and lost allelic variants identified in different tumors are selected and their polypeptide products will serve for the generation of variant specific mAbs using mAb production techniques. The discriminatory power of candidate mAbs will be assayed by double staining and flow cytometry experiments or immunohistochemistry, as determined by binding to recombinant cell-lines expressing the selected alleles.

[00552] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00553] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00554] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

WHAT IS CLAIMED IS:

1. A method of identifying a target for preparing an inhibitory chimeric antigen receptor (iCAR) or a protective chimeric antigen receptor (pCAR) capable of preventing or attenuating undesired activation of an effector immune cell, wherein the target is identified by a method comprising:

(i) identifying a gene with at least two expressed alleles that encodes a protein comprising an extracellular polymorphic epitope;

(ii) determining that at least one of the expressed alleles exhibits an amino acid sequence change in the extracellular polymorphic epitope sequence relative to an extracellular polymorphic epitope reference sequence;

(iii) determining that the gene is located in a chromosomal region which undergoes loss of heterozygosity (LOH) in a tumor type; and

(iv) determining that the gene is expressed in the tissue-of-origin of the tumor type in which the chromosomal region was found to undergo LOH.

2. The method of claim 1, wherein the LOH position is selected from the group consisting of a substitution, deletion, and insertion.

3. The method of claim 1, wherein the LOH position is a SNP.

4. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is an HLA gene.

5. The method of claim 4, wherein the gene comprising the extracellular polymorphic epitope is an HLA-A, HLA-B, HLA-C, HLA-G, HLA-E, HLA-F, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA_DQ, or HLA-DR gene.

6. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-A gene.

7. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-B gene.

8. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-C gene.

9. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-G gene.

10. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-E gene.

11. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-F gene.

12. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-K gene.

13. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-L gene.

14. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-DM gene.

15. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-DO gene.

16. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-DP gene.

17. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA_DQ gene.

18. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is an HLA-DR gene.

19. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA4, ADAM30, AQP10, ASTN1, C1orf101, CACNA1S, CATSPER4, CD101, CD164L2, CD1A, CD1C, CD244, CD34, CD46, CELSR2, CHRNB2, CLCA2, CLDN19, CLSTN1, CR1, CR2, CRB1, CSF3R, CSMD2, ECE1, ELTD1, EMC1, EPHA10, EPHA2, EPHA8, ERMAP, FCAMR, FCER1A, FCGR1B, FCGR2A, FCGR2B, FCGR3A, FCRL1, FCRL3, FCRL4, FCRL5, FCRL6, GJB4, GPA33, GPR157, GPR37L1, GPR88, HCRTR1, IGSF3, IGSF9, IL22RA1, IL23R, ITGA10, KIAA1324, KIAA2013, LDLRAD2, LEPR, LGR6, LRIG2, LRP8, LRRC52, LRRC8B,

LRRN2, LY9, MIA3, MR1, MUC1, MXRA8, NCSTN, NFASC, NOTCH2, NPR1, NTRK1, OPN3, OR10J1, OR10J4, OR10K1, OR10R2, OR10T2, OR10X1, OR11L1, OR14A16, OR14I1, OR14K1, OR2AK2, OR2C3, OR2G2, OR2G3, OR2L2, OR2M7, OR2T12, OR2T27, OR2T1, OR2T3, OR2T29, OR2T33, OR2T34, OR2T35, OR2T3, OR2T4, OR2T5, OR2T6, OR2T7, OR2T8, OR2W3, OR6F1, OR6K2, OR6K3, OR6K6, OR6N1, OR6P1, OR6Y1, PDPN, PEAR1, PIGR, PLXNA2, PTCH2, PTCHD2, PTGFRN, PTPRC, PTPRF, PVRL4, RHBG, RXFP4, S1PR1, SCNN1D, SDC3, SELE, SELL, SELP, SEMA4A, SEMA6C, SLAMF7, SLAMF9, SLC2A7, SLC5A9, TACSTD2, TAS1R2, TIE1, TLR5, TMEM81, TNFRSF14, TNFRSF1B, TRABD2B, USH2A, VCAM1, and ZP4.

20. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCG5, ALK, ASPRV1, ATRAID, CD207, CD8B, CHRNG, CLEC4F, CNTNAP5, CRIM1, CXCR1, DNER, DPP10, EDAR, EPCAM, GPR113, GPR148, GPR35, GPR39, GYPC, IL1RL1, ITGA4, ITGA6, ITGAV, LCT, LHCGR, LRP1B, LRP2, LY75, MARCO, MERTK, NRP2, OR6B2, PLA2R1, PLB1, PROKR1, PROM2, SCN7A, SDC1, SLC23A3, SLC5A6, TGOLN2, THSD7B, TM4SF20, TMEFF2, TMEM178A, TPO, and TRABD2A.

21. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ACKR2, ALCAM, ANO10, ATP13A4, BTLA, CACNA1D, CACNA2D2, CACNA2D3, CASR, CCRL2, CD200, CD200R1, CD86, CD96, CDCP1, CDHR4, CELSR3, CHL1, CLDN11, CLDN18, CLSTN2, CSPG5, CX3CR1, CXCR6, CYP8B1, DCBLD2, DRD3, EPHA6, EPHB3, GABRR3, GP5, GPR128, GPR15, GPR27, GRM2, GRM7, HEG1, HTR3C, HTR3D, HTR3E, IGSF11, IL17RC, IL17RD, IL17RE, IL5RA, IMPG2, ITGA9, ITGB5, KCNMB3, LRIG1, LRRC15, LRRN1, MST1R, NAALADL2, NRROS, OR5AC1, OR5H1, OR5H14, OR5H15, OR5H6, OR5K2, OR5K3, OR5K4, PIGX, PLXNB1, PLXND1, PRRT3, PTPRG, ROBO2, RYK, SEMA5B, SIDT1, SLC22A14, SLC33A1, SLC4A7, SLITRK3, STAB1, SUSD5, TFRC, TLR9, TMEM108, TMEM44, TMPRSS7, TNFSF10, UPK1B, VIPR1, and ZPLD1.

22. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANTXR2, BTC, CNGA1, CORIN, EGF, EMCN, ENPEP, EPHA5, ERVMER34-1, EVC2, FAT1, FAT4, FGFRL1, FRAS1, GPR125, GRID2, GYPA, GYPB, KDR, KIAA0922, KLB, MFSD8, PARM1, PDGFRA, RNF150, TENM3, TLR10, TLR1, TLR6, TMEM156, TMPRSS11A, TMPRSS11B, TMPRSS11E, TMPRSS11F, UGT2A1, and UNC5C.

23. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM19, ADRB2, BTNL3, BTNL8, BTNL9, C5orf15, CATSPER3, CD180, CDH12, CDHR2, COL23A1, CSF1R, F2RL2, FAM174A, FAT2, FGFR4, FLT4, GABRA6, GABRG2, GPR151, GPR98, GRM6, HAVCR1, HAVCR2, IL31RA, IL6ST, IL7R, IQGAP2, ITGA1, ITGA2, KCNMB1, LIFR, LNPEP, MEGF10, NIPAL4, NPR3, NRG2, OR2V1, OR2Y1, OSMR, PCDH12, PCDH1, PCDHA1, PCDHA2, PCDHA4, PCDHA8, PCDHA9, PCDHB10, PCDHB11, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHGA1, PCDHGA4, PDGFRB, PRLR, SEMA5A, SEMA6A, SGCD, SLC1A3, SLC22A4, SLC22A5, SLC23A1, SLC36A3, SLC45A2, SLC6A18, SLC6A19, SLCO6A1, SV2C, TENM2, TIMD4, and UGT3A1.

24. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of BAI3, BTN1A1, BTN2A1, BTN2A2, BTN3A1, BTN3A2, BTNL2, CD83, DCBLD1, DLL1, DPCR1, ENPP1, ENPP3, ENPP4, EPHA7, GABBR1, GABRR1, GCNT6, GFRAL, GJB7, GLP1R, GPR110, GPR111, GPR116, GPR126, GPR63, GPRC6A, HFE, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-E, HLA-F, HLA-G, IL20RA, ITPR3, KIAA0319, LMBRD1, LRFN2, LRP11, MAS1L, MEP1A, MICA, MICB, MOG, MUC21, MUC22, NCR2, NOTCH4, OPRM1, OR10C1, OR12D2, OR12D3, OR14J1, OR2B2, OR2B6, OR2J1, OR2W1, OR5V1, PDE10A, PI16, PKHD1, PTCRA, PTK7, RAET1E, RAET1G, ROS1, SDIM1, SLC16A10, SLC22A1, SLC44A4, TAAR2, TREM1, TREML1, and TREML2.

25. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of AQP1, C7orf50, CD36, CDHR3, CNTNAP2, DPP6, EGFR, EPHA1, EPHB6, ERVW-1, GHRHR, GJC3, GPNMB, GRM8, HUS1, HYAL4, KIAA1324L, LRRN3, MET, MUC12, MUC17, NPC1L1, NPSR1, OR2A12, OR2A14, OR2A25, OR2A42, OR2A7, OR2A2, OR2AE1, OR2F2, OR6V1, PILRA, PILRB, PKD1L1, PLXNA4, PODXL, PTPRN2, PTPRZ1, RAMP3, SLC29A4, SMO, TAS2R16, TAS2R40, TAS2R4, TFR2, THSD7A, TMEM213, TTYH3, ZAN, and ZP3.

26. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM18, ADAM28, ADAM32, ADAM7, ADAM9, ADRA1A, CDH17, CHRNA2, CSMD1, CSMD3,

DCSTAMP, FZD6, GPR124, NRG1, OR4F21, PKHD1L1, PRSS55, SCARA3, SCARA5, SDC2, SLC10A5, SLC39A14, SLC39A4, SLCO5A1, TNFRSF10A, and TNFRSF10B.

27. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA1, AQP7, ASTN2, C9orf135, CA9, CD72, CNTNAP3B, CNTNAP3, CRB2, ENTPD8, GPR144, GRIN3A, IZUMO3, KIAA1161, MAMDC4, MEGF9, MUSK, NOTCH1, OR13C2, OR13C3, OR13C5, OR13C8, OR13C9, OR13D1, OR13F1, OR1B1, OR1J2, OR1K1, OR1L1, OR1L3, OR1L6, OR1L8, OR1N1, OR1N2, OR1Q1, OR2S2, PCSK5, PDCD1LG2, PLGRKT, PTPRD, ROR2, SEMA4D, SLC31A1, TEK, TLR4, TMEM2, and VLDLR.

28. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCC2, ADAM8, ADRB1, ANTXRL, ATRNL1, C10orf54, CDH23, CDHR1, CNNM2, COL13A1, COL17A1, ENTPD1, FZD8, FGFR2, GPR158, GRID1, IL15RA, IL2RA, ITGA8, ITGB1, MRC1, NRG3, NPFFR1, NRP1, OPN4, PCDH15, PKD2L1, PLXDC2, PRLHR, RET, RGR, SLC16A9, SLC29A3, SLC39A12, TACR2, TCTN3, TSPAN15, UNC5B, and VSTM4.

29. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of AMICA1, ANO1, ANO3, APLP2, C11orf24, CCKBR, CD248, CD44, CD5, CD6, CD82, CDON, CLMP, CRTAM, DCHS1, DSCAML1, FAT3, FOLH1, GDPD4, GDPD5, GRIK4, HEPHL1, HTR3B, IFITM10, IL10RA, KIRREL3, LGR4, LRP4, LRP5, LRRC32, MCAM, MFRP, MMP26, MPEG1, MRGPRE, MRGPRF, MRGPRX2, MRGPRX3, MRGPRX4, MS4A4A, MS4A6A, MTNR1B, MUC15, NAALAD2, NAALADL1, NCAM1, NRXN2, OR10A2, OR10A5, OR10A6, OR10D3, OR10G4, OR10G7, OR10G8, OR10G9, OR10Q1, OR10S1, OR1S1, OR2AG1, OR2AG2, OR2D2, OR4A47, OR4A15, OR4A5, OR4C11, OR4C13, OR4C15, OR4C16, OR4C3, OR4C46, OR4C5, OR4D6, OR4A8P, OR4D9, OR4S2, OR4X1, OR51E1, OR51L1, OR52A1, OR52E1, OR52E2, OR52E4, OR52E6, OR52I1, OR52I2, OR52J3, OR52L1, OR52N1, OR52N2, OR52N4, OR52W1, OR56B1, OR56B4, OR5A1, OR5A2, OR5AK2, OR5AR1, OR5B17, OR5B3, OR5D14, OR5D16, OR5D18, OR5F1, OR5I1, OR5L2, OR5M11, OR5M3, OR5P2, OR5R1, OR5T2, OR5T3, OR5W2, OR6A2, OR6T1, OR6X1, OR8A1, OR8B12, OR8B2, OR8B3, OR8B4, OR8D1, OR8D2, OR8H1, OR8H2, OR8H3, OR8I2, OR8J1, OR8J2, OR8J3, OR8K1, OR8K3, OR8K5, OR8U1, OR9G1, OR9G4, OR9Q2, P2RX3, PTPRJ, ROBO3, SIGIRR, SLC22A10, SLC3A2, SLC5A12,

SLCO2B1, SORL1, ST14, SYT8, TENM4, TMEM123, TMEM225, TMPRSS4, TMPRSS5, TRIM5, TRPM5, TSPAN18, and ZP1.

30. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANO4, AVPR1A, BCL2L14, CACNA2D4, CD163, CD163L1, CD27, CD4, CLEC12A, CLEC1B, CLEC2A, CLEC4C, CLEC7A, CLECL1, CLSTN3, GPR133, GPRC5D, ITGA7, ITGB7, KLRB1, KLRC2, KLRC3, KLRC4, KLRF1, KLRF2, LRP1, LRP6, MANSC1, MANSC4, OLR1, OR10AD1, OR10P1, OR2AP1, OR6C1, OR6C2, OR6C3, OR6C4, OR6C6, OR6C74, OR6C76, OR8S1, OR9K2, ORAI1, P2RX4, P2RX7, PRR4, PTPRB, PTPRQ, PTPRR, SCNN1A, SELPLG, SLC2A14, SLC38A4, SLC5A8, SLC6A15, SLC8B1, SLCO1A2, SLCO1B1, SLCO1B7, SLCO1C1, SSPN, STAB2, TAS2R10, TAS2R13, TAS2R14, TAS2R20, TAS2R30, TAS2R31, TAS2R42, TAS2R43, TAS2R46, TAS2R7, TMEM119, TMEM132B, TMEM132C, TMEM132D, TMPRSS12, TNFRSF1A, TSPAN8, and VSIG10.

31. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP4B, ATP7B, FLT3, FREM2, HTR2A, KL, PCDH8, RXFP2, SGCG, SHISA2, SLC15A1, SLITRK6, and TNFRSF19.

32. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ADAM21, BDKRB2, C14orf37, CLEC14A, DLK1, FLRT2, GPR135, GPR137C, JAG2, LTB4R2, MMP14, OR11G2, OR11H12, OR11H6, OR4K1, OR4K15, OR4K5, OR4L1, OR4N2, OR4N5, SLC24A4, and SYNDIG1L.

33. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ANPEP, CD276, CHRNA7, CHRNB4, CSPG4, DUOX1, DUOX2, FAM174B, GLDN, IGDCC4, ITGA11, LCTL, LTK, LYSMD4, MEGF11, NOX5, NRG4, OCA2, OR4F4, OR4M2, OR4N4, PRTG, RHCG, SCAMP5, SEMA4B, SEMA6D, SLC24A1, SLC24A5, SLC28A1, SPG11, STRA6, TRPM1, and TYRO3.

34. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP2C2, CACNA1H, CD19, CDH11, CDH15, CDH16, CDH3, CDH5, CNGB1, CNTNAP4, GDPD3, GPR56, GPR97, IFT140, IL4R, ITFG3, ITGAL, ITGAM, ITGAX, KCNG4, MMP15, MSLNL, NOMO1,

NOMO3, OR2C1, PIEZO1, PKD1, PKD1L2, QPRT, SCNN1B, SEZ6L2, SLC22A31, SLC5A11, SLC7A6, SPN, TMC5, TMC7, TMEM204, TMEM219, and TMEM8A.

35. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCC3, ACE, AOC3, ARL17B, ASGR2, C17orf80, CD300A, CD300C, CD300E, CD300LF, CD300LG, CHRNB1, CLEC10A, CNTNAP1, CPD, CXCL16, ERBB2, FAM171A2, GCGR, GLP2R, GP1BA, GPR142, GUCY2D, ITGA2B, ITGA3, ITGAE, ITGB3, KCNJ12, LRRC37A2, LRRC37A3, LRRC37A, LRRC37B, MRC2, NGFR, OR1A2, OR1D2, OR1G1, OR3A1, OR3A2, OR4D1, OR4D2, RNF43, SCARF1, SCN4A, SDK2, SECTM1, SEZ6, SHPK, SLC26A11, SLC5A10, SPACA3, TMEM102, TMEM132E, TNFSF12, TRPV3, TTYH2, and TUSC5.

36. The method of claim 5, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of APCDD1, CDH19, CDH20, CDH7, COLEC12, DCC, DSC1, DSG1, DSG3, DYNAP, MEP1B, PTPRM, SIGLEC15, and TNFRSF11A.

37. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABCA7, ACPT, BCAM, C19orf38, C19orf59, C5AR1, CATSPERD, CATSPERG, CD22, CD320, CD33, CD97, CEACAM19, CEACAM1, CEACAM21, CEACAM3, CEACAM4, CLEC4M, DLL3, EMR1, EMR2, EMR3, ERVV-1, ERVV-2, FAM187B, FCAR, FFAR3, FPR1, FXYD5, GFY, GP6, GPR42, GRIN3B, ICAM3, IGFLR1, IL12RB1, IL27RA, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIRREL2, KISS1R, LAIR1, LDLR, LILRA1, LILRA2, LILRA4, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LINGO3, LPHN1, LRP3, MADCAM1, MAG, MEGF8, MUC16, NCR1, NOTCH3, NPHS1, OR10H1, OR10H2, OR10H3, OR10H4, OR1I1, OR2Z1, OR7A10, OR7C1, OR7D4, OR7E24, OR7G1, OR7G2, OR7G3, PLVAP, PTGIR, PTPRH, PTPRS, PVR, SCN1B, SHISA7, SIGLEC10, SIGLEC11, SIGLEC12, SIGLEC5, SIGLEC6, SIGLEC8, SIGLEC9, SLC44A2, SLC5A5, SLC7A9, SPINT2, TARM1, TGFBR3L, TMC4, TMEM91, TMEM161A, TMPRSS9, TNFSF14, TNFSF9, TRPM4, VN1R2, VSIG10L, VSTM2B, and ZNRF4.

38. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ABHD12, ADAM33, ADRA1D, APMAP, ATRN, CD40, CD93, CDH22, CDH26, CDH4, FLRT3, GCNT7,

GGT7, JAG1, LRRN4, NPBWR2, OCSTAMP, PTPRA, PTPRT, SEL1L2, SIGLEC1, SIRPA, SIRPB1, SIRPG, SLC24A3, SLC2A10, SLC4A11, SSTR4, and THBD.

39. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of CLDN8, DSCAM, ICOSLG, IFNAR1, IFNGR2, IGSF5, ITGB2, KCNJ15, NCAM2, SLC19A1, TMPRSS15, TMPRSS2, TMPRSS3, TRPM2, and UMODL1.

40. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of CACNA1I, CELSR1, COMT, CSF2RB, GGT1, GGT5, IL2RB, KREMEN1, MCHR1, OR11H1, P2RX6, PKDREJ, PLXNB2, SCARF2, SEZ6L, SSTR3, SUSD2, TMPRSS6, and TNFRSF13C.

41. The method of claim 1, wherein the gene comprising the extracellular polymorphic epitope is selected from the group consisting of ATP6AP2, ATP7A, CNGA2, EDA2R, FMR1NB, GLRA4, GPR112, GUCY2F, HEPH, P2RY10, P2RY4, PLXNA3, PLXNB3, TLR8, VSIG4, and XG.

42. The method of any of claims 1 through 41, wherein the tumor is selected from the group consisting of a breast tumor, a prostate tumor, an ovarian tumor, a cervical tumor, a skin tumor, a pancreatic tumor, a colorectal tumor, a renal tumor, a liver tumor, a brain tumor, a lymphoma, a leukemia, a lung tumor, and a glioma.

43. The method of any of claims 1 through 41, wherein the tumor is selected from the group consisting of an adrenal gland tumor, a kidney tumor, a melanoma, DLBC, a breast tumor, a sarcoma, an ovary tumor, a lung tumor, a bladder tumor, and a liver tumor.

44. The method of claim 43, wherein the adrenal gland tumor is an adrenocortical carcinoma.

45. The method of claim 43, wherein the kidney tumor is a chromophobe renal cell carcinoma.

46. The method of claim 43, wherein the melanoma is uveal melanoma.

47. A safe effector immune cell expressing (i) an iCAR or pCAR according to any of claims 1 through 46 and (ii) an activating chimeric antigen receptor (aCAR).

48. The safe effector immune cell of claim 47, wherein the aCAR is directed against or specifically binds to a tumor-associated antigen or a non-polymorphic cell surface epitope.

49. The safe effector immune cell of claim 47, wherein the aCAR is directed against or specifically binds to a tumor associated protein, a CAR target as listed in table 1, any cell surface protein that is expressed in a tumor tissue in which the iCAR is also expressed.

50. The safe effector immune cell of claim 49, wherein the non-polymorphic cell surface epitope is selected from the group consisting of CD19, CD20, CD22, CD10, CD7, CD49f, CD56, CD74, CAIX Igκ, ROR1, ROR2, CD30, LewisY, CD33, CD34, CD38, CD123, CD28, CD44v6, CD44, CD41, CD133, CD138, NKG2D-L, CD139, BCMA, GD2, GD3, hTERT, FBP, EGP-2, EGP-40, FR-α, L1-CAM, ErbB2,3,4, EGFRvIII, VEGFR-2, IL-13Rα2, FAP, Mesothelin, c-MET, PSMA, CEA, kRas, MAGE-A1, MUC1 MUC16, PDL1, PSCA, EpCAM, FSHR, AFP, AXL, CD80, CD89, CDH17, CLD18, GPC3, TEM8, TGFB1, NY-ESO-1, WT-1 and EGFR.

51. The safe effector immune cell of any of claims 47 to 50, wherein the safe effector immune cell is an autologous or a universal (allogeneic) effector cell.

52. The safe effector immune cell of any of claims 47 to 51, wherein the safe effector immune cell is selected from the group consisting of a T cell, a natural killer cell and a cytokine-induced killer cell.

53. The safe effector immune cell of any of claims 47 to 52, wherein the expression level of the iCAR or pCAR is greater than or equal to the expression level of the aCAR.

54. The safe effector immune cell of any of claims 47 to 53, wherein the iCAR or pCAR is expressed by a first vector and the aCAR is expressed by a second vector.

55. The safe effector immune cell of any of claims 47 to 53, wherein the iCAR or pCAR and the aCAR are both expressed by the same vector.

56. The safe effector immune cell of claim 55, wherein the nucleotide sequence encoding for the aCAR is downstream of the nucleotide sequence encoding for the iCAR or pCAR.

57. The safe effector immune cell of claim 55, wherein the nucleotide sequence comprises a viral self-cleaving 2A peptide between the nucleotide sequence encoding for the aCAR and the nucleotide sequence encoding for the iCAR or pCAR.

58. The safe effector immune cell of claim 57, wherein the viral self-cleaving 2A peptide is selected from the group consisting of T2A from *Thosea asigna* virus (TaV), F2A from Foot-and-mouth disease virus (FMDV), E2A from *Equine rhinitis A* virus (ERAV) and P2A from *Porcine teschovirus-1* (PTV1).

59. The safe effector immune cell of claim 55, wherein the nucleotide sequence encoding the aCAR is linked via a flexible linker to the iCAR or pCAR.

60. The safe effector immune cell of any of claims 47 to 59, wherein the aCAR comprises at least one signal transduction element that activates or co-stimulates an effector immune cell

61. The safe effector immune cell of claim 60, wherein the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to an immunoreceptor tyrosine-based activation motif (ITAM) of for example CD3ζ or FcRγ chains.

62. The safe effector immune cell of claim 60, wherein the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to an activating killer cell immunoglobulin-like receptor (KIR), such as KIR2DS and KIR3DS.

63. The safe effector immune cell of any of claim 60, wherein the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to or an adaptor molecule, such as DAP12.

64. The safe effector immune cell of calim 60, wherein the at least one signal transduction element that activates or co-stimulates an effector immune cell is homolgous to or a co-stimulatory signal transduction element of CD27, CD28, ICOS, CD137 (4-1BB), CD134 (OX40) or GITR.

65. A method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient a safe effector immune cell expressing the iCAR according to any of claims 1 through 64.

66. A method for treating cancer in a patient having a tumor characterized by LOH, comprising administering to the patient a safe effector immune cell according to any of claims 47 through 64.

Figure 1

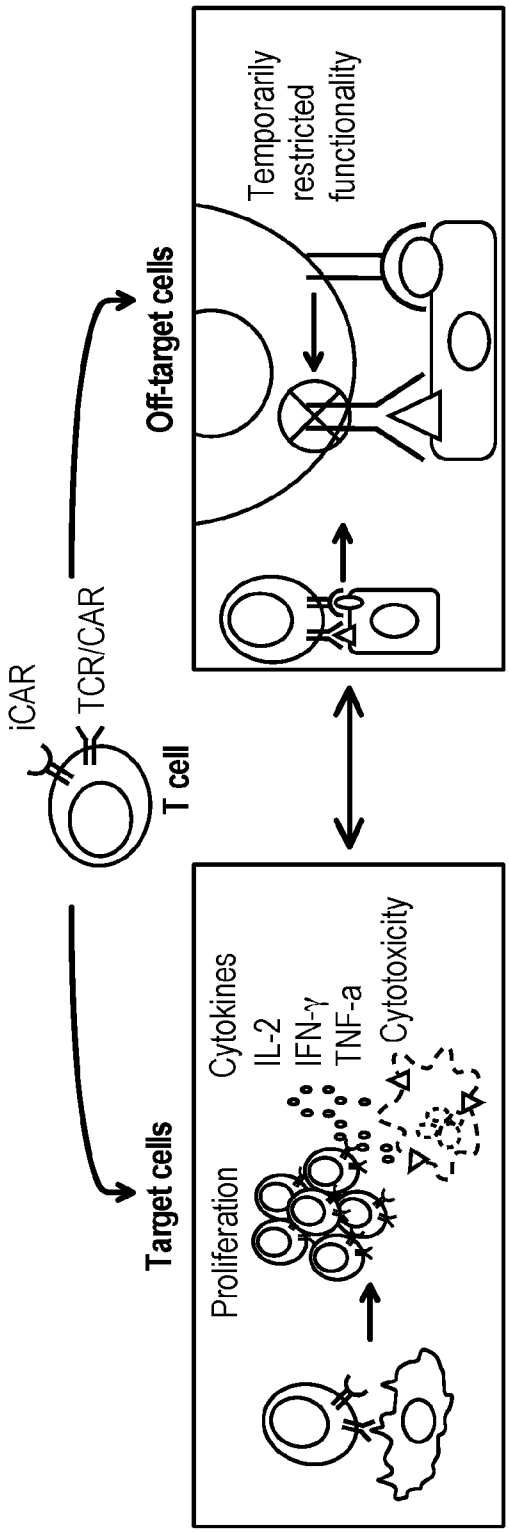

Off-target cells
Temporarily restricted functionality
iCAR
TCR/CAR
T cell
Target cells
Proliferation
Cytokines
IL-2
IFN-γ
TNF-a
Cytotoxicity

Figure 2

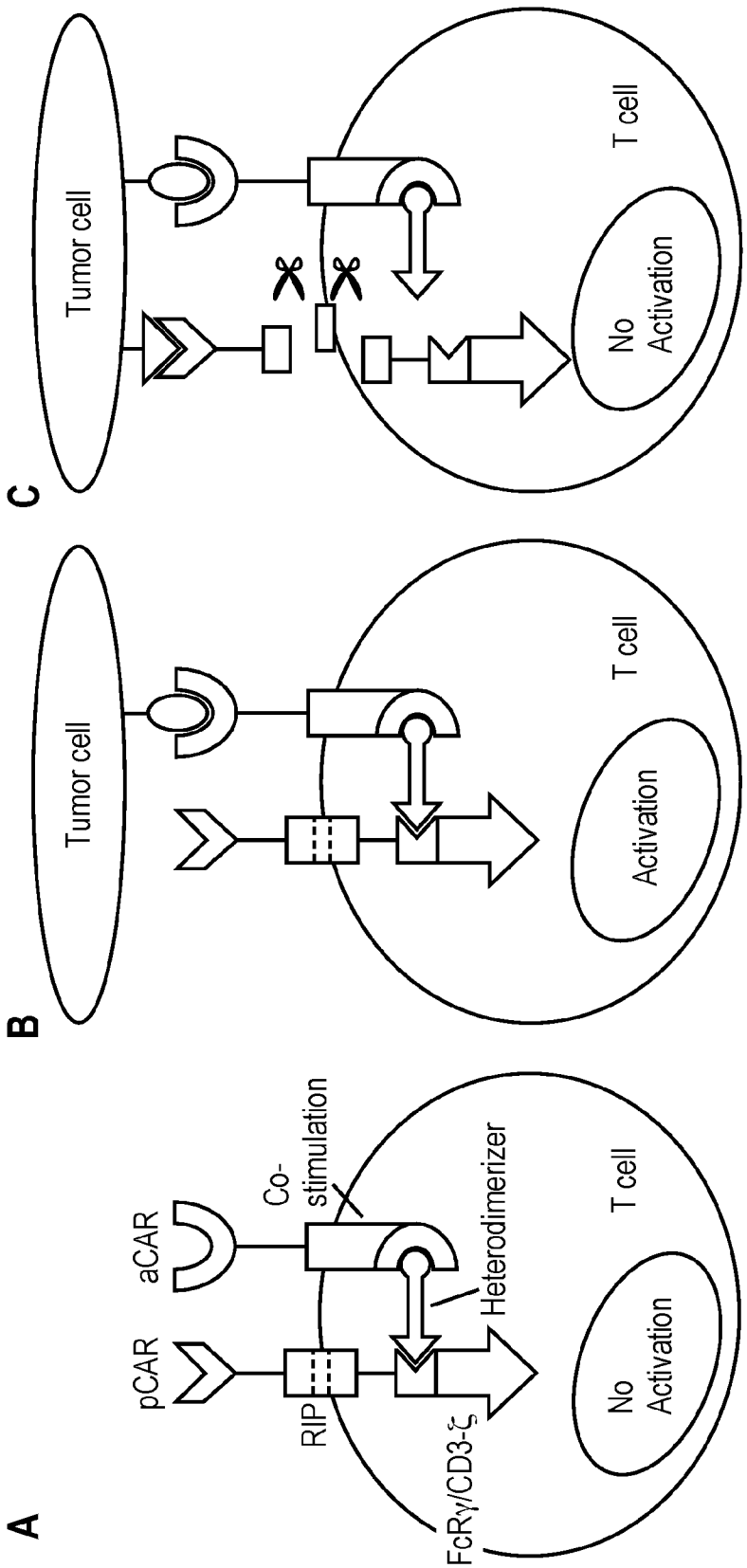
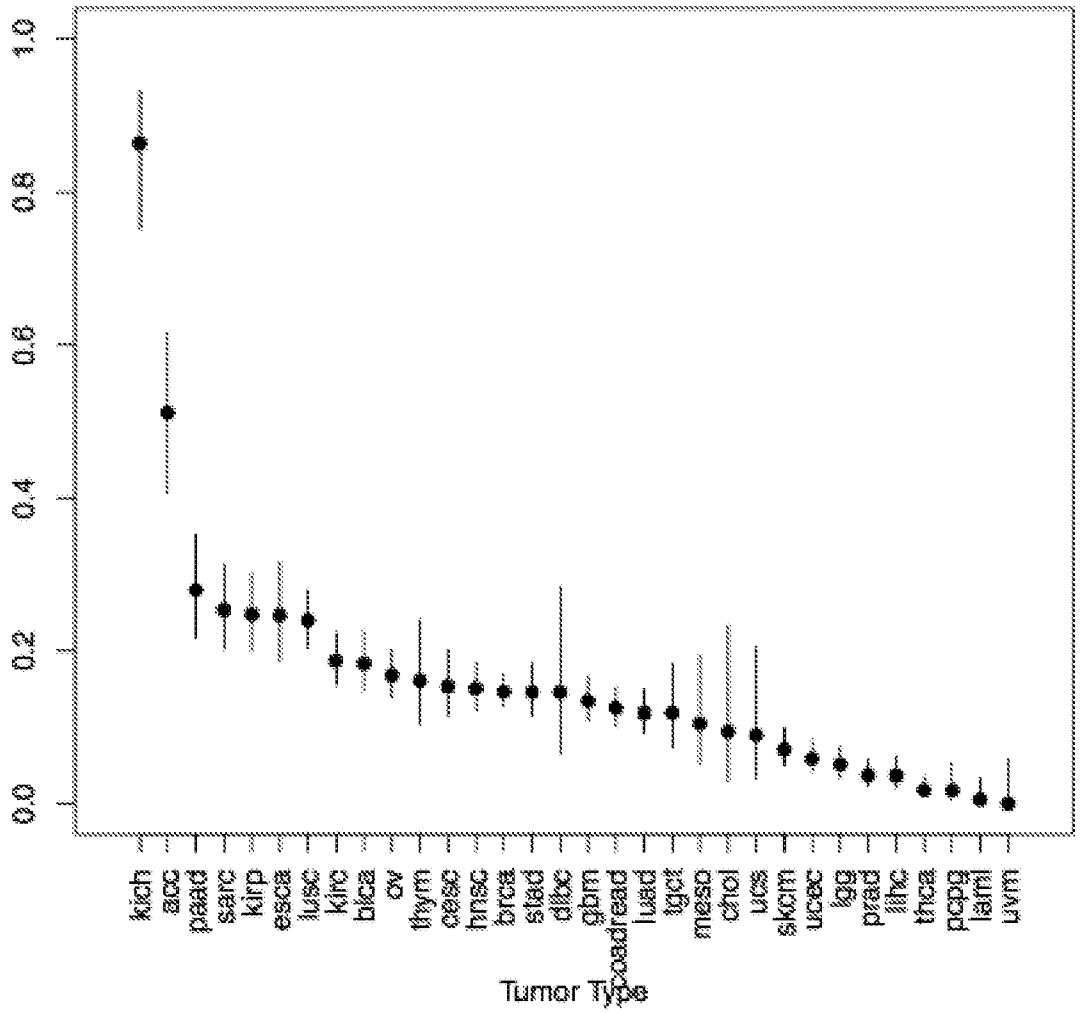
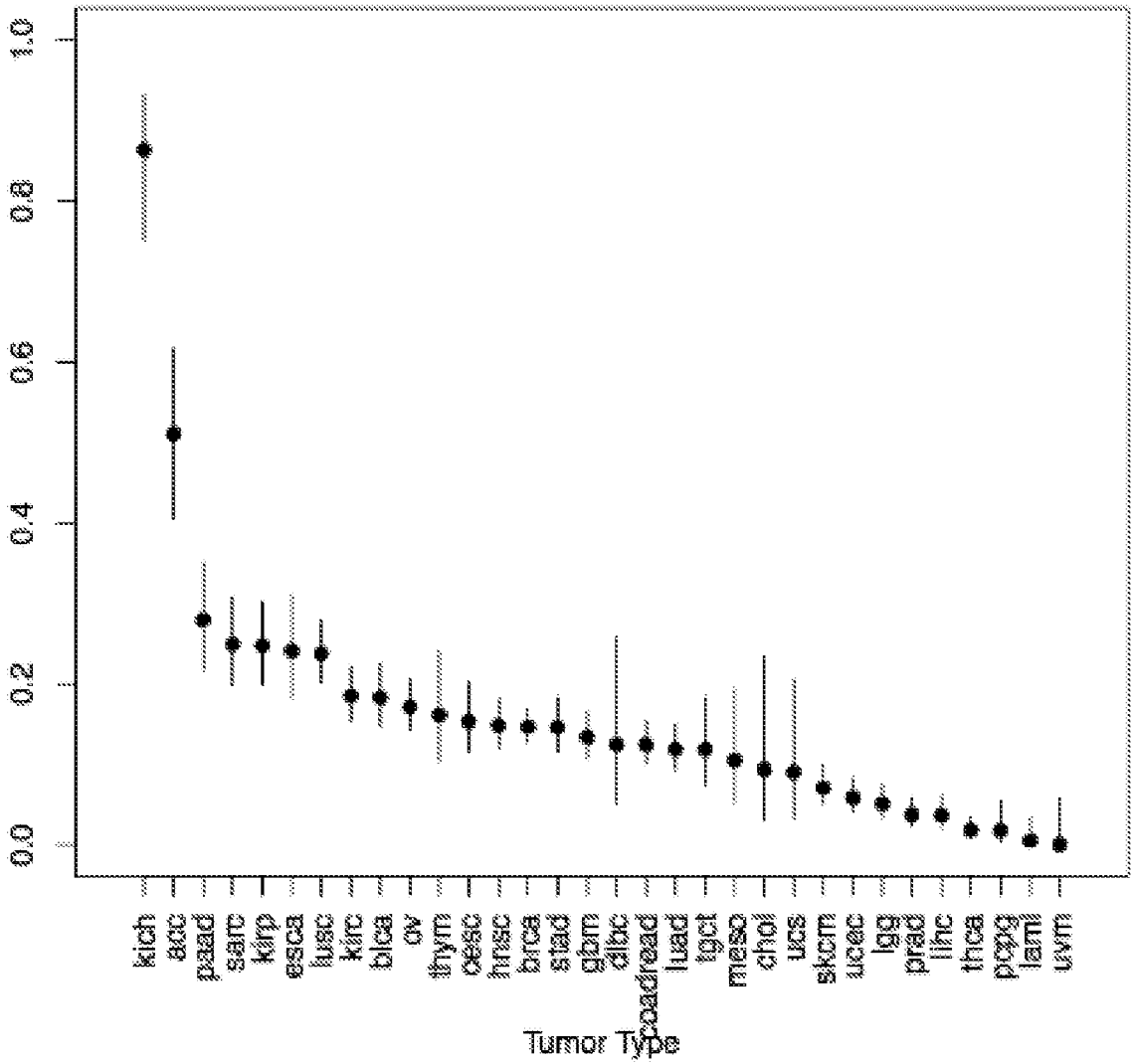

A
pCAR
aCAR
Co-
stimulation
RIP
Heterodimerizer
FcRγ/CD3-ζ
T cell
No
Activation
B
Tumor cell
T cell
Activation
C
Tumor cell
T cell
No
Activation

Figure 3A



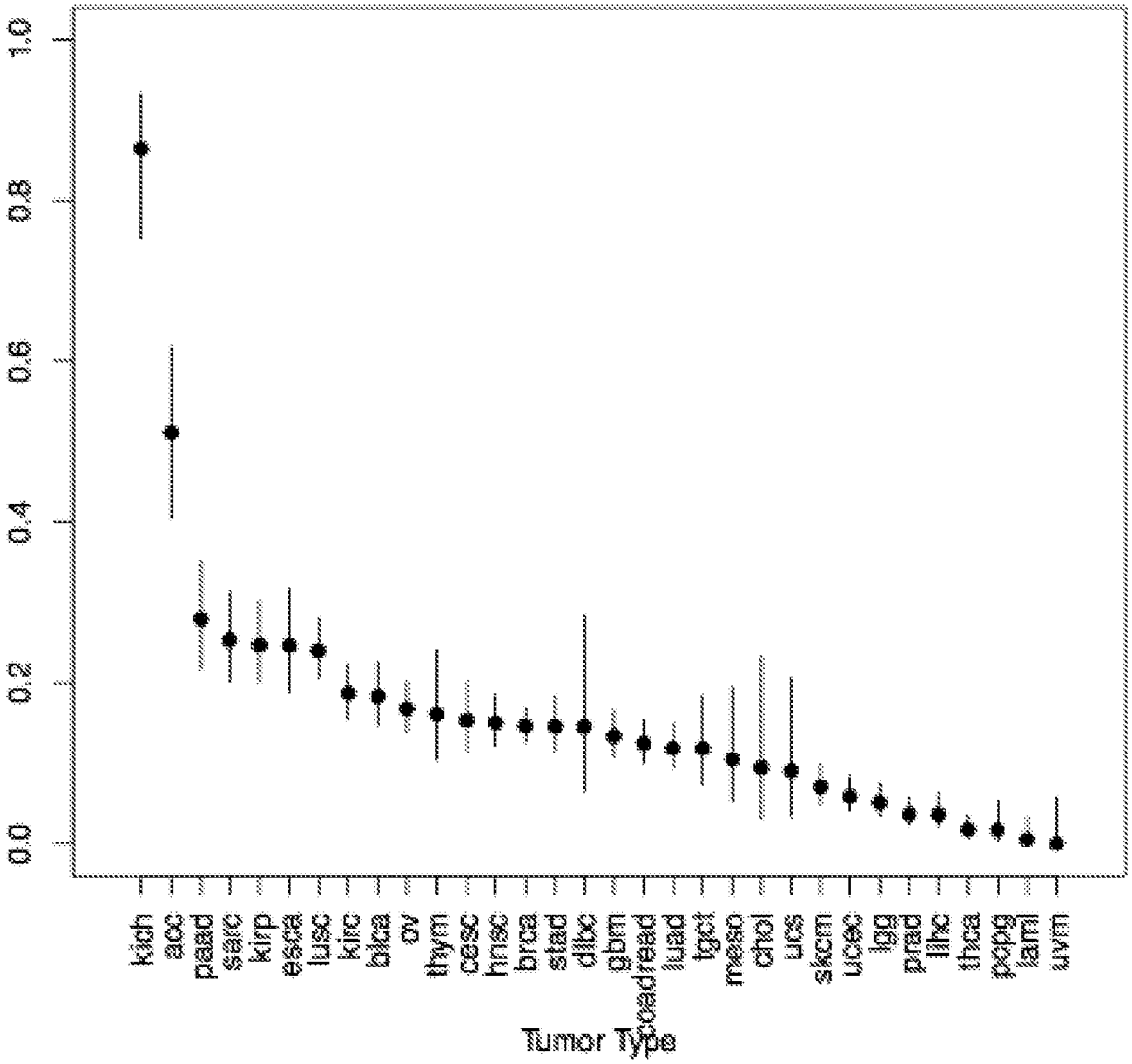
HLA-G (gene upstream)

Figure 3B



HLA-A

Figure 3C



ZNRD1 (gene downstream)

Figure 4

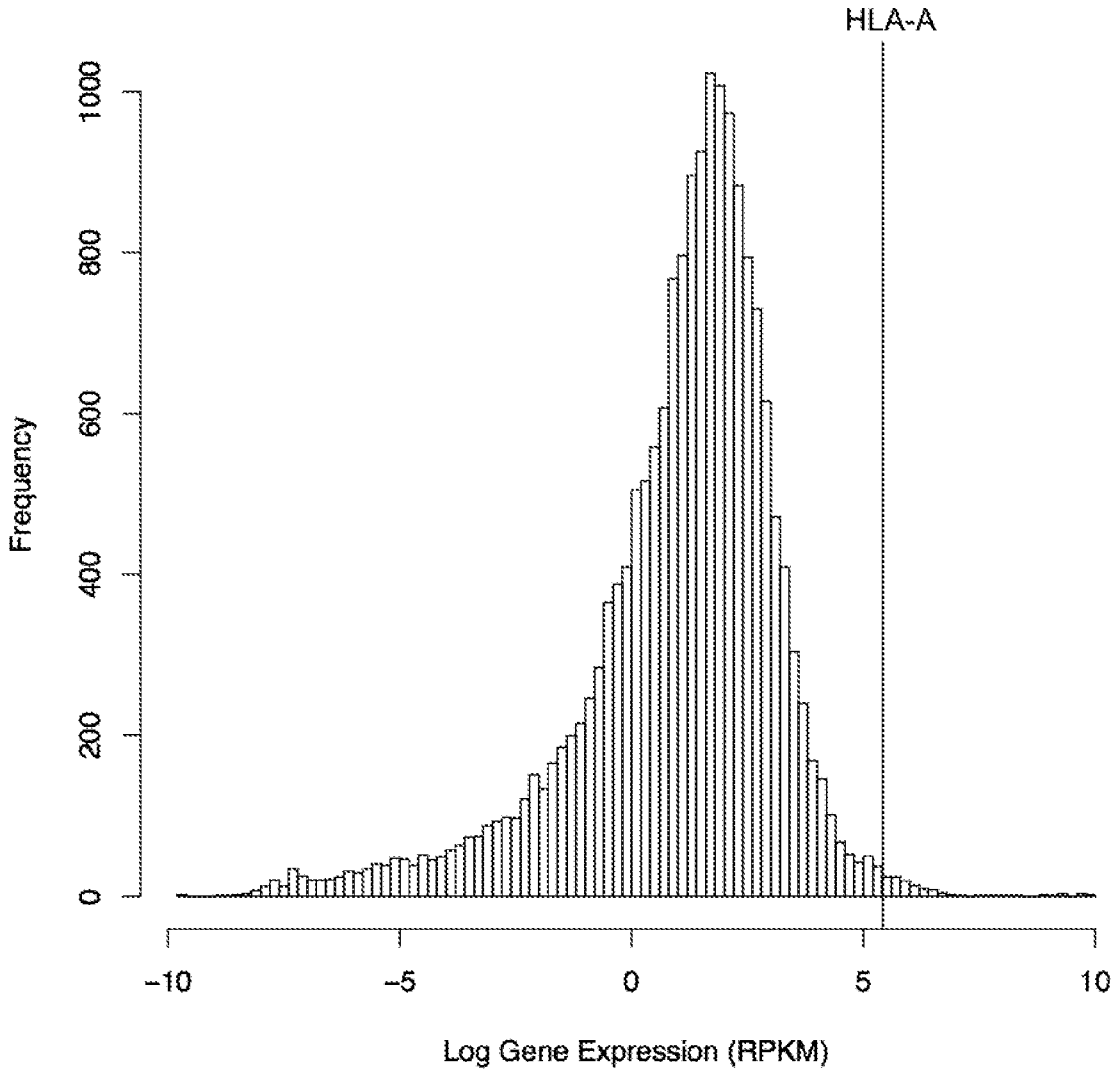

HLA-A
Frequency
0
200
400
600
800
1000
−10
−5
0
5
10
Log Gene Expression (RPKM)

Figure 5

Loss of Heterozygosity of HLA proteins across cancers

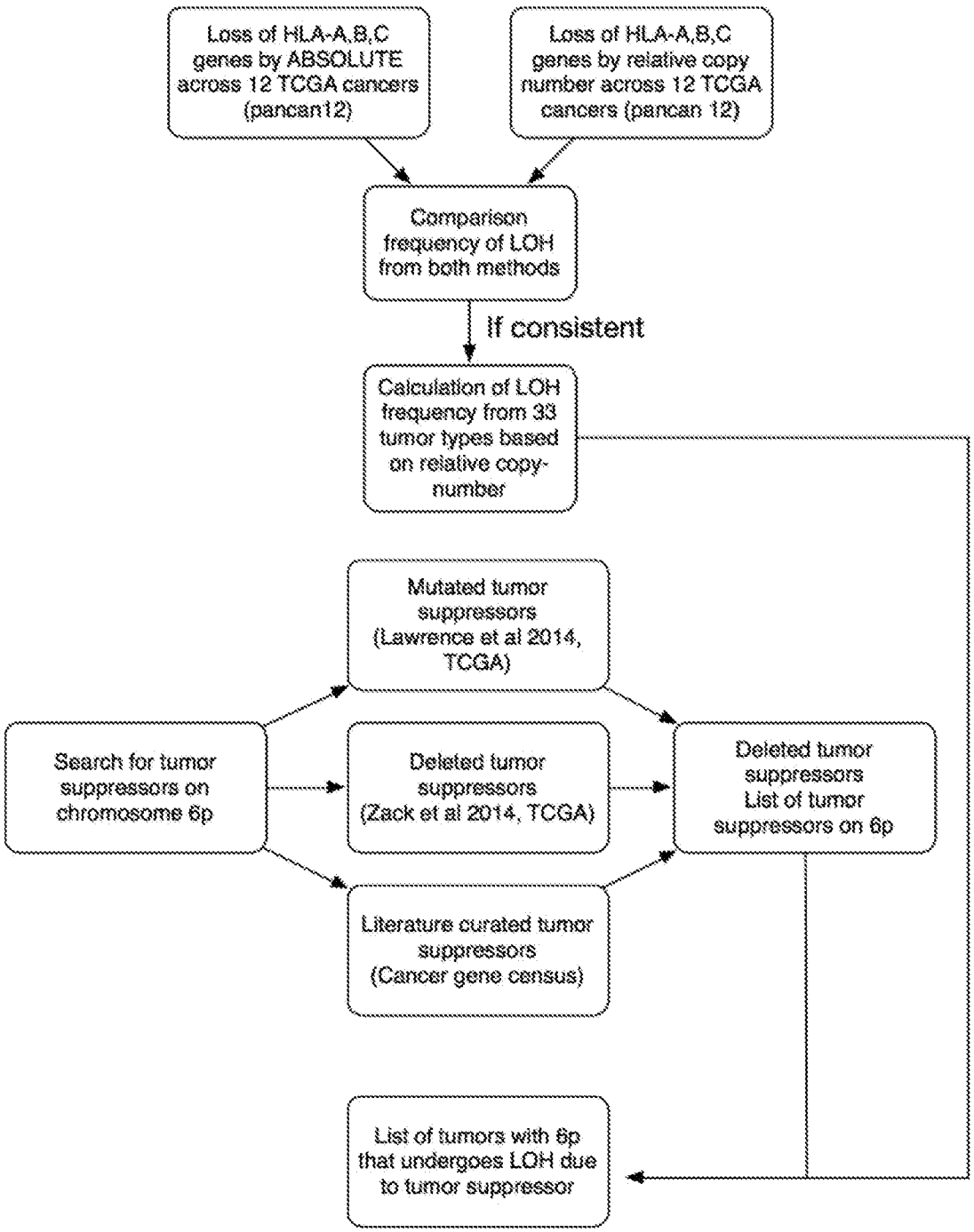


Figure 6

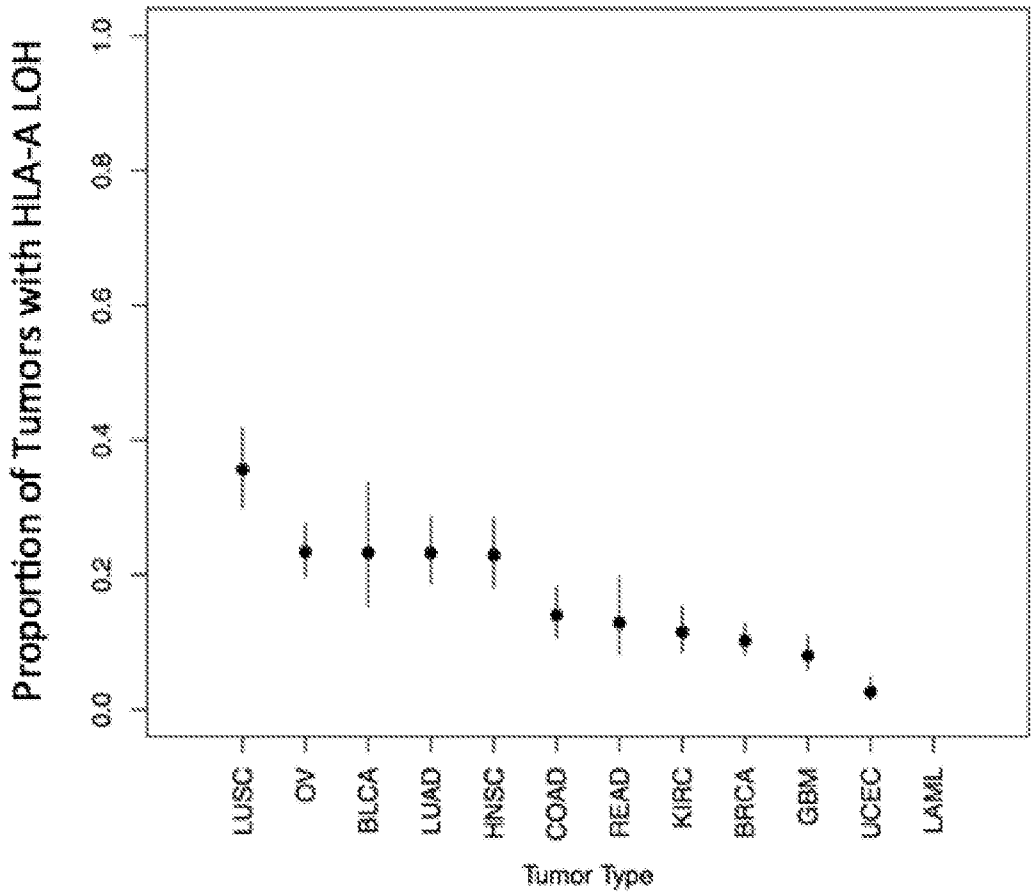

Proportion of Tumors with HLA-A LOH
0.0
0.2
0.4
0.6
0.8
1.0
LUSC
OV
BLCA
LUAD
HNSC
COAD
READ
KIRC
BRCA
GBM
UCEC
LAML
Tumor Type

Figure 7

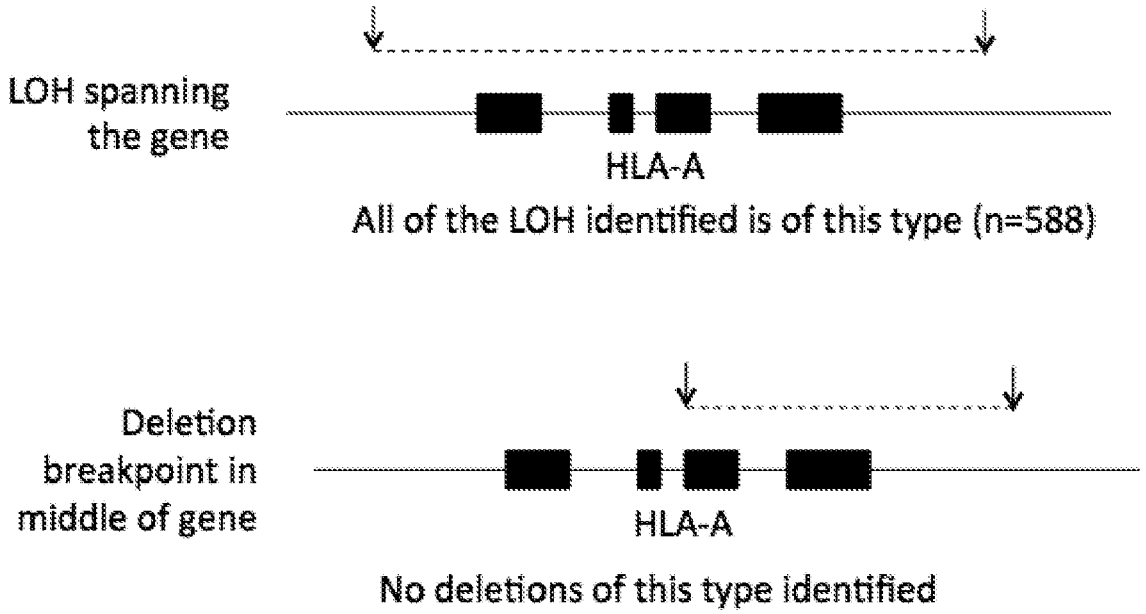

LOH spanning the gene
HLA-A
All of the LOH identified is of this type (n=588)
Deletion breakpoint in middle of gene
HLA-A
No deletions of this type identified

Figure 8

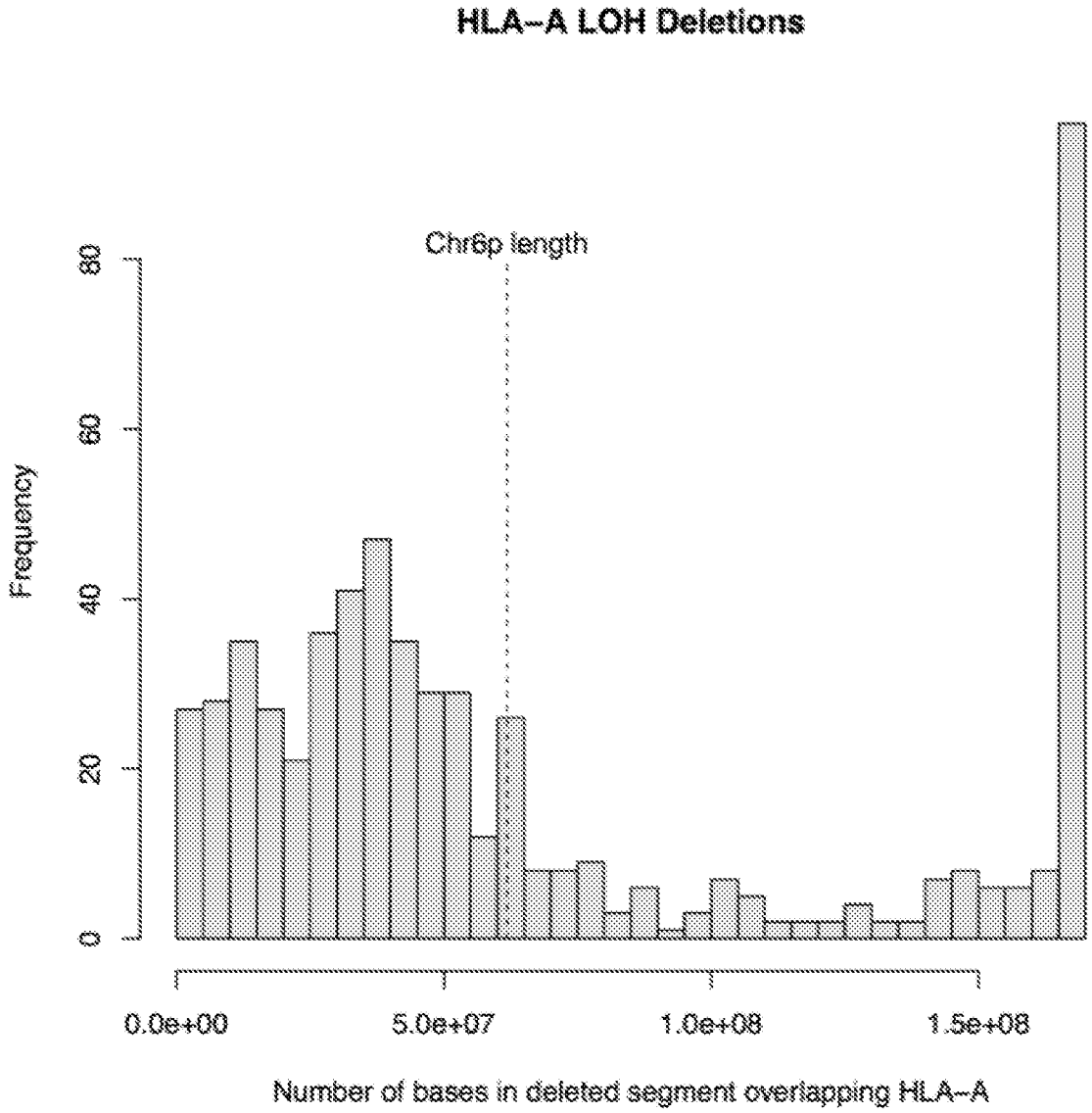

HLA-A LOH Deletions
Chr6p length
Frequency
0
20
40
60
80
0.0e+00
5.0e+07
1.0e+08
1.5e+08
Number of bases in deleted segment overlapping HLA-A

Figure 9

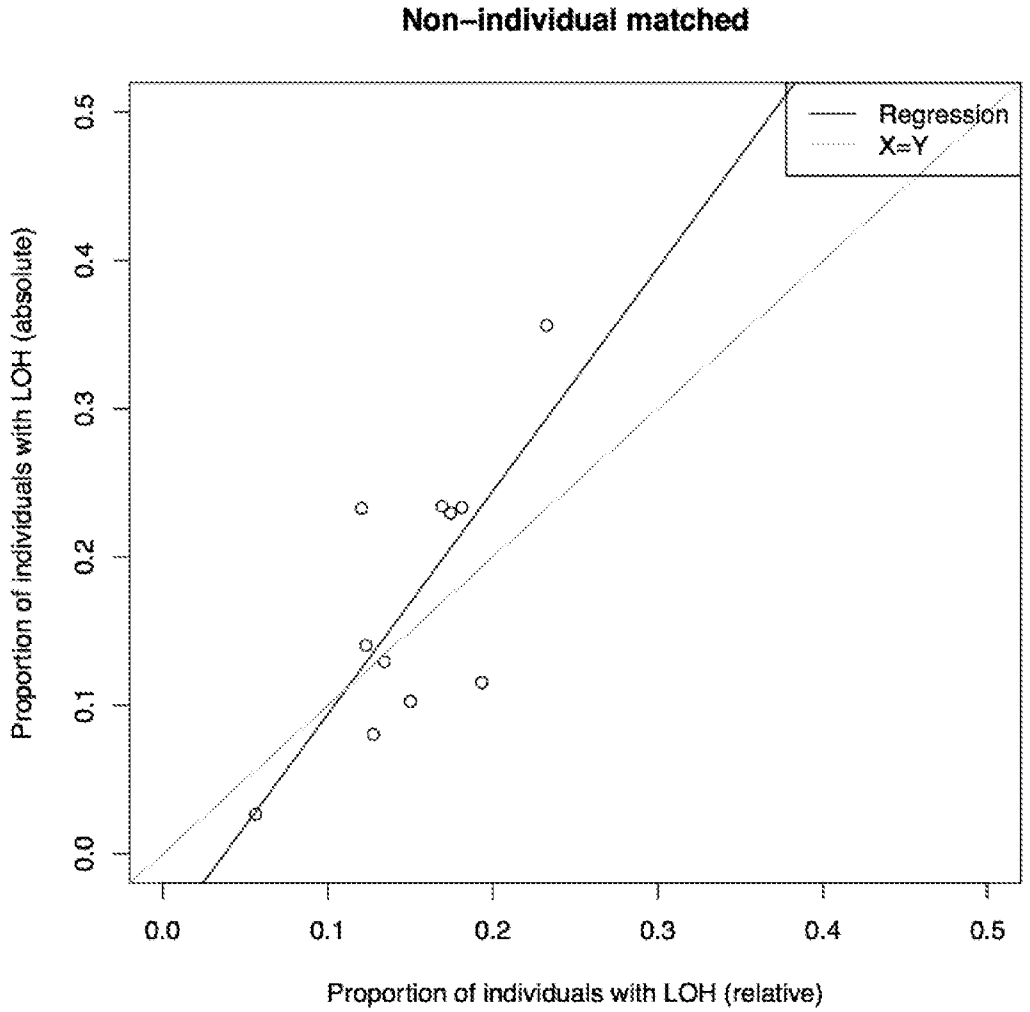
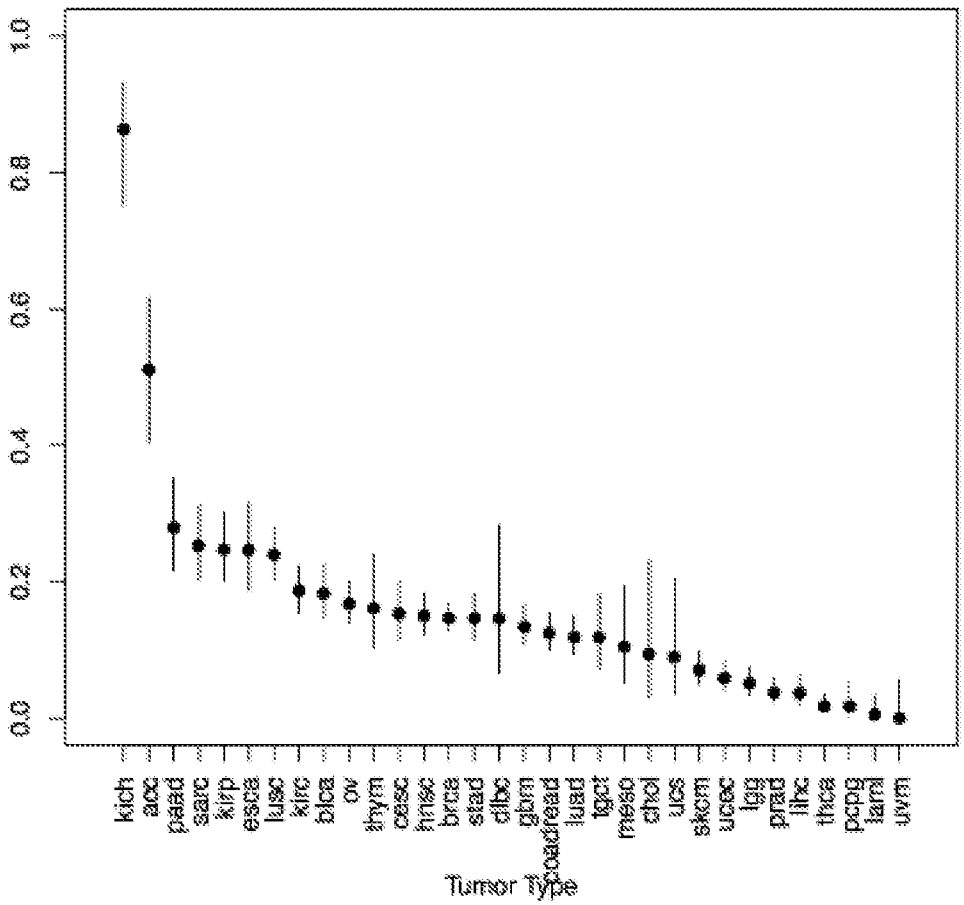
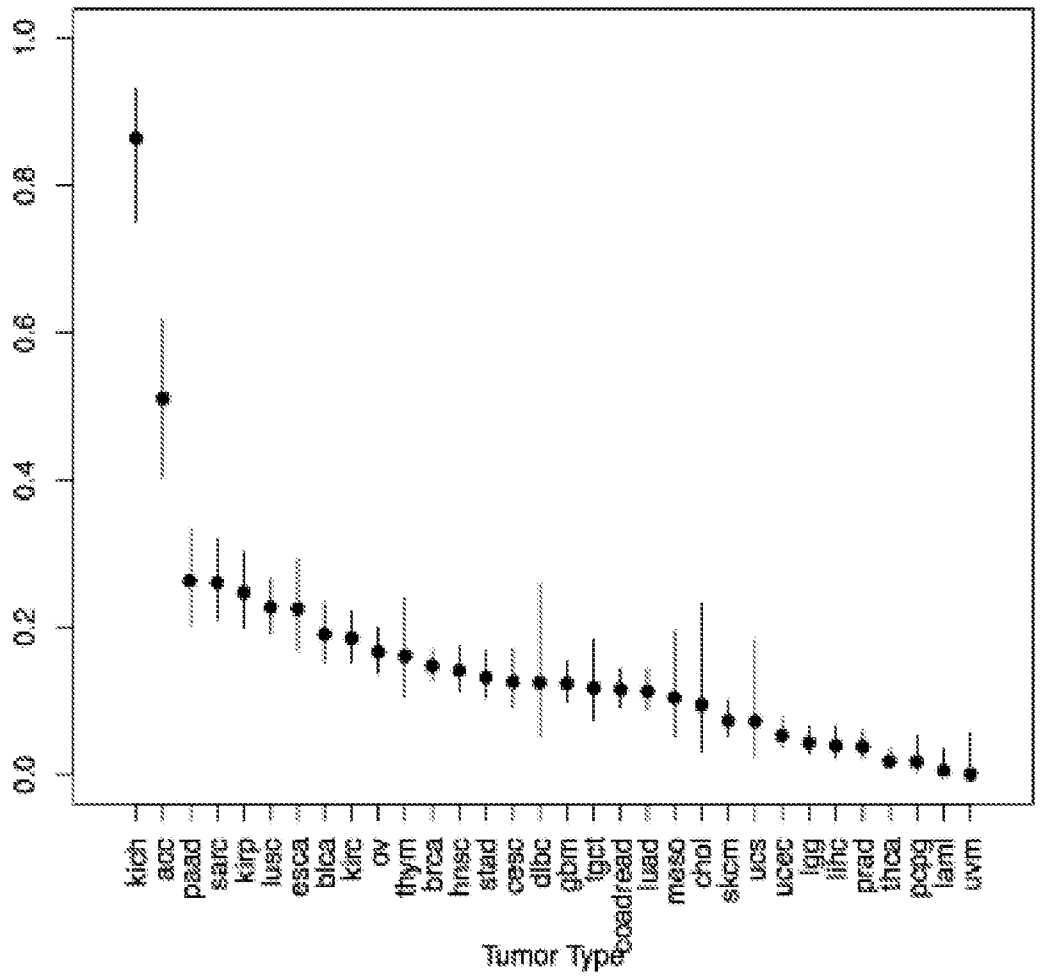

Non-individual matched
Regression
X=Y
Proportion of individuals with LOH (absolute)
Proportion of individuals with LOH (relative)
0.0
0.1
0.2
0.3
0.4
0.5

Figure 10A



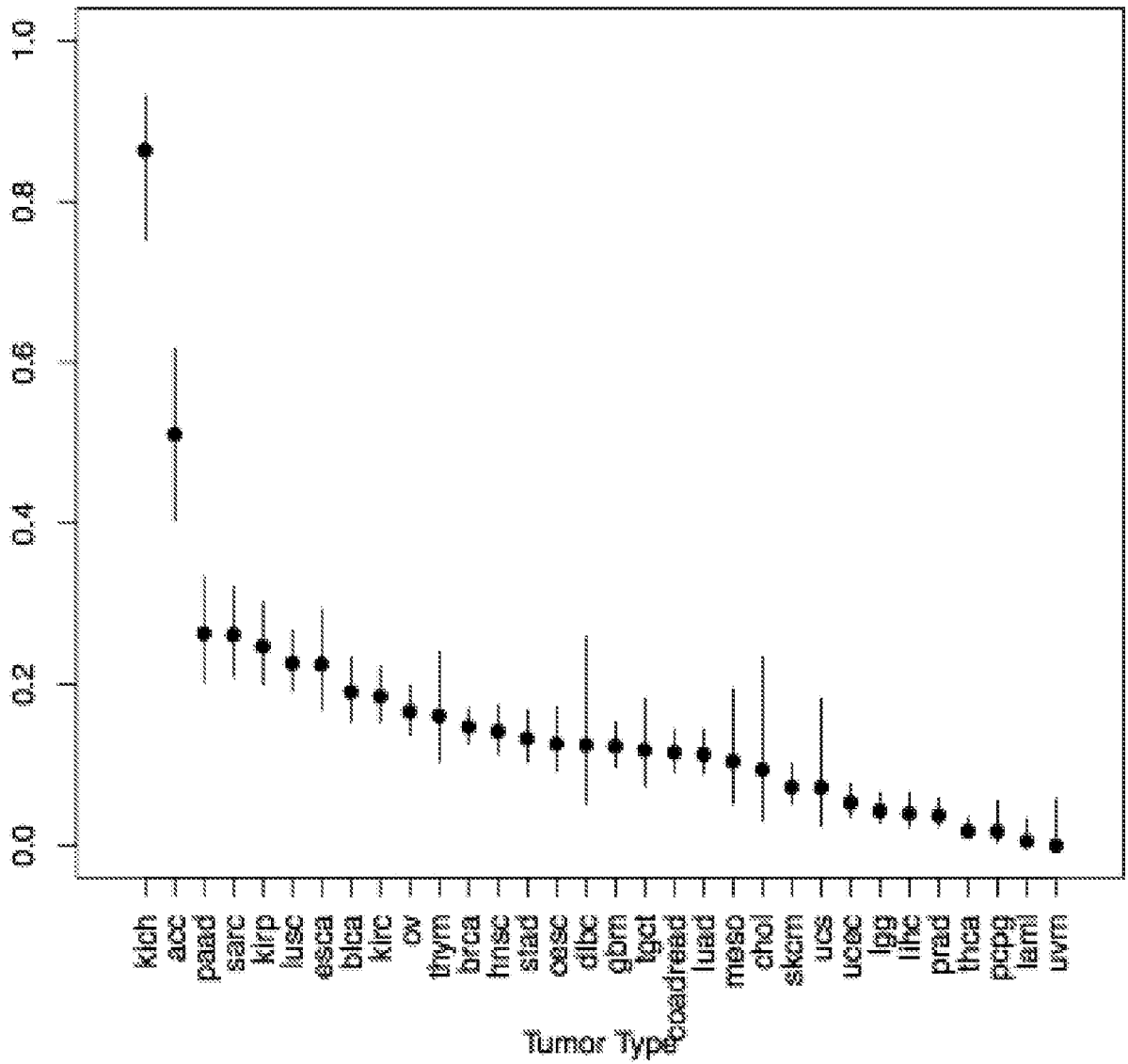
HLA-A

Figure 10B



HLA-B

Figure 10C



HLA-C

Figure 11

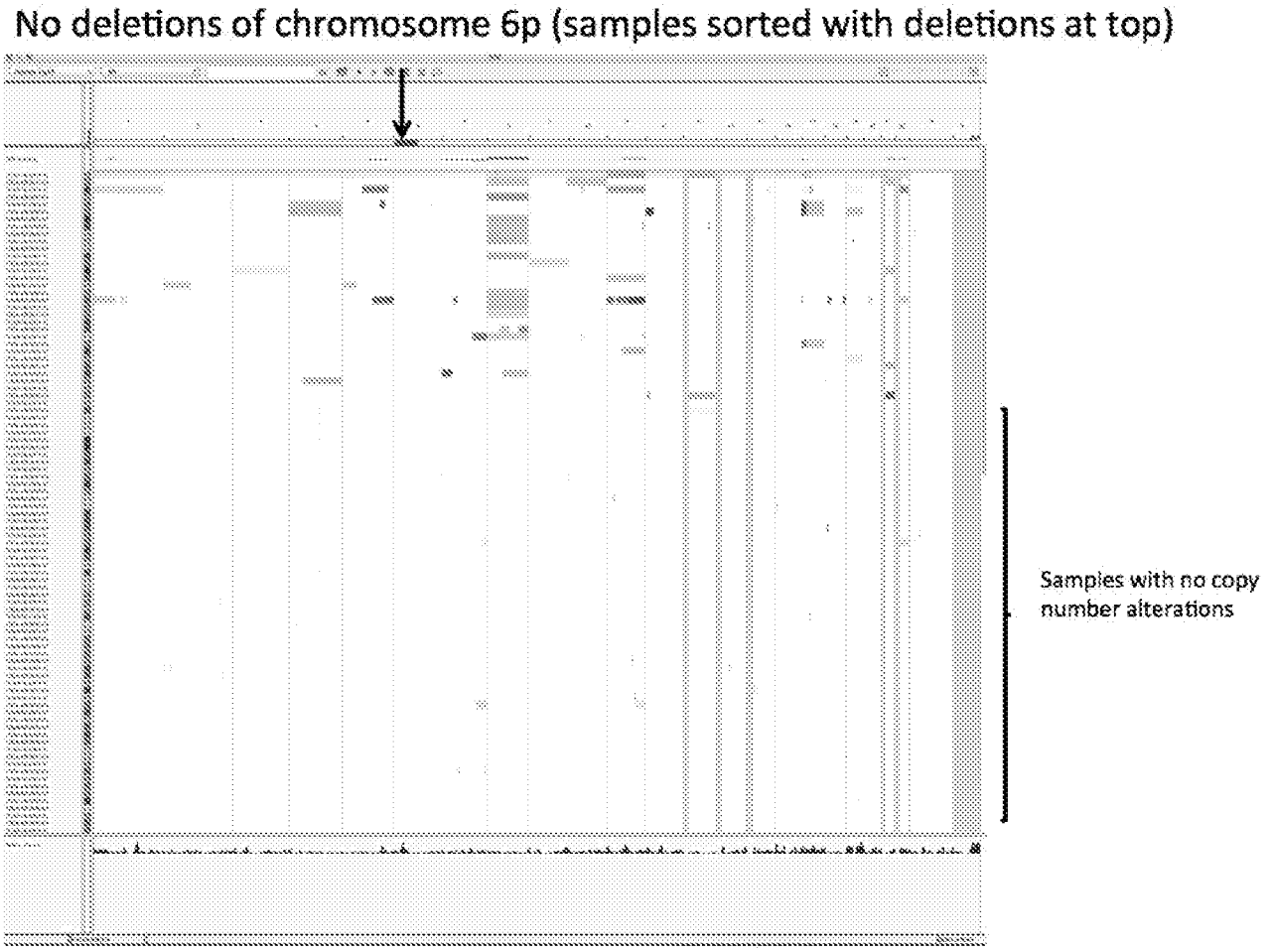

No deletions of chromosome 6p (samples sorted with deletions at top)
Samples with no copy number alterations

Figure 12

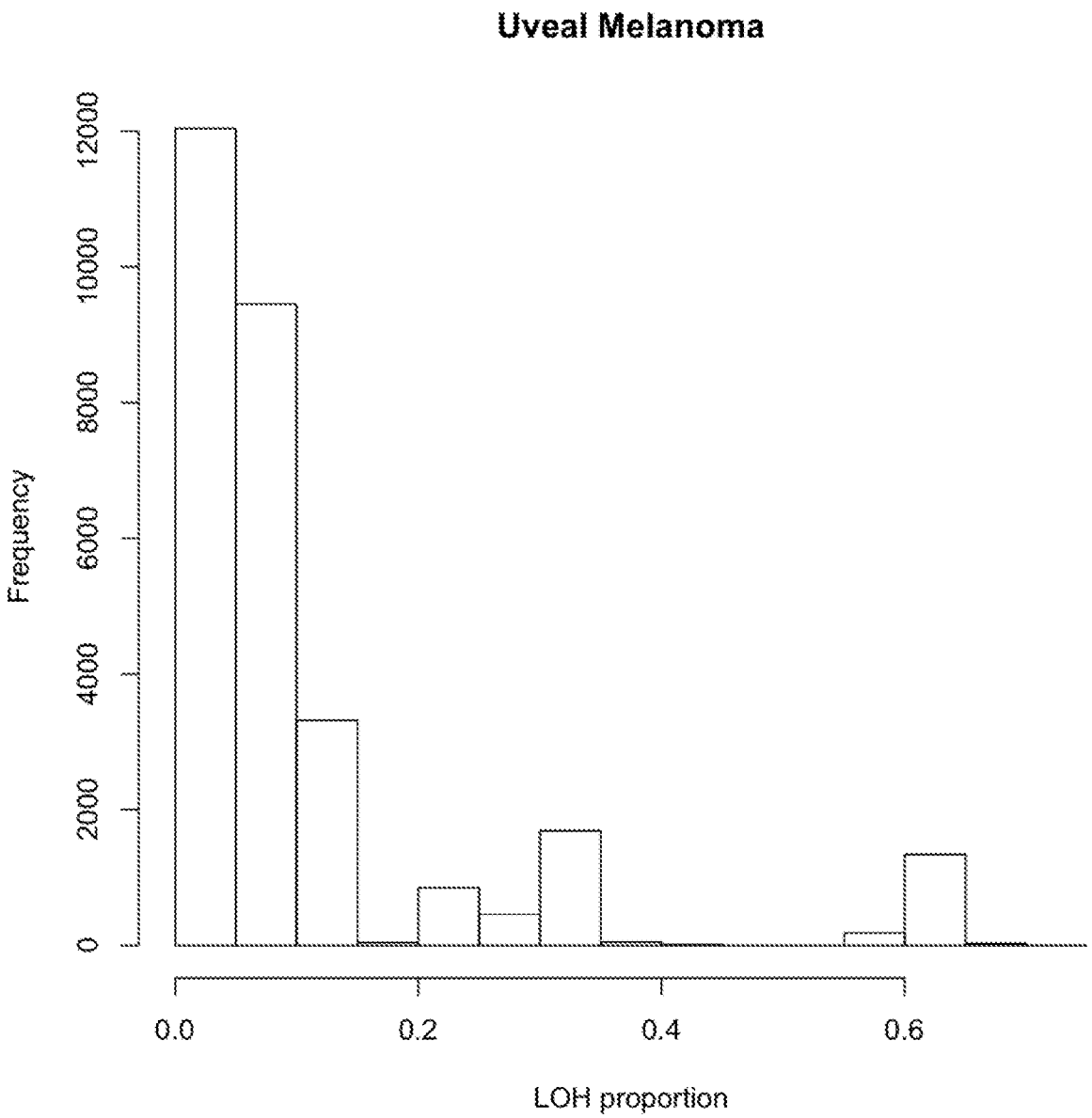

Uveal Melanoma
Frequency
0
2000
4000
6000
8000
10000
12000
0.0
0.2
0.4
0.6
LOH proportion

Figure 13

TCGA Study Abbreviations

Study Abbreviation	Study Name
LAML	Acute Myeloid Leukemia
ACC	Adrenocortical carcinoma
BLCA	Bladder Urothelial Carcinoma
LGG	Brain Lower Grade Glioma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
LCML	Chronic Myelogenous Leukemia
COAD	Colon adenocarcinoma
CNTL	Controls
ESCA	Esophageal carcinoma
FPPP FFPE	Pilot Phase II
GBM	Glioblastoma multiforme
HNSC	Head and Neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
MESO	Mesothelioma
MISC	Miscellaneous
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular Germ Cell Tumors
THYM	Thymoma
THCA	Thyroid carcinoma
UCS	Uterine Carcinosarcoma
UCEC	Uterine Corpus Endometrial Carcinoma
UVM	Uveal Melanoma

Figure 14

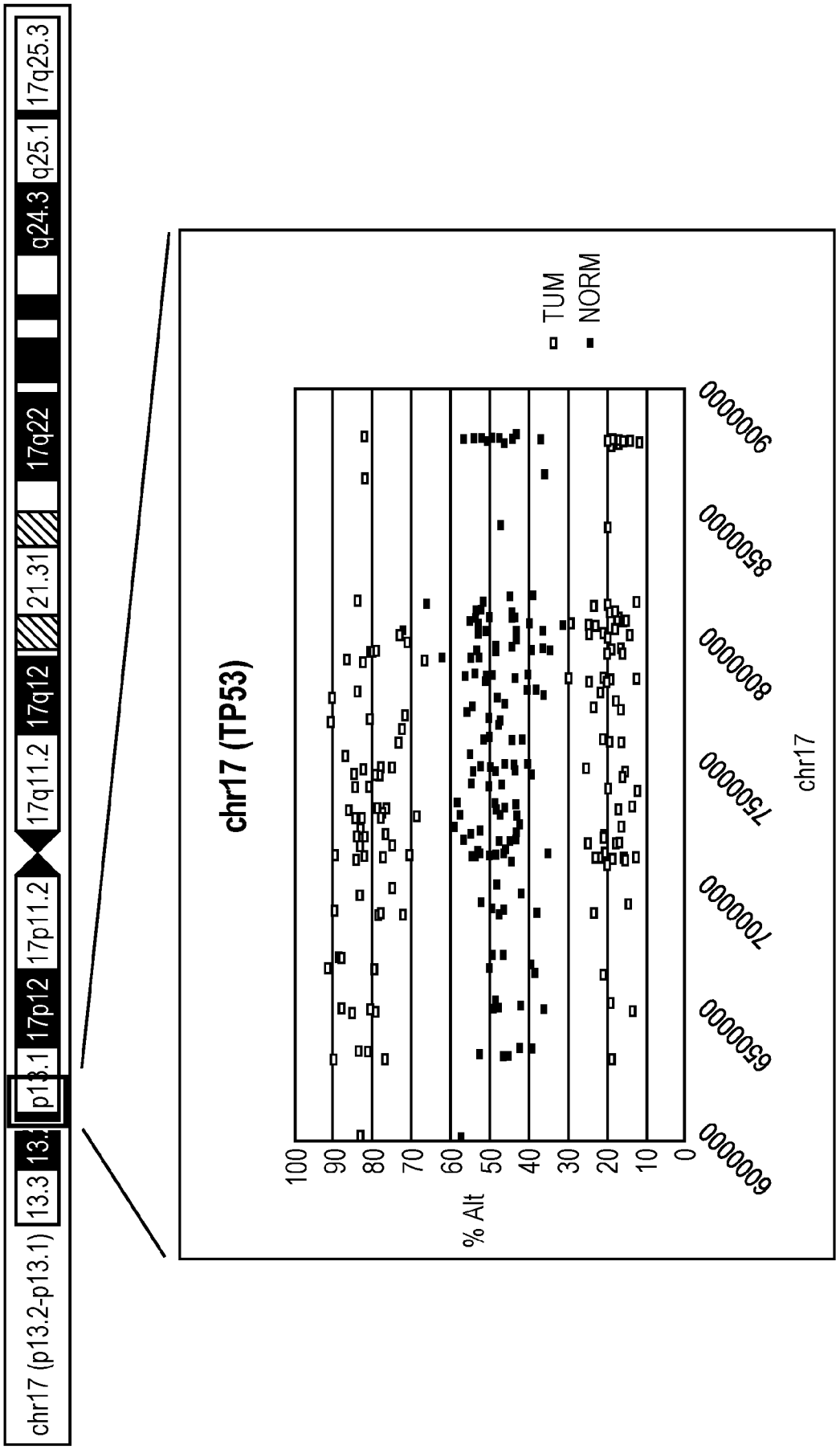

chr17 (p13.2-p13.1)
13.3
13.2
p13.1
17p12
17p11.2
17q11.2
17q12
21.31
17q22
q24.3
q25.1
17q25.3
chr17 (TP53)
TUM
NORM
% Alt
100
90
80
70
60
50
40
30
20
10
0
0000009
6500000
7000000
7500000
0000008
8500000
0000009
chr17

Figure 15

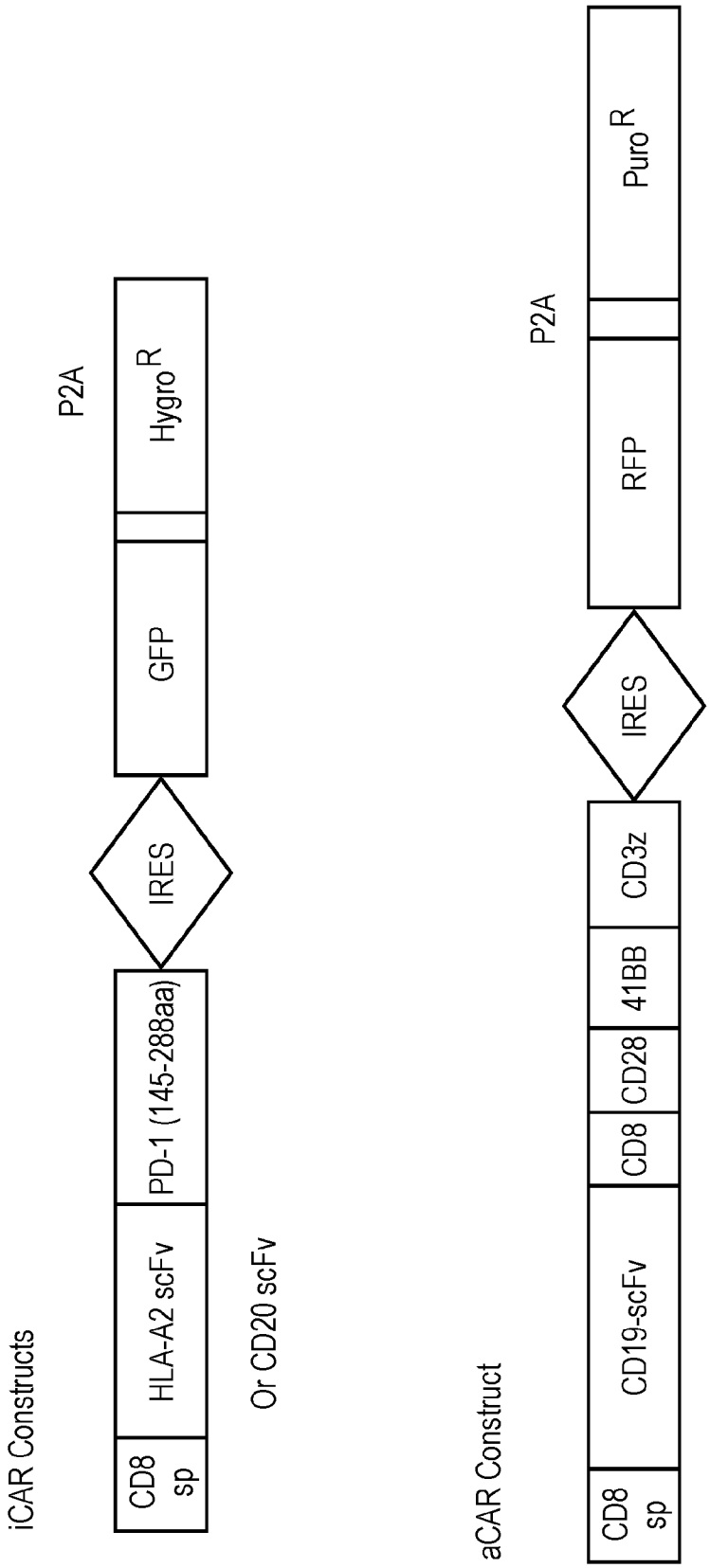

iCAR Constructs
CD8 sp
HLA-A2 scFv
PD-1 (145-288aa)
IRES
GFP
P2A
Hygro R
Or CD20 scFv
aCAR Construct
CD8 sp
CD19-scFv
CD8
CD28
41BB
CD3z
IRES
RFP
P2A
Puro R

Figure 16

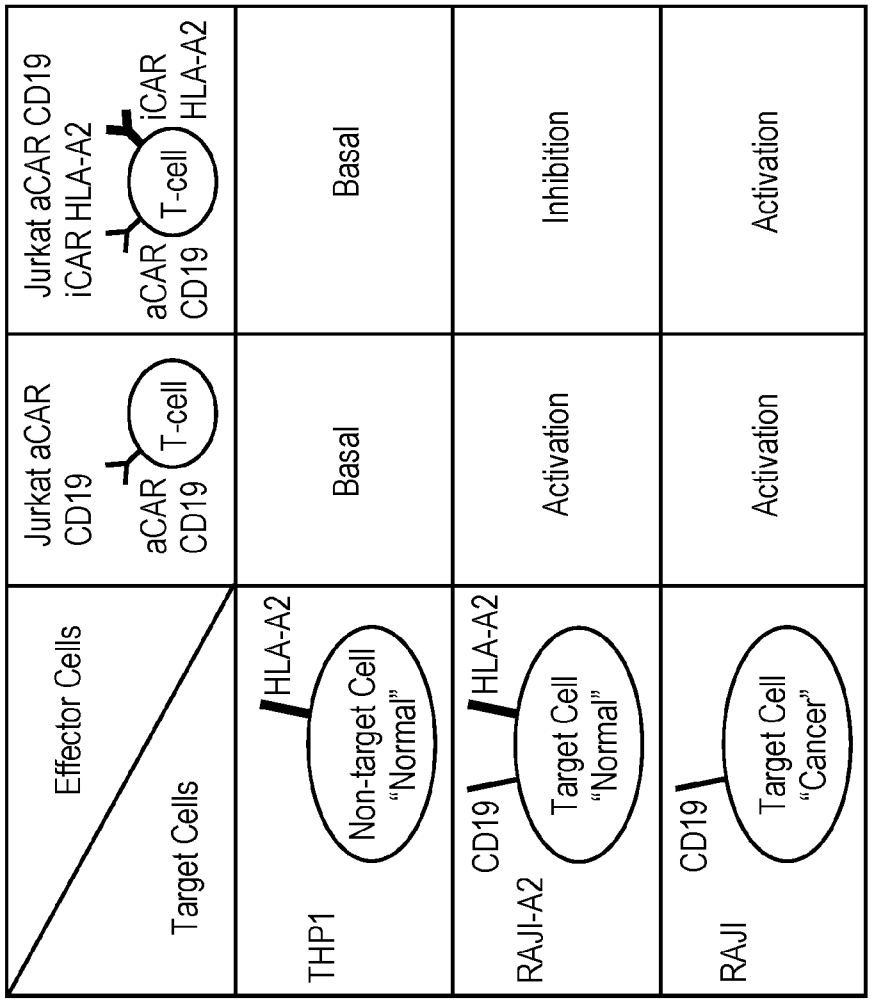

A Schematic illustration of the assay
Effector Cells
Target Cells
Jurkat aCAR CD19
aCAR CD19
T-cell
Jurkat aCAR CD19 iCAR HLA-A2
aCAR CD19
T-cell
iCAR HLA-A2
THP1
HLA-A2
Non-target Cell "Normal"
Basal
Basal
RAJI-A2
CD19
HLA-A2
Target Cell "Normal"
Activation
Inhibition
RAJI
CD19
Target Cell "Cancer"
Activation
Activation
B IL-2 secretion as measured by ELISA
IL-2 secretion-ELISA analysis
Jurkat aCAR-CD19_iCAR-HLA-A2 (E:T 1:1)
Jurkat aCAR-CD19 (E:T 1:1)
THP1-
Raji-A2
Raji
0
0.05
11
60
89
63
0
20
40
60
80
100
120
IL2 [pg/ml]

Figure 17

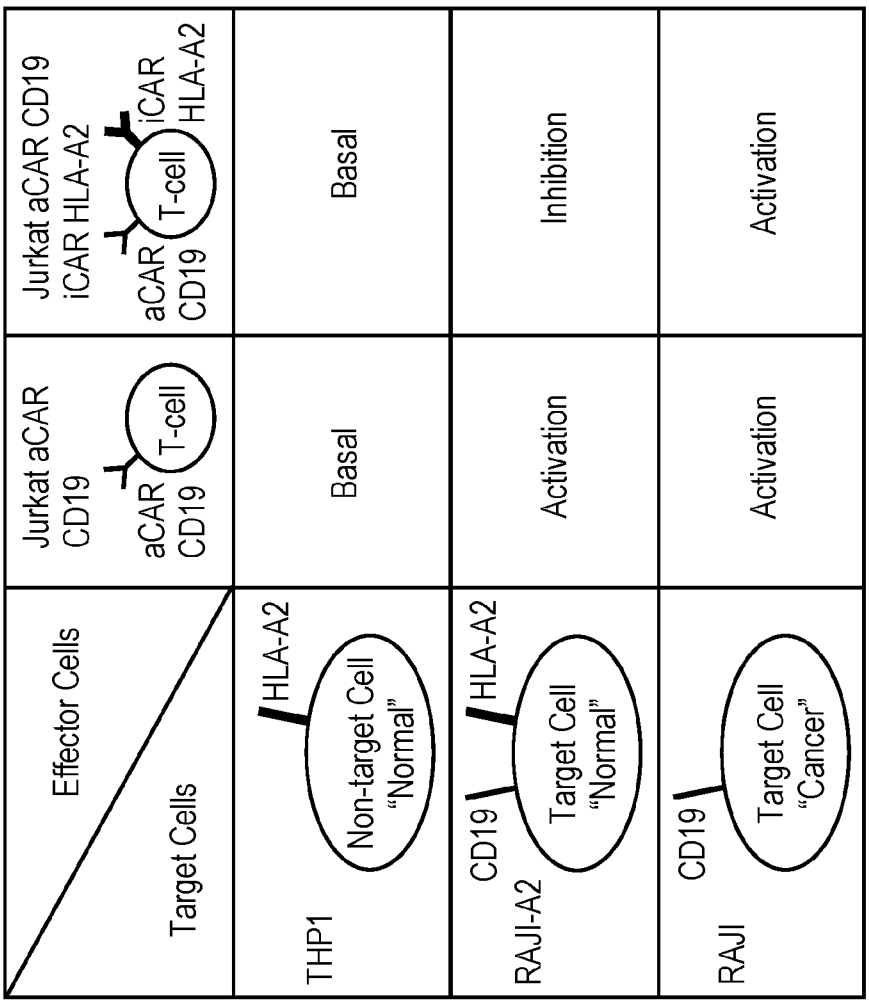

A Schematic illustration of the assay
Effector Cells
Target Cells
Jurkat aCAR CD19
aCAR CD19
T-cell
Jurkat aCAR CD19 iCAR HLA-A2
aCAR CD19
T-cell
iCAR HLA-A2
THP1
HLA-A2
Non-target Cell "Normal"
Basal
Basal
RAJI-A2
CD19
HLA-A2
Target Cell "Normal"
Activation
Inhibition
RAJI
CD19
Target Cell "Cancer"
Activation
Activation
B IL-2 secretion as measured by CBA
IL-2 secretion-CBA analysis
Jurkat aCAR-CD19_iCAR-HLA-A2 (E:T 1:1)
Jurkat aCAR-CD19 (E:T 1:1)
THP1-
Raji-A2
Raji
1
9
44
64
47
0
10
20
30
40
50
60
70
Averaged IL2 Conc [pg/ml]

Figure 18

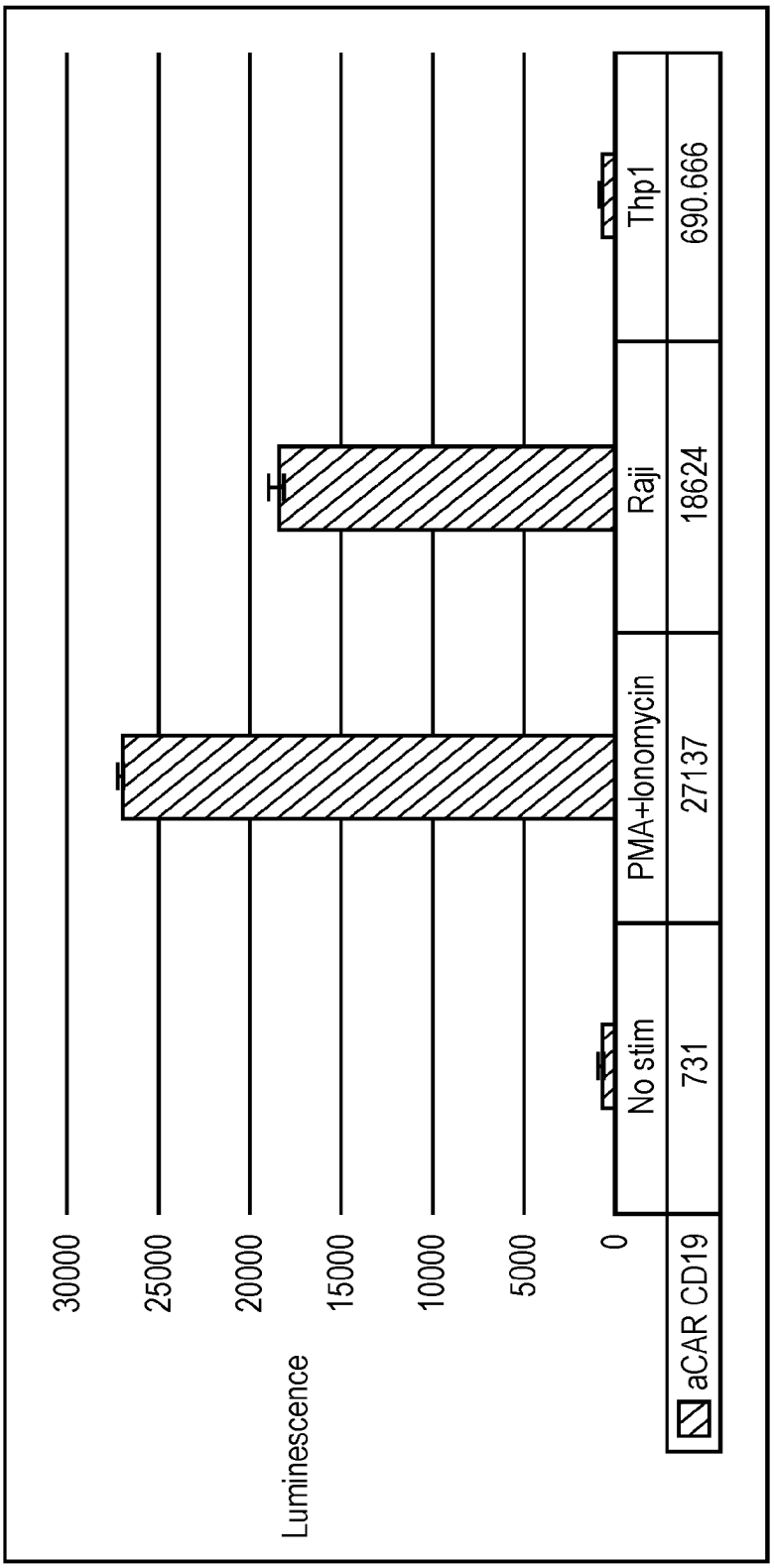

30000
25000
20000
15000
10000
5000
0
Luminescence
aCAR CD19
No stim
731
PMA+Ionomycin
27137
Raji
18624
Thp1
690.666

Figure 19

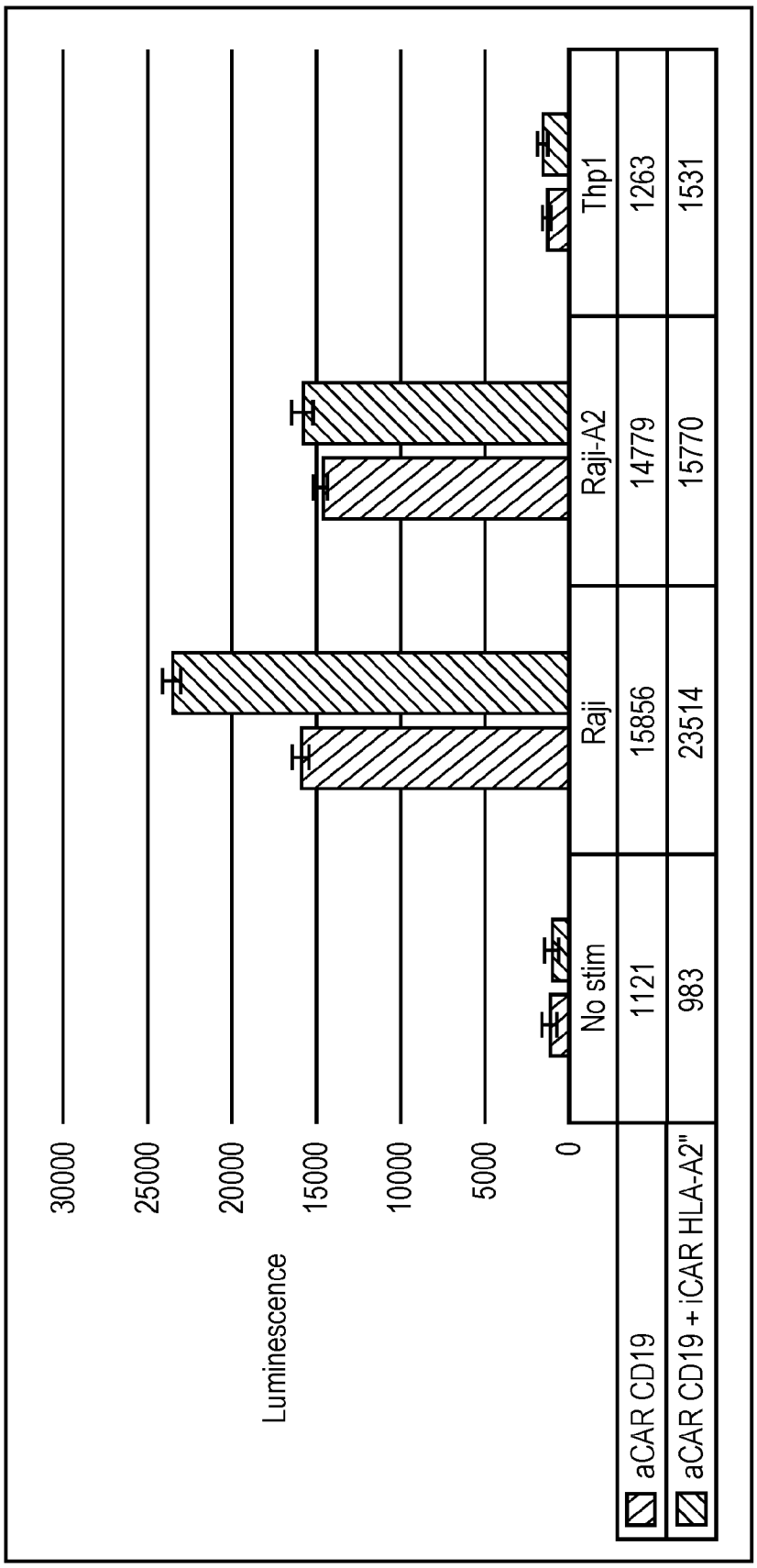

30000
25000
20000
15000
10000
5000
0
Luminescence
No stim
Raji
Raji-A2
Thp1
aCAR CD19
1121
15856
14779
1263
aCAR CD19 + iCAR HLA-A2"
983
23514
15770
1531

Figure 20

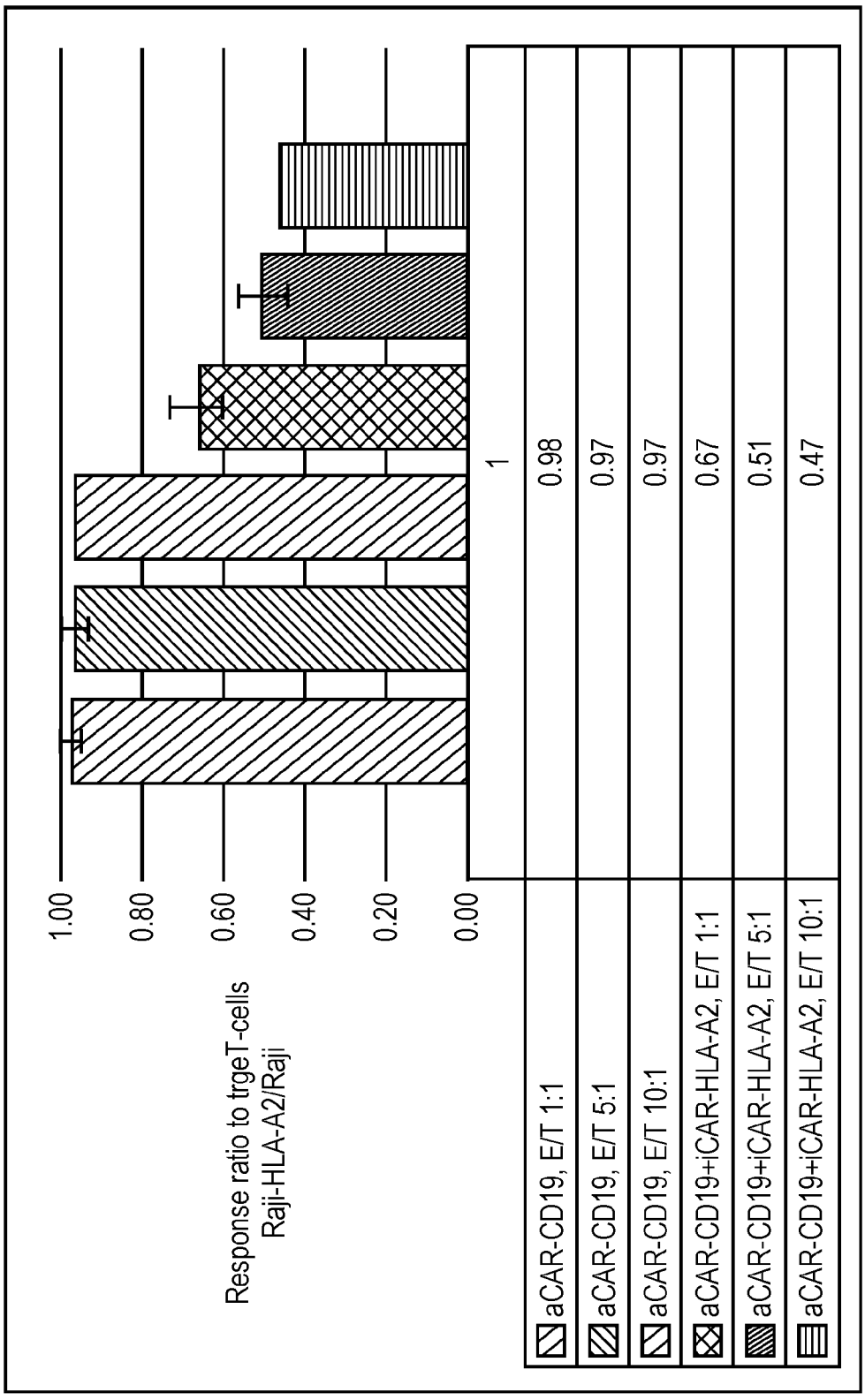

Response ratio to trgeT-cells
Raji-HLA-A2/Raji
1.00
0.80
0.60
0.40
0.20
0.00
1
0.98
0.97
0.97
0.67
0.51
0.47
aCAR-CD19, E/T 1:1
aCAR-CD19, E/T 5:1
aCAR-CD19, E/T 10:1
aCAR-CD19+iCAR-HLA-A2, E/T 1:1
aCAR-CD19+iCAR-HLA-A2, E/T 5:1
aCAR-CD19+iCAR-HLA-A2, E/T 10:1

FIGURE 21

CD19 aCAR_IRES_RFP_P2A_Puro- DNA sequence

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGG
CCGGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAA
CCAGATGGAACTGTTAAACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAG
CAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTACACGTTCGGA
GGGGGGACCAAGCTGGAGATCACAGGTGGCGGTGGCTCGGGCGGTGGTGGGTCGGGTGGC
GGCGGATCTGAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGC
CTGTCCGTCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATT
CGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACA
TACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACTCCAAGAGCCAA
GTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCAAA
CATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGAACCTCAGTCACC
GTCTCCTCAACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCC
CAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACC
CGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGG
GTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTG
TACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGT
TCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGC
AGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTT
GGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC
GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAG
ATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCAC
GACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG
CAGGCCCTGCCGCCTCGGTGAGCGGCCGCAAATTCCGCCCCTCTCCCTCCCCCCCCCCTA
ACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTT
CCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGA
CGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCG
TGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTT
GCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTAT
AAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGG
AAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGG
TACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGT
CGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAA
CACGATAATACCATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAG
CTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGC
AAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCC
TTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAACCAC
ACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGA
GTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGAC
GGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTG
ATGCAGAAGAAAACACTCGGCTGGGAGGCCAACACCGAGATGCTGTACCCCGCTGACGGC
GGCCTGGAAGGCAGAAGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGC
AACTTCAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTC
TACTATGTGGACCACAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTCGAG
CAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTT
AATGGATCCGGCGCGACAAACTTTAGCTTGCTGAAGCAAGCTGGTGACGTGGAGGAGAAT
CCCGGCCCTATGGCCACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGCGACGACGTC
CCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGCCACACC
GTCGATCCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCGC
GTCGGGCTCGACATCGGCAAGGTGTGGGTCGCGGACGACGGCGCCGCGGTGGCGGTCTGG

ACCACGCCGGAGAGCGTCGAAGCGGGGGCGGTGTTCGCCGAGATCGGCCCGCGCATGGCC
GAGTTGAGCGGTTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCAC
CGGCCCAAGGAGCCCGCGTGGTTCCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGC
AAGGGTCTGGGCAGCGCCGTCGTGCTCCCCGGAGTGGAGGCGGCCGAGCGCGCCGGGGTG
CCCGCCTTCCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTC
ACCGTCACCGCCGACGTCGAGGTGCCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAG
CCCGGTGCCTGA

CD19 aCAR- protein sequence
MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE
QEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQES
GPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETT
YYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAM
DYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL
GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

RFP-protein sequence
MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVV
EGGPLPFAFDILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTY
EDGGVLTATQDTSLQDGCLIYNVKIRGVNFPSNGPVMQKKTLGWEANTEM
LYPADGGLEGRSDMALKLVGGGHLICNFKTTYRSKKPAKNLKMPGVYYVD
HRLERIKEADKETYVEQHEVAVARYCDLPSKLGHKLN

Puromycin resistance-protein sequence
MATEYKPTVRLATRDDVPRAVRTLAAAFADYPATRHTVDPDRHIERVTEL
QELFLTRVGLDIGKVWVADDGAAVAVWTTPESVEAGAVFAEIGPRMAELS
GSRLAAQQQMEGLLAPHRPKEPAWFLATVGVSPDHQGKGLGSAVVLPGVE
AAERAGVPAFLETSAPRNLPFYERLGFTVTADVEVPEGPRTWCMTRKPGA

CD20 iCAR_IRES_GFP_P2A_Hygro- DNA sequence
ATGGCACTGCCTGTGACCGCCCTGCTGCTGCCACTGGCCCTGCTGCTGCACGCAGCCAGG
CCCGACATCGTGCTGACACAGAGCCCAGCAATCCTGTCCGCCTCTCCTGGAGAGAAGGTG
ACCATGACATGCCGCGCCAGCTCCTCTGTGAACTACATGGATTGGTATCAGAAGAAGCCT
GGCAGCTCCCCAAAGCCCTGGATCTACGCCACCAGCAATCTGGCCTCCGGCGTGCCAGCA
CGGTTCAGCGGCTCCGGCTCTGGCACCAGCTATTCCCTGACAATCTCCAGAGTGGAGGCA
GAGGACGCAGCAACCTACTATTGCCAGCAGTGGTCTTTCAACCCCCCTACCTTTGGCGGC
GGCACAAAGCTGGAGATCAAGGGCTCTACAAGCGGAGGAGGCTCTGGAGGAGGCAGCGGA
GGCGGCGGCTCTAGCGAGGTGCAGCTGCAGCAGAGCGGAGCAGAGCTGGTGAAGCCTGGA
GCCTCCGTGAAGATGTCTTGTAAGGCCAGCGGCTACACCTTCACATCCTATAATATGCAC
TGGGTGAAGCAGACCCCAGGACAGGGCCTGGAGTGGATCGGAGCAATCTACCCAGGAAAC
GGCGACACAAGCTATAATCAGAAGTTTAAGGGCAAGGCCACCCTGACAGCCGATAAGTCC
TCTAGCACCGCCTACATGCAGCTGTCCTCTCTGACATCCGAGGACTCTGCCGATTACTAT
TGTGCCCGGTCCAACTACTATGGCAGCTCCTACTGGTTCTTTGACGTGTGGGGAGCAGGC
ACCACAGTGACCGTGTCTAGCACCGAGAGGAGAGCAGAGGTGCCCACAGCACACCCATCT
CCAAGCCCTAGGCCAGCAGGACAGTTCCAGACCCTGGTGGTGGGAGTGGTGGGAGGCCTG
CTGGGCTCTCTGGTGCTGCTGGTGTGGGTGCTGGCCGTGATCTGCAGCAGGGCCGCCCGC
GGCACCATCGGCGCCAGGCGCACAGGCCAGCCTCTGAAGGAGGACCCTTCCGCCGTGCCA
GTGTTCTCTGTGGACTACGGCGAGCTGGATTTTCAGTGGCGGGAGAAAACCCCAGAGCCA
CCTGTGCCCTGCGTGCCTGAGCAGACCGAGTATGCCACAATCGTGTTTCCATCCGGAATG
GGCACAAGCTCCCCTGCAAGGAGAGGCAGCGCCGACGGACCACGGTCCGCCCAGCCACTG
CGGCCCGAGGATGGCCACTGTTCTTGGCCCCTGTGACGCCCCTCTCCCCCCCCCCCCTCT
CCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTT
GTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCT

GGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAA
GGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACG
TCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGC
CAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTG
AGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTG
AAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGC
TTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTG
GTTTTCCTTTGAAAAACACGATGATAAGGCTTGCCACAACCCGTACCAAAGATGGTGTCC
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GACTCTGTGAGCGATCTGATGCAGCTGTCCGAGGGAGAGGAGTCCAGGGCCTTCTCTTTT
GATGTGGGCGGCAGGGGATACGTGCTGAGGGTGAATAGCTGCGCCGACGGCTTCTATAAG
GATAGATACGTGTATAGACACTTTGCCTCCGCCGCCCTGCCAATCCCAGAGGTGCTGGAC
ATCGGCGAGTTTTCCGAGTCTCTGACCTACTGTATCAGCCGGAGAGCCCAGGGAGTGACC
CTGCAGGATCTGCCTGAGACAGAGCTGCCAGCCGTGCTGCAGCCAGTGGCAGAGGCTATG
GACGCAATCGCCGCCGCCGACCTGTCTCAGACAAGCGGCTTCGGCCCTTTTGGCCCACAG
GGCATCGGCCAGTACACCACATGGAGGGACTTCATCTGCGCCATCGCCGATCCTCACGTG
TATCACTGGCAGACCGTGATGGACGATACAGTGAGCGCCTCCGTGGCACAGGCCCTGGAC
GAGCTGATGCTGTGGGCCGAGGATTGTCCAGAGGTGCGCCACCTGGTGCACGCAGACTTT
GGCAGCAACAATGTGCTGACCGATAATGGCCGGATCACAGCCGTGATCGACTGGTCCGAG
GCCATGTTCGGCGATTCTCAGTACGAGGTGGCCAACATCTTCTTTTGGAGGCCTTGGCTG
GCCTGCATGGAGCAGCAGACCCGCTATTTTGAGAGGCGCCACCCTGAGCTGGCCGGCTCT
CCACGGCTGAGAGCATACATGCTGCGCATCGGCCTGGACCAGCTGTATCAGAGCCTGGTG
GATGGCAATTTCGACGATGCAGCATGGGCACAGGGCCGGTGCGACGCAATCGTGAGATCC
GGCGCCGGCACCGTGGGCCGGACACAGATCGCACGGCGGAGCGCCGCCGTGTGGACCGAC
GGATGCGTGGAGGTGCTGGCCGATTCTGGCAACAGGCGCCCAAGCACAAGGCCCCGCGCC
AAGGAGTGA

CD20 iCAR -protein sequence

MALPVTALLLPLALLLHAARPDIVLTQSPAILSASPGEKVTMTCRASSSV
NYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEA
EDAATYYCQQWSFNPPTFGGGTKLEIKGSTSGGGSGGGSGGGGSSEVQLQ
QSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGN
GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSS
YWFFDVWGAGTTVTVSSTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGL
LGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELD
FQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPL
RPEDGHCSWPL

GFP--protein sequence

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICT
TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN
VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

Hygromycin resistance--protein sequence
MDRSGKPELTATSVEKFLIEKFDSVSDLMQLSEGEESRAFSFDVGGRGYV
LRVNSCADGFYKDRYVYRHFASAALPIPEVLDIGEFSESLTYCISRRAQG
VTLQDLPETELPAVLQPVAEAMDAIAAADLSQTSGFGPFGPQGIGQYTTW
RDFICAIADPHVYHWQTVMDDTVSASVAQALDELMLWAEDCPEVRHLVHA
DFGSNNVLTDNGRITAVIDWSEAMFGDSQYEVANIFFWRPWLACMEQQTR
YFERRHPELAGSPRLRAYMLRIGLDQLYQSLVDGNFDDAAWAQGRCDAIV
RSGAGTVGRTQIARRSAAVWTDGCVEVLADSGNRRPSTRPRAKE

HLA-A2 iCAR_IRES_ GFP_P2A_Hygro- DNA sequence
ATGGCACTGCCAGTGACCGCCCTGCTGCTGCCTCTGGCCCTGCTGCTGCACGCAGCCAGA
CCCGAGCAGAAGCTGATCTCCGAGGAGGACCTGCAGGTGCAGCTGCAGCAGTCTGGACCT
GAGCTGGTGAAGCCAGGAGCCTCCGTGAAGATGTCTTGCAAGGCCAGCGGCTACACCTTC
ACATCTTATCACATCCAGTGGGTGAAGCAGCGGCCCGGACAGGGCCTGGAGTGGATCGGA
TGGATCTACCCAGGCGACGGCTCCACACAGTATAACGAGAAGTTCAAGGGCAAGACCACA
CTGACCGCCGATAAGAGCAGCAGCACCGCCTACATGCTGCTGAGCAGCCTGACCAGCGAG
GACAGCGCCATCTACTTTTGCGCCAGGGAGGGCACATACTATGCTATGGACTATTGGGGC
CAGGGCACCAGCGTGACAGTGTCTAGCGGAGGAGGAGGCTCCGGAGGAGGAGGCTCTGGC
GGCGGCGGCAGCGACGTGCTGATGACCCAGACACCACTGAGCCTGCCCGTGAGCCTGGGC
GATCAGGTGAGCATCTCCTGTAGATCCTCTCAGAGCATCGTGCACTCCAACGGCAATACC
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TCTAATCGGTTCAGCGGCGTGCCTGACAGATTTTCTGGCAGCGGCTCCGGCACCGACTTC
ACCCTGAAGATCAGCCGGGTGGAGGCAGAGGATCTGGGCGTGTACTATTGTTTCCAGGGC
TCCCACGTGCCACGCACCTTTGGCGGCGGCACAAAGCTGGAGATCAAGACCGAGAGGAGA
GCAGAGGTGCCCACAGCACACCCATCTCCAAGCCCTAGGCCAGCAGGACAGTTCCAGACC
CTGGTGGTGGGAGTGGTGGGAGGCCTGCTGGGCTCTCTGGTGCTGCTGGTGTGGGTGCTG
GCCGTGATCTGCAGCAGGGCCGCCCGCGGCACCATCGGCGCCAGGCGCACAGGCCAGCCT
CTGAAGGAGGACCCTTCCGCCGTGCCAGTGTTCTCTGTGGACTACGGCGAGCTGGATTTT
CAGTGGCGGGAGAAAACCCCAGAGCCACCTGTGCCCTGCGTGCCTGAGCAGACCGAGTAT
GCCACAATCGTGTTTCCATCCGGAATGGGCACAAGCTCCCCTGCAAGGAGAGGCAGCGCC
GACGGACCACGGTCCGCCCAGCCACTGCGGCCCGAGGATGGCCACTGTTCTTGGCCCCTG
TGACGCCCCTCTCCCCCCCCCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGC
CGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCT
TTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGT
CTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCT
CTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCC
CCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAG
GCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTC
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CCACAACCCGTACCAAAGATGGTGTCCAAGGGAGAGGAGCTGTTCACCGGAGTGGTGCCC
ATCCTGGTGGAGCTGGACGGCGATGTGAATGGCCACAAGTTTAGCGTGTCCGGAGAGGGA
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CCCGTGCCTTGGCCAACCCTGGTGACCACACTGACATACGGCGTGCAGTGTTTTTCTCGC
TATCCCGACCACATGAAGCAGCACGATTTCTTTAAGAGCGCCATGCCTGAGGGCTACGTG
CAGGAGCGGACCATCTTCTTTAAGGACGATGGCAACTATAAGACCAGAGCCGAGGTGAAG
TTCGAGGGCGACACACTGGTGAACAGGATCGAGCTGAAGGGCATCGACTTTAAGGAGGAT
GGCAATATCCTGGGCCACAAGCTGGAGTACAACTATAATTCCCACAACGTGTACATCATG
GCCGATAAGCAGAAGAACGGCATCAAGGTCAATTTCAAGATCAGACACAATATCGAGGAC
GGCTCTGTGCAGCTGGCCGATCACTACCAGCAGAACACCCCAATCGGCGACGGACCCGTG
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AAGAGGGATCACATGGTGCTGCTGGAGTTTGTGACCGCAGCAGGAATCACACTGGGAATG
GACGAGCTGTATAAGGGCAGCGGCGCCACCAACTTCTCCCTGCTGAAGCAGGCAGGCGAC
GTGGAGGAGAATCCAGGACCTATGGATAGAAGCGGCAAGCCAGAGCTGACCGCCACATCC
GTGGAGAAGTTCCTGATCGAGAAGTTTGACTCTGTGAGCGATCTGATGCAGCTGTCCGAG

GGAGAGGAGTCCAGGGCCTTCTCTTTTGATGTGGGCGGCAGGGGATACGTGCTGAGGGTG
AATAGCTGCGCCGACGGCTTCTATAAGGATAGATACGTGTATAGACACTTTGCCTCCGCC
GCCCTGCCAATCCCAGAGGTGCTGGACATCGGCGAGTTTTCCGAGTCTCTGACCTACTGT
ATCAGCCGGAGAGCCCAGGGAGTGACCCTGCAGGATCTGCCTGAGACAGAGCTGCCAGCC
GTGCTGCAGCCAGTGGCAGAGGCTATGGACGCAATCGCCGCCGCCGACCTGTCTCAGACA
AGCGGCTTCGGCCCTTTTGGCCCACAGGGCATCGGCCAGTACACCACATGGAGGGACTTC
ATCTGCGCCATCGCCGATCCTCACGTGTATCACTGGCAGACCGTGATGGACGATACAGTG
AGCGCCTCCGTGGCACAGGCCCTGGACGAGCTGATGCTGTGGGCCGAGGATTGTCCAGAG
GTGCGCCACCTGGTGCACGCAGACTTTGGCAGCAACAATGTGCTGACCGATAATGGCCGG
ATCACAGCCGTGATCGACTGGTCCGAGGCCATGTTCGGCGATTCTCAGTACGAGGTGGCC
AACATCTTCTTTTGGAGGCCTTGGCTGGCCTGCATGGAGCAGCAGACCCGCTATTTTGAG
AGGCGCCACCCTGAGCTGGCCGGCTCTCCACGGCTGAGAGCATACATGCTGCGCATCGGC
CTGGACCAGCTGTATCAGAGCCTGGTGGATGGCAATTTCGACGATGCAGCATGGGCACAG
GGCCGGTGCGACGCAATCGTGAGATCCGGCGCCGGCACCGTGGGCCGGACACAGATCGCA
CGGCGGAGCGCCGCCGTGTGGACCGACGGATGCGTGGAGGTGCTGGCCGATTCTGGCAAC
AGGCGCCCAAGCACAAGGCCCCGCGCCAAGGAGTGA

HLA-A2 iCAR --protein sequence
MALPVTALLLPLALLLHAARPEQKLISEEDLQVQLQQSGPELVKPGASVK
MSCKASGYTFTSYHIQWVKQRPGQGLEWIGWIYPGDGSTQYNEKFKGKTT
LTADKSSSTAYMLLSSLTSEDSAIYFCAREGTYYAMDYWGQGTSVTVSSG
GGGSGGGGSGGGGSDVLMTQTPLSLPVSLGDQVSISCRSSQSIVHSNGNT
YLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAE
DLGVYYCFQGSHVPRTFGGGTKLEIKTERRAEVPTAHPSPSPRPAGQFQT
LVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPV
FSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSA
DGPRSAQPLRPEDGHCSWPL

Figure 22

Chr	Gene
1	ABCA4
1	ADAM30
1	ASTN1
1	C1orf101
1	CACNA1S
1	CATSPER4
1	CD101
1	CD164L2
1	CD1A
1	CD1C
1	CD244
1	CD34
1	CELSR2
1	CHRNB2
1	CLCA2
1	CLSTN1
1	CR1
1	CR2
1	CRB1
1	CSF3R
1	CSMD2
1	ECE1
1	ELTD1
1	EMC1
1	EPHA10
1	EPHA2
1	ERMAP
1	FCAMR
1	FCER1A
1	FCGR1B
1	FCGR2A
1	FCGR2B
1	FCGR3A
1	FCRL1
1	FCRL3
1	FCRL4
1	FCRL5
1	FCRL6
1	GJB4
1	GPA33
1	GPR157
1	GPR37L1
1	GPR88
1	HCRTR1

Chr	Gene
1	IGSF3
1	IGSF9
1	IL22RA1
1	ITGA10
1	KIAA1324
1	KIAA2013
1	LDLRAD2
1	LEPR
1	LRIG2
1	LRP8
1	LRRC52
1	LRRC8B
1	LRRN2
1	LY9
1	MR1
1	MUC1
1	MXRA8
1	NCSTN
1	NFASC
1	NOTCH2
1	NPR1
1	NTRK1
1	OPN3
1	OR10J1
1	OR10J4
1	OR10K1
1	OR10R2
1	OR10T2
1	OR10X1
1	OR11L1
1	OR14A16
1	OR14I1
1	OR14K1
1	OR2AK2
1	OR2C3
1	OR2G2
1	OR2G3
1	OR2L2
1	OR2M7
1	OR2T1
1	OR2T12
1	OR2T27
1	OR2T29
1	OR2T3

Chr	Gene
1	OR2T33
1	OR2T34
1	OR2T35
1	OR2T4
1	OR2T5
1	OR2T6
1	OR2T7
1	OR2T8
1	OR2W3
1	OR6F1
1	OR6K2
1	OR6K3
1	OR6K6
1	OR6N1
1	OR6P1
1	OR6Y1
1	PEAR1
1	PIGR
1	PLXNA2
1	PTCH2
1	PTCHD2
1	PTGFRN
1	PTPRC
1	PTPRF
1	PVRL4
1	RXFP4
1	S1PR1
1	SCNN1D
1	SDC3
1	SELE
1	SELL
1	SELP
1	SEMA4A
1	SEMA6C
1	SLAMF7
1	SLAMF9
1	SLC2A7
1	SLC5A9
1	TACSTD2
1	TAS1R2
1	TIE1
1	TLR5
1	TMEM81
1	TNFRSF14

Figure 22 (Continued)

Chr	Gene
1	TNFRSF1B
1	TRABD2B
1	USH2A
1	VCAM1
1	ZP4
2	ABCG5
2	ALK
2	ASPRV1
2	ATRAID
2	CD207
2	CHRNG
2	CLEC4F
2	CNTNAP5
2	CRIM1
2	CXCR1
2	DNER
2	DPP10
2	EDAR
2	EPCAM
2	GPR113
2	GPR148
2	GPR35
2	GPR39
2	IL1RL1
2	ITGA4
2	ITGA6
2	ITGAV
2	LCT
2	LHCGR
2	LRP1B
2	LRP2
2	LY75
2	MARCO
2	MERTK
2	NRP2
2	OR6B2
2	PLA2R1
2	PLB1
2	PROKR1
2	PROM2
2	SCN7A
2	SDC1
2	TGOLN2
2	THSD7B

Chr	Gene
2	TMEFF2
2	TMEM178A
2	TPO
2	TRABD2A
3	ACKR2
3	ALCAM
3	ANO10
3	ATP13A4
3	CACNA1D
3	CACNA2D2
3	CACNA2D3
3	CASR
3	CCRL2
3	CD200
3	CD200R1
3	CD86
3	CD96
3	CDCP1
3	CDHR4
3	CELSR3
3	CHL1
3	CLDN11
3	CLDN18
3	CLSTN2
3	CSPG5
3	CX3CR1
3	CXCR6
3	DCBLD2
3	DRD3
3	EPHB3
3	GABRR3
3	GP5
3	GPR128
3	GPR15
3	GPR27
3	GRM2
3	GRM7
3	HEG1
3	HTR3C
3	HTR3D
3	HTR3E
3	IGSF11
3	IL17RC
3	IL17RD

Chr	Gene
3	IL17RE
3	IL5RA
3	IMPG2
3	ITGA9
3	ITGB5
3	KCNMB3
3	LRIG1
3	LRRC15
3	LRRN1
3	MST1R
3	NAALADL2
3	NRROS
3	OR5AC1
3	OR5H1
3	OR5H14
3	OR5H15
3	OR5H6
3	OR5K2
3	OR5K3
3	OR5K4
3	PLXNB1
3	PLXND1
3	PRRT3
3	PTPRG
3	ROBO2
3	RYK
3	SEMA5B
3	SIDT1
3	SLC22A14
3	SLC33A1
3	SLC4A7
3	SLITRK3
3	STAB1
3	SUSD5
3	TFRC
3	TLR9
3	TMEM44
3	TMPRSS7
3	TNFSF10
3	UPK1B
3	VIPR1
3	ZPLD1
4	ANTXR2
4	BTC

Figure 22 (Continued)

Chr	Gene
4	CNGA1
4	CORIN
4	EGF
4	EMCN
4	ENPEP
4	EPHA5
4	ERVMER34-1
4	EVC2
4	FAT1
4	FAT4
4	FGFRL1
4	FRAS1
4	GPR125
4	GRID2
4	GYPA
4	GYPB
4	KDR
4	KIAA0922
4	KLB
4	MFSD8
4	PARM1
4	PDGFRA
4	RNF150
4	TENM3
4	TLR1
4	TLR10
4	TLR6
4	TMEM156
4	TMPRSS11A
4	TMPRSS11B
4	TMPRSS11E
4	TMPRSS11F
4	UNC5C
5	ADAM19
5	ADRB2
5	BTNL3
5	BTNL8
5	BTNL9
5	C5orf15
5	CATSPER3
5	CD180
5	CDH12
5	CDHR2

Chr	Gene
5	COL23A1
5	CSF1R
5	F2RL2
5	FAM174A
5	FAT2
5	FGFR4
5	FLT4
5	GABRA6
5	GABRG2
5	GPR151
5	GPR98
5	GRM6
5	HAVCR1
5	HAVCR2
5	IL31RA
5	IL6ST
5	IL7R
5	ITGA1
5	ITGA2
5	KCNMB1
5	LIFR
5	LNPEP
5	MEGF10
5	NIPAL4
5	OR2V1
5	OR2Y1
5	OSMR
5	PCDH1
5	PCDH12
5	PCDHA1
5	PCDHA2
5	PCDHA4
5	PCDHA8
5	PCDHA9
5	PCDHB10
5	PCDHB11
5	PCDHB13
5	PCDHB14
5	PCDHB15
5	PCDHB16
5	PCDHB2
5	PCDHB3
5	PCDHB4
5	PCDHB5

Chr	Gene
5	PCDHB6
5	PCDHGA1
5	PCDHGA4
5	PDGFRB
5	PRLR
5	SEMA5A
5	SEMA6A
5	SGCD
5	SLC1A3
5	SLC22A4
5	SLC22A5
5	SLC36A3
5	SLC6A18
5	SLC6A19
5	SLCO6A1
5	SV2C
5	TENM2
5	TIMD4
5	UGT3A1
6	BAI3
6	BTN1A1
6	BTN2A1
6	BTN2A2
6	BTN3A2
6	BTNL2
6	CD83
6	DCBLD1
6	DLL1
6	DPCR1
6	ENPP1
6	ENPP3
6	ENPP4
6	EPHA7
6	GABBR1
6	GABRR1
6	GCNT6
6	GFRAL
6	GJB7
6	GLP1R
6	GPR110
6	GPR111
6	GPR116
6	GPR126
6	GPR63

Figure 22 (Continued)

Chr	Gene
6	GPRC6A
6	HFE
6	HLA-A
6	HLA-B
6	HLA-C
6	HLA-DPA1
6	HLA-DPB1
6	HLA-DQA1
6	HLA-DQA2
6	HLA-DQB1
6	HLA-DQB2
6	HLA-DRB1
6	HLA-DRB5
6	HLA-E
6	HLA-F
6	HLA-G
6	IL20RA
6	ITPR3
6	KIAA0319
6	LMBRD1
6	LRFN2
6	LRP11
6	MAS1L
6	MEP1A
6	MICA
6	MICB
6	MUC21
6	MUC22
6	NCR2
6	NOTCH4
6	OPRM1
6	OR10C1
6	OR12D2
6	OR12D3
6	OR14J1
6	OR2B2
6	OR2B6
6	OR2J1
6	OR2W1
6	OR5V1
6	PKHD1
6	PTCRA
6	RAET1E
6	RAET1G

Chr	Gene
6	ROS1
6	SDIM1
6	SLC22A1
6	SLC44A4
6	TAAR2
6	TREM1
6	TREML1
6	TREML2
7	AQP1
7	CD36
7	CDHR3
7	CNTNAP2
7	DPP6
7	EGFR
7	EPHA1
7	EPHB6
7	ERVW-1
7	GHRHR
7	GJC3
7	GPNMB
7	GRM8
7	HYAL4
7	KIAA1324L
7	LRRN3
7	MET
7	MUC12
7	MUC17
7	NPC1L1
7	NPSR1
7	OR2A12
7	OR2A14
7	OR2A2
7	OR2A25
7	OR2A42
7	OR2A7
7	OR2AE1
7	OR2F2
7	OR6V1
7	PILRA
7	PKD1L1
7	PLXNA4
7	PODXL
7	PTPRN2
7	PTPRZ1

Chr	Gene
7	RAMP3
7	SLC29A4
7	SMO
7	TAS2R16
7	TAS2R4
7	TAS2R40
7	TFR2
7	THSD7A
7	TMEM213
7	TTYH3
7	ZAN
7	ZP3
8	ADAM18
8	ADAM28
8	ADAM32
8	ADAM7
8	ADAM9
8	CDH17
8	CHRNA2
8	CSMD1
8	CSMD3
8	DCSTAMP
8	FZD6
8	GPR124
8	NRG1
8	OR4F21
8	PKHD1L1
8	PRSS55
8	SCARA3
8	SCARA5
8	SDC2
8	SLC10A5
8	SLC39A14
8	SLC39A4
8	SLCO5A1
8	TNFRSF10A
8	TNFRSF10B
9	ABCA1
9	AQP7
9	C9orf135
9	CA9
9	CD72
9	CNTNAP3
9	CNTNAP3B

Figure 22 (Continued)

Chr	Gene
9	ENTPD8
9	GPR144
9	GRIN3A
9	IZUMO3
9	KIAA1161
9	MAMDC4
9	MEGF9
9	MUSK
9	NOTCH1
9	OR13C2
9	OR13C3
9	OR13C5
9	OR13C8
9	OR13C9
9	OR13D1
9	OR13F1
9	OR1B1
9	OR1J2
9	OR1K1
9	OR1L1
9	OR1L3
9	OR1L6
9	OR1L8
9	OR1N1
9	OR1N2
9	OR1Q1
9	OR2S2
9	PCSK5
9	PLGRKT
9	PTPRD
9	ROR2
9	SEMA4D
9	SLC31A1
9	TEK
9	TLR4
9	TMEM2
9	VLDLR
10	ABCC2
10	ADAM8
10	ADRB1
10	ANTXRL
10	ATRNL1
10	C10orf54
10	CDH23

Chr	Gene
10	CDHR1
10	CNNM2
10	COL13A1
10	COL17A1
10	ENTPD1
10	FGFR2
10	FZD8
10	GPR158
10	GRID1
10	IL15RA
10	IL2RA
10	ITGA8
10	ITGB1
10	MRC1
10	NPFFR1
10	NRP1
10	OPN4
10	PCDH15
10	PKD2L1
10	PLXDC2
10	PRLHR
10	RGR
10	SLC29A3
10	SLC39A12
10	TACR2
10	TCTN3
10	TSPAN15
10	UNC5B
10	VSTM4
11	AMICA1
11	ANO3
11	APLP2
11	C11orf24
11	CCKBR
11	CD248
11	CD44
11	CD5
11	CD6
11	CDON
11	CLMP
11	CRTAM
11	DCHS1
11	DSCAML1
11	FAT3

Chr	Gene
11	FOLH1
11	GDPD4
11	GDPD5
11	GRIK4
11	HEPHL1
11	HTR3B
11	IFITM10
11	IL10RA
11	KIRREL3
11	LGR4
11	LRP4
11	LRP5
11	LRRC32
11	MCAM
11	MFRP
11	MPEG1
11	MRGPRE
11	MRGPRF
11	MRGPRG
11	MRGPRX2
11	MRGPRX3
11	MRGPRX4
11	MS4A4A
11	MTNR1B
11	MUC15
11	NAALAD2
11	NAALADL1
11	NCAM1
11	NRXN2
11	OR10A2
11	OR10A5
11	OR10A6
11	OR10D3
11	OR10G4
11	OR10G7
11	OR10G8
11	OR10G9
11	OR10Q1
11	OR10S1
11	OR1S1
11	OR2AG1
11	OR2AG2
11	OR2D2
11	OR4A15

Figure 22 (Continued)

Chr	Gene
11	OR4A47
11	OR4A5
11	OR4A8P
11	OR4C11
11	OR4C13
11	OR4C15
11	OR4C16
11	OR4C3
11	OR4C46
11	OR4C5
11	OR4D6
11	OR4D9
11	OR4S2
11	OR4X1
11	OR51E1
11	OR51L1
11	OR52A1
11	OR52E1
11	OR52E2
11	OR52E4
11	OR52E6
11	OR52I1
11	OR52I2
11	OR52J3
11	OR52L1
11	OR52N1
11	OR52N2
11	OR52N4
11	OR52W1
11	OR56B1
11	OR56B4
11	OR5A1
11	OR5A2
11	OR5AK2
11	OR5AR1
11	OR5B17
11	OR5B3
11	OR5D14
11	OR5D16
11	OR5D18
11	OR5F1
11	OR5I1
11	OR5L2
11	OR5M11

Chr	Gene
11	OR5M3
11	OR5P2
11	OR5R1
11	OR5T2
11	OR5T3
11	OR5W2
11	OR6A2
11	OR6T1
11	OR6X1
11	OR8A1
11	OR8B12
11	OR8B2
11	OR8B3
11	OR8B4
11	OR8D1
11	OR8D2
11	OR8H1
11	OR8H2
11	OR8H3
11	OR8I2
11	OR8J1
11	OR8J2
11	OR8J3
11	OR8K1
11	OR8K3
11	OR8K5
11	OR8U1
11	OR9G1
11	OR9G4
11	OR9Q2
11	P2RX3
11	PTPRJ
11	ROBO3
11	SIGIRR
11	SLC22A10
11	SLC3A2
11	SLC5A12
11	SLCO2B1
11	SORL1
11	ST14
11	SYT8
11	TENM4
11	TMEM123
11	TMPRSS4

Chr	Gene
11	TMPRSS5
11	TRPM5
11	TSPAN18
11	ZP1
12	ANO4
12	AVPR1A
12	CACNA2D4
12	CD163
12	CD163L1
12	CD27
12	CD4
12	CLEC12A
12	CLEC2A
12	CLEC4C
12	CLEC7A
12	CLECL1
12	CLSTN3
12	GPR133
12	GPRC5D
12	ITGA7
12	ITGB7
12	KLRB1
12	KLRC2
12	KLRC3
12	KLRC4
12	KLRF1
12	KLRF2
12	LRP1
12	LRP6
12	MANSC1
12	MANSC4
12	OLR1
12	OR10AD1
12	OR10P1
12	OR2AP1
12	OR6C1
12	OR6C2
12	OR6C3
12	OR6C4
12	OR6C6
12	OR6C74
12	OR6C76
12	OR8S1
12	OR9K2

Figure 22 (Continued)

Chr	Gene
12	ORAI1
12	P2RX4
12	P2RX7
12	PTPRB
12	PTPRQ
12	SCNN1A
12	SELPLG
12	SLC38A4
12	SLC5A8
12	SLC6A15
12	SLC8B1
12	SLCO1B1
12	SLCO1B7
12	SSPN
12	STAB2
12	TAS2R10
12	TAS2R13
12	TAS2R20
12	TAS2R30
12	TAS2R31
12	TAS2R42
12	TAS2R43
12	TAS2R46
12	TAS2R7
12	TMEM119
12	TMEM132B
12	TMEM132C
12	TMEM132D
12	TMPRSS12
12	TNFRSF1A
12	TSPAN8
12	VSIG10
13	ATP4B
13	ATP7B
13	FLT3
13	FREM2
13	KL
13	PCDH8
13	SGCG
13	SHISA2
13	SLC15A1
13	SLITRK6
13	TNFRSF19
14	ADAM21

Chr	Gene
14	BDKRB2
14	C14orf37
14	CLEC14A
14	DLK1
14	FLRT2
14	GPR135
14	GPR137C
14	JAG2
14	LTB4R2
14	MMP14
14	OR11G2
14	OR11H12
14	OR11H6
14	OR4K1
14	OR4K15
14	OR4K5
14	OR4L1
14	OR4N2
14	OR4N5
14	OR4Q2
14	SLC24A4
14	SYNDIG1L
15	ANPEP
15	CD276
15	CHRNA7
15	CHRNB4
15	CSPG4
15	DUOX1
15	DUOX2
15	FAM174B
15	GLDN
15	IGDCC4
15	ITGA11
15	LCTL
15	LTK
15	LYSMD4
15	MEGF11
15	NRG4
15	OCA2
15	OR4F4
15	OR4M2
15	OR4N4
15	PRTG
15	RHCG

Chr	Gene
15	SCAMP5
15	SEMA4B
15	SEMA6D
15	SLC24A1
15	SLC28A1
15	TRPM1
15	TYRO3
16	ATP2C2
16	CACNA1H
16	CD19
16	CDH11
16	CDH16
16	CDH3
16	CDH5
16	CNGB1
16	CNTNAP4
16	GDPD3
16	GPR56
16	GPR97
16	IL4R
16	ITFG3
16	ITGAL
16	ITGAM
16	ITGAX
16	KCNG4
16	MMP15
16	MSLNL
16	NOMO1
16	NOMO3
16	OR2C1
16	PKD1
16	PKD1L2
16	SCNN1B
16	SEZ6L2
16	SLC22A31
16	SLC5A11
16	SLC7A6
16	SPN
16	TMC5
16	TMC7
16	TMEM204
16	TMEM219
16	TMEM8A
17	ABCC3

Figure 22 (Continued)

Chr	Gene
17	ACE
17	AOC3
17	ASGR2
17	C17orf80
17	CD300A
17	CD300C
17	CD300E
17	CD300LG
17	CHRNB1
17	CLEC10A
17	CNTNAP1
17	CPD
17	CXCL16
17	FAM171A2
17	GCGR
17	GLP2R
17	GP1BA
17	GPR142
17	GUCY2D
17	ITGA2B
17	ITGA3
17	ITGAE
17	ITGB3
17	KCNJ12
17	LRRC37A
17	LRRC37A2
17	LRRC37A3
17	LRRC37B
17	MRC2
17	NGFR
17	OR1A2
17	OR1D2
17	OR1G1
17	OR3A1
17	OR3A2
17	OR4D1
17	OR4D2
17	RNF43
17	SCN4A
17	SDK2
17	SECTM1
17	SEZ6
17	SLC26A11
17	SPACA3

Chr	Gene
17	TMEM102
17	TMEM132E
17	TNFSF12
17	TRPV3
17	TTYH2
17	TUSC5
18	APCDD1
18	CDH19
18	CDH20
18	CDH7
18	COLEC12
18	DCC
18	DSC1
18	DSG1
18	DSG3
18	DYNAP
18	MEP1B
18	PTPRM
18	SIGLEC15
18	TNFRSF11A
19	ABCA7
19	ACPT
19	BCAM
19	C19orf38
19	C19orf59
19	C5AR1
19	CATSPERD
19	CATSPERG
19	CD320
19	CD33
19	CD97
19	CEACAM1
19	CEACAM19
19	CEACAM21
19	CEACAM3
19	CEACAM4
19	CLEC4M
19	DLL3
19	EMR1
19	EMR2
19	EMR3
19	ERVV-1
19	ERVV-2
19	FAM187B

Chr	Gene
19	FCAR
19	FFAR3
19	FPR1
19	GFY
19	GP6
19	GPR42
19	GRIN3B
19	ICAM3
19	IGFLR1
19	IL12RB1
19	IL27RA
19	KIR2DL1
19	KIR2DL3
19	KIR2DL4
19	KIR3DL1
19	KIR3DL2
19	KIR3DL3
19	KIRREL2
19	KISS1R
19	LAIR1
19	LDLR
19	LILRA1
19	LILRA2
19	LILRA4
19	LILRA6
19	LILRB1
19	LILRB2
19	LILRB3
19	LILRB4
19	LILRB5
19	LINGO3
19	LPHN1
19	LRP3
19	MADCAM1
19	MAG
19	MEGF8
19	MUC16
19	NCR1
19	NOTCH3
19	NPHS1
19	OR10H1
19	OR10H2
19	OR10H3
19	OR10H4

Figure 22 (Continued)

Chr	Gene
19	OR1I1
19	OR2Z1
19	OR7A10
19	OR7C1
19	OR7D4
19	OR7E24
19	OR7G1
19	OR7G2
19	OR7G3
19	PLVAP
19	PTGIR
19	PTPRH
19	PTPRS
19	PVR
19	SCN1B
19	SHISA7
19	SIGLEC10
19	SIGLEC11
19	SIGLEC12
19	SIGLEC5
19	SIGLEC6
19	SIGLEC8
19	SIGLEC9
19	SLC44A2
19	SLC5A5
19	SLC7A9
19	TARM1
19	TGFBR3L
19	TMC4
19	TMEM91
19	TMPRSS9
19	TNFSF14
19	TNFSF9
19	TRPM4
19	VN1R2
19	VSIG10L
19	VSTM2B
20	ABHD12

Chr	Gene
20	ADAM33
20	ADRA1D
20	APMAP
20	ATRN
20	CD40
20	CD93
20	CDH22
20	CDH26
20	CDH4
20	FLRT3
20	GCNT7
20	GGT7
20	JAG1
20	LRRN4
20	NPBWR2
20	OCSTAMP
20	PTPRA
20	PTPRT
20	SEL1L2
20	SIGLEC1
20	SIRPA
20	SIRPB1
20	SIRPG
20	SLC24A3
20	SLC2A10
20	SSTR4
20	THBD
21	CLDN8
21	DSCAM
21	ICOSLG
21	IFNAR1
21	IFNGR2
21	IGSF5
21	ITGB2
21	KCNJ15
21	NCAM2
21	TMPRSS15
21	TMPRSS2

Chr	Gene
21	TMPRSS3
21	TRPM2
21	UMODL1
22	CACNA1I
22	CELSR1
22	COMT
22	CSF2RB
22	GGT1
22	GGT5
22	IL2RB
22	KREMEN1
22	MCHR1
22	OR11H1
22	P2RX6
22	PKDREJ
22	PLXNB2
22	SCARF2
22	SEZ6L
22	SSTR3
22	SUSD2
22	TMPRSS6
22	TNFRSF13C
X	ATP6AP2
X	ATP7A
X	EDA2R
X	FMR1NB
X	GLRA4
X	GPR112
X	GUCY2F
X	HEPH
X	P2RY10
X	P2RY4
X	PLXNA3
X	PLXNB3
X	VSIG4
X	XG

Figure 23

Chr	Position	ref	alt	Gene
1	101005090	G	A	GPR88
1	101197012	A	G	VCAM1
1	101704572	C	A	S1PR1
1	109735398	A	C	KIAA1324
1	109801674	A	C	CELSR2
1	109806313	T	C	CELSR2
1	113650307	G	A	LRIG2
1	11561593	G	A	PTCHD2
1	11584109	C	G	PTCHD2
1	11585308	C	T	PTCHD2
1	117122130	T	C	IGSF3
1	117142613	C	T	IGSF3
1	117142641	G	A	IGSF3
1	117503940	G	A	PTGFRN
1	117554421	A	G	CD101
1	117559726	A	G	CD101
1	117560058	C	G	CD101
1	117568200	G	A	CD101
1	11986011	T	C	KIAA2013
1	120437884	A	G	ADAM30
1	120469147	T	C	NOTCH2
1	120572547	T	C	NOTCH2
1	120572572	C	T	NOTCH2
1	120934449	C	T	FCGR1B
1	120934573	C	T	FCGR1B
1	1221001	A	G	SCNN1D
1	1222598	C	T	SCNN1D
1	12252955	T	G	TNFRSF1B
1	1291085	C	T	MXRA8
1	145533877	C	G	ITGA10
1	145535814	C	T	ITGA10
1	145536082	G	A	ITGA10
1	145541460	C	T	ITGA10
1	151107304	C	G	SEMA6C
1	151108137	T	G	SEMA6C
1	151112483	T	C	SEMA6C
1	153653757	G	C	NPR1
1	154541971	T	G	CHRNB2
1	155161702	GT GT CC GG GG CC	G	MUC1
		GA GG TG AC AC CG TG GG CT GG GG GG GC GG TG GA GC CC GG GG CT GG CT TG T		
1	155161714	G	A	MUC1
1	155911517	C	A	RXFP4
1	156144971	G	A	SEMA4A
1	156838432	C	T	NTRK1
1	156877457	T	C	PEAR1
1	156878044	G	A	PEAR1
1	156878473	C	T	PEAR1
1	157504422	C	T	FCRL5
1	157508882	C	T	FCRL5
1	157508908	T	C	FCRL5
1	157508997	G	T	FCRL5
1	157509025	C	T	FCRL5
1	157514091	C	T	FCRL5
1	157514097	A	G	FCRL5
1	157559122	C	T	FCRL4
1	157668194	A	C	FCRL3
1	157668390	T	C	FCRL3
1	157772404	C	T	FCRL1
1	158225019	C	G	CD1A
1	158261071	A	C	CD1C

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
1	158263011	T	C	CD1C
1	158368964	C	T	OR10T2
1	158368974	A	G	OR10T2
1	158369210	A	G	OR10T2
1	158435928	G	A	OR10K1
1	158449749	A	G	OR10R2
1	158450238	G	A	OR10R2
1	158450314	A	G	OR10R2
1	158517585	G	A	OR6Y1
1	158517891	G	A	OR6Y1
1	158532605	C	A	OR6P1
1	158532614	G	A	OR6P1
1	158532908	T	C	OR6P1
1	158549082	A	T	OR10X1
1	158670426	C	T	OR6K2
1	158687896	C	T	OR6K3
1	158724722	G	C	OR6K6
1	158724750	C	T	OR6K6
1	158725194	T	C	OR6K6
1	158725237	C	T	OR6K6
1	158735691	T	C	OR6N1
1	158735892	A	G	OR6N1
1	158736445	C	T	OR6N1
1	159273892	A	G	FCER1A
1	159273943	G	A	FCER1A
1	159275786	C	G	FCER1A
1	159402547	T	C	OR10J4
1	159409857	A	G	OR10J1
1	159409870	T	G	OR10J1
1	159778910	G	A	FCRL6
1	159779464	T	C	FCRL6
1	159779465	C	T	FCRL6
1	159912899	C	T	IGSF9
1	159922175	C	T	SLAMF9
1	159923111	T	G	SLAMF9
1	159923234	C	T	SLAMF9
1	160318829	G	C	NCSTN
1	160719757	C	T	SLAMF7
1	160719843	A	C	SLAMF7
1	160784275	A	C	LY9
1	160811488	T	C	CD244
1	161049509	G	T	PVRL4
1	161049662	A	G	PVRL4
1	161476204	C	T	FCGR2A

Chr	Position	ref	alt	Gene
1	161476205	A	G	FCGR2A
1	161479745	A	G	FCGR2A
1	161518214	T	C	FCGR3A
1	161518336	C	T	FCGR3A
1	161642774	T	G	FCGR2B
1	16464489	C	T	EPHA2
1	165532746	T	A	LRRC52
1	167032895	C	A	GPA33
1	169563951	T	G	SELP
1	169565346	C	A	SELP
1	169566313	C	T	SELP
1	169580885	C	T	SELP
1	169581595	G	A	SELP
1	169582317	C	T	SELP
1	169676486	A	G	SELL
1	169696946	G	A	SELE
1	177030344	T	C	ASTN1
1	181018236	A	G	MR1
1	19175846	T	C	TAS1R2
1	19181015	G	C	TAS1R2
1	19181393	T	C	TAS1R2
1	19186093	G	A	TAS1R2
1	19565338	C	T	EMC1
1	19565344	C	G	EMC1
1	19566382	A	G	EMC1
1	197396761	G	A	CRB1
1	198671653	A	G	PTPRC
1	201052310	A	T	CACNA1S
1	202092332	C	G	GPR37L1
1	202092360	G	A	GPR37L1
1	204588700	C	G	LRRN2
1	204589066	C	T	LRRN2
1	204924020	C	T	NFASC
1	204944474	T	G	NFASC
1	204966402	A	G	NFASC
1	205053150	A	G	TMEM81
1	205053219	C	T	TMEM81
1	207106478	G	A	PIGR
1	207107806	G	A	PIGR
1	207109116	C	T	PIGR
1	207134226	C	T	FCAMR
1	207134410	T	G	FCAMR
1	207646462	G	A	CR2
1	207646923	G	A	CR2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
1	207707637	A	C	CR1
1	207715740	T	C	CR1
1	207715866	G	A	CR1
1	207741193	T	C	CR1
1	207753621	A	G	CR1
1	207760773	C	T	CR1
1	208073205	C	A	CD34
1	208252715	A	C	PLXNA2
1	208252777	G	C	PLXNA2
1	208252778	C	A	PLXNA2
1	208272313	A	C	PLXNA2
1	208390162	T	C	PLXNA2
1	208390469	C	T	PLXNA2
1	208391098	T	C	PLXNA2
1	208391131	C	T	PLXNA2
1	21573855	G	A	ECE1
1	215808007	G	A	USH2A
1	215808022	T	C	USH2A
1	215821909	C	T	USH2A
1	215916563	G	A	USH2A
1	215960153	A	C	USH2A
1	215960167	T	G	USH2A
1	215987222	T	C	USH2A
1	216011361	T	C	USH2A
1	216011408	T	C	USH2A
1	216051125	G	A	USH2A
1	216172299	C	G	USH2A
1	216172380	A	G	USH2A
1	216219781	A	G	USH2A
1	216258213	A	G	USH2A
1	216348764	C	T	USH2A
1	216373416	A	C	USH2A
1	216462662	T	A	USH2A
1	216595306	C	T	USH2A
1	22141206	A	C	LDLRAD2
1	223284528	A	G	TLR5
1	223284599	T	C	TLR5
1	223285200	G	A	TLR5
1	223285833	G	T	TLR5
1	223285946	T	G	TLR5
1	238046056	G	A	ZP4
1	238053226	G	A	ZP4
1	241767708	C	T	OPN3
1	24454688	C	T	IL22RA1
1	24460844	A	G	IL22RA1
1	244723989	C	A	C1orf101
1	244724277	C	T	C1orf101
1	247695756	T	C	OR2C3
1	247751731	C	G	OR2G2
1	247752161	T	C	OR2G2
1	247768948	C	T	OR2G3
1	247769425	G	A	OR2G3
1	247876020	A	G	OR6F1
1	247902448	G	A	OR14K1
1	247902693	G	T	OR14K1
1	247978517	A	G	OR14A16
1	247978541	AA GG	A	OR14A16
1	248004660	T	G	OR11L1
1	248004687	C	G	OR11L1
1	248059150	T	C	OR2W3
1	248059151	G	A	OR2W3
1	248059394	G	C	OR2W3
1	248059423	C	T	OR2W3
1	248059703	T	A	OR2W3
1	248084579	G	A	OR2T8
1	248084825	C	T	OR2T8
1	248084854	A	G	OR2T8
1	248084909	T	G	OR2T8
1	248085124	G	A	OR2T8
1	248128745	A	G	OR2AK2
1	248128929	G	A	OR2AK2
1	248129240	G	A	OR2AK2
1	248129486	C	T	OR2AK2
1	248202344	G	C	OR2L2
1	248436582	T	C	OR2T33
1	248436611	G	A	OR2T33
1	248436857	C	T	OR2T33
1	248458875	CT	C	OR2T12
1	248487300	C	T	OR2M7
1	248487338	C	A	OR2M7
1	248487834	G	T	OR2M7
1	248524937	AT GG GA CT CT TC	A	OR2T4

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
		AG AC AA TC CA AA CA TC CA AT GG CC AA TA TC AC CT GG AT GG CC AA CC AC AC TG GA TG GT CG GA TT TC AT CC TG T		
1	248524974	A	G	OR2T4
1	248550976	T	G	OR2T6
1	248569369	A	G	OR2T1
1	248604542	C	T	OR2T7
1	248604971	T	C	OR2T7
1	248605007	AC TT	A	OR2T7
1	248636713	G	C	OR2T3
1	248636934	G	T	OR2T3
1	248637480	A	G	OR2T3
1	248651905	A	T	OR2T5
1	248652175	G	A	OR2T5
1	248722507	C	T	OR2T29
1	248722723	G	A	OR2T29
1	248722777	T	A	OR2T29
1	248737230	T	C	OR2T34
1	248801778	A	T	OR2T35
1	248813650	CA GA	C	OR2T27
1	248813695	A	G	OR2T27
1	248845097	C	T	OR14I1
1	248845356	G	T	OR14I1
1	2491306	G	A	TNFRSF14
1	26520292	G	T	CATSPER4
1	27709020	T	C	CD164L2
1	27709044	T	C	CD164L2
1	31347320	G	A	SDC3
1	31349647	C	T	SDC3
1	31381313	CC AG	C	SDC3
1	32084861	T	C	HCRTR1
1	34238283	T	C	CSMD2
1	34401453	A	G	CSMD2
1	35227005	CT	C	GJB4
1	35227007	TT GT C	T	GJB4
1	36937701	T	C	CSF3R
1	38197152	C	T	EPHA10
1	38227086	A	T	EPHA10
1	38227268	G	T	EPHA10
1	43296456	G	A	ERMAP
1	43770692	C	T	TIE1
1	44057042	A	G	PTPRF
1	45292937	T	G	PTCH2
1	48241061	G	A	TRABD2B
1	48694594	G	A	SLC5A9
1	48697277	C	T	SLC5A9
1	53746346	T	G	LRP8
1	53792651	A	C	LRP8
1	59042311	T	G	TACSTD2
1	59042388	C	G	TACSTD2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
1	66036441	A	G	LEPR
1	66058513	A	G	LEPR
1	66075952	G	C	LEPR
1	79392756	C	A	ELTD1
1	86900372	C	G	CLCA2
1	86909582	G	A	CLCA2
1	90049071	G	A	LRRC8B
1	9079301	C	T	SLC2A7
1	9083094	G	T	SLC2A7
1	9188876	T	C	GPR157
1	94496053	G	A	ABCA4
1	94544234	T	C	ABCA4
1	94564483	C	T	ABCA4
1	9804693	C	T	CLSTN1
2	102955468	C	A	IL1RL1
2	109545691	T	C	EDAR
2	112686988	G	A	MERTK
2	112722854	G	A	MERTK
2	112751928	G	A	MERTK
2	112754942	A	G	MERTK
2	116503671	G	A	DPP10
2	116510817	G	C	DPP10
2	116525960	G	A	DPP10
2	116548675	G	A	DPP10
2	119739063	T	C	MARCO
2	125281910	C	T	CNTNAP5
2	125320888	A	C	CNTNAP5
2	131486765	C	G	GPR148
2	133175250	A	G	GPR39
2	136555659	T	C	LCT
2	136567203	T	C	LCT
2	136575534	T	C	LCT
2	137872746	G	A	THSD7B
2	137917922	T	A	THSD7B
2	137928320	C	T	THSD7B
2	137988626	C	T	THSD7B
2	137990678	C	T	THSD7B
2	138420996	A	G	THSD7B
2	141116447	G	T	LRP1B
2	141242918	T	C	LRP1B
2	141259399	A	C	LRP1B
2	141457962	C	T	LRP1B
2	142567910	T	C	LRP1B
2	1481155	G	T	TPO

Chr	Position	ref	alt	Gene
2	1481231	G	C	TPO
2	1488616	A	C	TPO
2	1497657	G	A	TPO
2	1499870	A	G	TPO
2	1499927	A	C	TPO
2	160673519	G	A	LY75
2	160673526	A	G	LY75
2	160676350	T	C	LY75
2	160676427	C	A	LY75
2	160710153	T	G	LY75
2	160711045	G	C	LY75
2	160729005	C	T	LY75
2	160743040	T	A	LY75
2	160808075	C	T	PLA2R1
2	160843746	GC	G	PLA2R1
2	160843748	T	G	PLA2R1
2	160879259	C	T	PLA2R1
2	160885418	G	C	PLA2R1
2	160885442	T	C	PLA2R1
2	160898583	G	T	PLA2R1
2	167279922	C	A	SCN7A
2	170003432	T	G	LRP2
2	170010985	T	C	LRP2
2	170053505	C	T	LRP2
2	170060512	C	G	LRP2
2	170070172	C	T	LRP2
2	170097707	T	G	LRP2
2	170175334	T	C	LRP2
2	173339808	G	A	ITGA6
2	173352507	G	T	ITGA6
2	173355958	G	A	ITGA6
2	182376480	T	A	ITGA4
2	182395345	G	A	ITGA4
2	187466842	A	C	ITGAV
2	187511466	A	G	ITGAV
2	192922499	G	A	TMEFF2
2	20403794	A	T	SDC1
2	206581033	G	A	NRP2
2	219029108	C	G	CXCR1
2	219029843	A	C	CXCR1
2	230341874	T	G	DNER
2	233406178	G	A	CHRNG
2	240969312	A	G	OR6B2
2	241558397	G	A	GPR35

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
2	26534801	G	A	GPR113
2	26534813	A	G	GPR113
2	26534872	C	T	GPR113
2	26536694	C	T	GPR113
2	26536727	C	T	GPR113
2	26539204	C	A	GPR113
2	26539806	A	G	GPR113
2	27435165	G	A	ATRAID
2	27435250	A	G	ATRAID
2	28814033	A	G	PLB1
2	28827625	C	T	PLB1
2	28843148	G	A	PLB1
2	28854958	C	T	PLB1
2	29448410	T	G	ALK
2	36744583	A	C	CRIM1
2	39893305	A	C	TMEM178A
2	44040401	G	C	ABCG5
2	47601106	T	C	EPCAM
2	47604176	C	T	EPCAM
2	48921375	T	C	LHCGR
2	48921438	T	C	LHCGR
2	68873071	A	G	PROKR1
2	70188531	C	T	ASPRV1
2	71036417	T	C	CLEC4F
2	71036482	T	C	CLEC4F
2	71043407	A	G	CLEC4F
2	71043461	C	T	CLEC4F
2	71043728	T	A	CLEC4F
2	71044076	T	C	CLEC4F
2	71058230	T	A	CD207
2	71058306	T	C	CD207
2	71060111	G	A	CD207
2	71060936	G	C	CD207
2	71061001	T	A	CD207
2	71061145	C	T	CD207
2	85059227	C	T	TRABD2A
2	85097590	C	T	TRABD2A
2	85554080	G	A	TGOLN2
2	95947085	A	G	PROM2
2	95947099	G	A	PROM2
3	100354524	A	G	GPR128
3	100368546	A	G	GPR128
3	100963154	G	A	IMPG2
3	102157417	A	G	ZPLD1

Chr	Position	ref	alt	Gene
3	102181131	A	G	ZPLD1
3	105258861	A	G	ALCAM
3	111286375	G	C	CD96
3	111780727	C	A	TMPRSS7
3	111780741	C	T	TMPRSS7
3	111799845	A	T	TMPRSS7
3	112059768	C	G	CD200
3	112063850	C	A	CD200
3	112647832	A	C	CD200R1
3	112648127	T	G	CD200R1
3	112648222	T	C	CD200R1
3	113251935	G	A	SIDT1
3	113251962	C	A	SIDT1
3	113890815	C	T	DRD3
3	118649060	G	T	IGSF11
3	118909159	A	G	UPK1B
3	118909892	A	G	UPK1B
3	121825197	G	A	CD86
3	121981215	A	G	CASR
3	122631896	A	T	SEMA5B
3	122632436	A	G	SEMA5B
3	122634665	G	C	SEMA5B
3	122640851	G	C	SEMA5B
3	122646828	A	G	SEMA5B
3	122694930	C	T	SEMA5B
3	124515498	T	C	ITGB5
3	124515636	C	T	ITGB5
3	124728626	A	G	HEG1
3	124731485	C	G	HEG1
3	124731689	T	A	HEG1
3	124731944	T	G	HEG1
3	124732618	A	G	HEG1
3	129293256	T	C	PLXND1
3	129304797	G	A	PLXND1
3	129305459	C	A	PLXND1
3	129305460	C	T	PLXND1
3	133941320	C	T	RYK
3	137743508	A	T	CLDN18
3	140178381	T	C	CLSTN2
3	140178485	G	A	CLSTN2
3	155571275	T	C	SLC33A1
3	164906806	T	C	SLITRK3
3	170136972	A	C	CLDN11
3	172232780	G	T	TNFSF10

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
3	174951756	T	C	NAALADL2
3	175345143	C	G	NAALADL2
3	175455116	A	G	NAALADL2
3	175473047	T	C	NAALADL2
3	178961025	G	C	KCNMB3
3	178961038	T	C	KCNMB3
3	183753777	G	A	HTR3D
3	183754278	C	G	HTR3D
3	183774762	C	A	HTR3C
3	183822145	C	T	HTR3E
3	183824398	G	A	HTR3E
3	184293716	A	C	EPHB3
3	193171979	T	A	ATP13A4
3	193209178	T	C	ATP13A4
3	194080916	G	A	LRRC15
3	194080983	C	T	LRRC15
3	194117911	C	G	GP5
3	194337901	T	C	TMEM44
3	194353875	G	T	TMEM44
3	195800811	C	T	TFRC
3	196388099	G	C	NRROS
3	27472936	C	T	SLC4A7
3	27473156	T	A	SLC4A7
3	3139957	T	C	IL5RA
3	33194990	C	A	SUSD5
3	33195309	A	G	SUSD5
3	33255592	A	G	SUSD5
3	37559040	A	G	ITGA9
3	37574951	G	A	ITGA9
3	37695260	A	G	ITGA9
3	38347851	A	G	SLC22A14
3	38350543	A	G	SLC22A14
3	3886665	G	A	LRRN1
3	391100	A	G	CHL1
3	39323163	A	C	CX3CR1
3	42555275	A	C	VIPR1
3	42906116	T	C	ACKR2
3	43602803	C	T	ANO10
3	439963	A	G	CHL1
3	45134822	T	C	CDCP1
3	45987980	G	A	CXCR6
3	46449164	G	A	CCRL2
3	46449175	G	A	CCRL2
3	46449581	A	G	CCRL2
3	46450070	T	A	CCRL2
3	46450072	G	A	CCRL2
3	47618953	C	A	CSPG5
3	47619025	G	C	CSPG5
3	48461437	G	A	PLXNB1
3	48461530	G	A	PLXNB1
3	48463856	G	A	PLXNB1
3	48691316	T	C	CELSR3
3	48697654	C	G	CELSR3
3	49832788	G	A	CDHR4
3	49933240	G	A	MST1R
3	49933461	C	T	MST1R
3	49936102	T	C	MST1R
3	49936608	T	C	MST1R
3	49940078	C	T	MST1R
3	50513613	C	T	CACNA2D2
3	51747288	G	A	GRM2
3	52258319	G	A	TLR9
3	52551973	C	T	STAB1
3	53700459	T	G	CACNA1D
3	54420749	T	G	CACNA2D3
3	54420752	A	G	CACNA2D3
3	57138419	G	A	IL17RD
3	61975382	T	C	PTPRG
3	62189189	G	A	PTPRG
3	62189385	A	G	PTPRG
3	66434643	T	C	LRIG1
3	66436546	C	T	LRIG1
3	71803448	CG GC CG CG GC GG G	C	GPR27
3	7494417	A	T	GRM7
3	75986717	G	A	ROBO2
3	75986741	G	T	ROBO2
3	97726747	T	A	GABRR3
3	97783335	T	C	OR5AC1
3	97783823	C	G	OR5AC1
3	97783851	T	C	OR5AC1
3	97852083	C	T	OR5H1
3	97868795	A	G	OR5H14

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
3	97888322	T	TG	OR5H15
3	97888337	C	T	OR5H15
3	97983231	C	T	OR5H6
3	97983664	C	T	OR5H6
3	97983699	C	A	OR5H6
3	97983711	T	C	OR5H6
3	97983723	C	T	OR5H6
3	97983981	G	A	OR5H6
3	98072715	C	A	OR5K4
3	98109529	C	T	OR5K3
3	98110287	C	T	OR5K3
3	98217050	C	A	OR5K2
3	98251211	A	G	GPR15
3	98600385	T	C	DCBLD2
3	9953258	C	T	IL17RE
3	9959410	G	A	IL17RC
3	9960070	C	T	IL17RC
3	9991101	C	G	PRRT3
3	9991163	A	G	PRRT3
3	9991388	G	C	PRRT3
3	9991429	G	A	PRRT3
4	101386643	C	T	EMCN
4	101401161	TAAC	T	EMCN
4	1018705	C	A	FGFRL1
4	110864533	C	T	EGF
4	110883121	G	A	EGF
4	110901198	G	A	EGF
4	110902111	A	T	EGF
4	110908933	T	C	EGF
4	110914427	A	T	EGF
4	111398208	A	G	ENPEP
4	111409705	T	C	ENPEP
4	126241335	C	G	FAT4
4	126371814	C	T	FAT4
4	126372742	G	A	FAT4
4	126373570	C	T	FAT4
4	126373653	A	G	FAT4
4	128842876	C	G	MFSD8
4	142053488	G	A	RNF150
4	142053711	C	G	RNF150
4	144920596	G	A	GYPB
4	145041720	A	G	GYPA
4	154513627	A	G	KIAA0922

Chr	Position	ref	alt	Gene
4	154514965	T	C	KIAA0922
4	183601761	G	A	TENM3
4	187524464	G	A	FAT1
4	187531022	A	G	FAT1
4	187539323	C	T	FAT1
4	187540635	T	G	FAT1
4	187542755	T	C	FAT1
4	187542927	T	C	FAT1
4	187549428	C	T	FAT1
4	187557893	T	C	FAT1
4	187627792	T	C	FAT1
4	187629140	G	C	FAT1
4	187629770	A	C	FAT1
4	187630590	G	A	FAT1
4	187630597	C	G	FAT1
4	187630905	C	T	FAT1
4	22404391	G	A	GPR125
4	38776070	C	T	TLR10
4	38776235	A	G	TLR10
4	38776320	C	T	TLR10
4	38776491	T	G	TLR10
4	38776725	C	A	TLR10
4	38799399	G	T	TLR1
4	38799710	T	C	TLR1
4	38800214	C	G	TLR1
4	38800323	A	G	TLR1
4	38829675	C	T	TLR6
4	38829702	C	T	TLR6
4	38829815	A	G	TLR6
4	38830116	C	T	TLR6
4	38830350	A	G	TLR6
4	38830736	A	G	TLR6
4	39000305	A	G	TMEM156
4	39000475	T	C	TMEM156
4	39448529	G	A	KLB
4	39448586	C	T	KLB
4	47663760	T	G	CORIN
4	47663799	G	A	CORIN
4	47667064	T	C	CORIN
4	47695024	G	T	CORIN
4	47945295	C	T	CNGA1
4	53610402	G	A	ERVMER34-1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
4	53611484	C	T	ERVMER34-1
4	55133806	G	A	PDGFRA
4	55139771	T	C	PDGFRA
4	55972974	T	A	KDR
4	55979558	C	T	KDR
4	5690902	T	C	EVC2
4	66467418	A	C	EPHA5
4	66509085	T	G	EPHA5
4	68784774	T	C	TMPRSS11A
4	68938185	C	T	TMPRSS11F
4	69094507	T	A	TMPRSS11B
4	69095197	T	C	TMPRSS11B
4	69337325	C	G	TMPRSS11E
4	69343287	A	G	TMPRSS11E
4	75695301	G	A	BTC
4	75937971	C	T	PARM1
4	78987157	A	G	FRAS1
4	79158715	G	C	FRAS1
4	79166478	A	T	FRAS1
4	79186200	C	T	FRAS1
4	79186257	T	C	FRAS1
4	79188027	A	G	FRAS1
4	79188584	C	T	FRAS1
4	79205699	T	A	FRAS1
4	79284694	C	T	FRAS1
4	79285146	C	G	FRAS1
4	79300993	G	A	FRAS1
4	79373353	C	T	FRAS1
4	79373436	G	A	FRAS1
4	79373499	G	A	FRAS1
4	79387464	A	G	FRAS1
4	79394702	G	A	FRAS1
4	79434685	T	G	FRAS1
4	79443837	A	T	FRAS1
4	79443850	G	A	FRAS1
4	79447763	T	C	FRAS1
4	80977133	G	A	ANTXR2
4	94006294	T	G	GRID2
4	96171659	T	G	UNC5C
5	101724448	G	C	SLCO6A1
5	101748741	G	C	SLCO6A1
5	115811248	G	T	SEMA6A
5	115811273	C	T	SEMA6A

Chr	Position	ref	alt	Gene
5	1213668	G	A	SLC6A19
5	1240749	A	G	SLC6A18
5	1245997	C	A	SLC6A18
5	126732256	A	C	MEGF10
5	126732427	G	A	MEGF10
5	131630568	G	C	SLC22A4
5	131630571	A	C	SLC22A4
5	131705926	A	C	SLC22A5
5	133295334	T	C	C5orf15
5	134343766	T	G	CATSPER3
5	140166953	A	G	PCDHA1
5	140167175	T	C	PCDHA1
5	140174622	G	A	PCDHA2
5	140174865	G	C	PCDHA2
5	140186937	G	T	PCDHA4
5	140187322	C	T	PCDHA4
5	140187664	A	G	PCDHA4
5	140188354	C	G	PCDHA4
5	140221098	C	G	PCDHA8
5	140221139	G	A	PCDHA8
5	140229086	C	G	PCDHA9
5	140229368	G	C	PCDHA9
5	140229581	G	C	PCDHA9
5	140229794	G	C	PCDHA9
5	140230122	G	A	PCDHA9
5	140474756	G	A	PCDHB2
5	140480852	G	A	PCDHB3
5	140481333	T	G	PCDHB3
5	140481475	A	T	PCDHB3
5	140481594	T	C	PCDHB3
5	140482013	G	A	PCDHB3
5	140502083	A	G	PCDHB4
5	140502343	C	T	PCDHB4
5	140502344	C	T	PCDHB4
5	140502841	G	A	PCDHB4
5	140503237	A	G	PCDHB4
5	140503531	C	T	PCDHB4
5	140515483	T	C	PCDHB5
5	140516407	G	A	PCDHB5
5	140530529	G	A	PCDHB6
5	140531592	C	T	PCDHB6
5	140531667	T	C	PCDHB6
5	140531746	C	G	PCDHB6
5	140562739	G	A	PCDHB16

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
5	140563173	G	T	PCDHB16
5	140563656	G	A	PCDHB16
5	140564048	G	C	PCDHB16
5	140564088	C	T	PCDHB16
5	140572763	C	G	PCDHB10
5	140573754	A	C	PCDHB10
5	140574079	C	T	PCDHB10
5	140574103	T	G	PCDHB10
5	140580931	C	G	PCDHB11
5	140581076	C	T	PCDHB11
5	140595625	G	A	PCDHB13
5	140604419	G	A	PCDHB14
5	140625897	G	A	PCDHB15
5	140626566	A	C	PCDHB15
5	140626627	G	A	PCDHB15
5	140626855	C	T	PCDHB15
5	140627020	G	T	PCDHB15
5	140627184	C	G	PCDHB15
5	140710705	G	A	PCDHGA1
5	140710810	G	A	PCDHGA1
5	140735215	G	A	PCDHGA4
5	140736689	G	A	PCDHGA4
5	140736814	G	C	PCDHGA4
5	141244356	C	T	PCDH1
5	141335498	C	T	PCDH12
5	145895558	G	A	GPR151
5	148206440	G	A	ADRB2
5	148206473	G	C	ADRB2
5	149450132	T	C	CSF1R
5	149509508	G	A	PDGFRB
5	150666907	A	G	SLC36A3
5	150897236	A	G	FAT2
5	150901261	C	T	FAT2
5	150901613	A	G	FAT2
5	150908812	C	T	FAT2
5	150908813	G	A	FAT2
5	150930186	C	T	FAT2
5	150930345	C	T	FAT2
5	150942969	G	A	FAT2
5	150945483	C	T	FAT2
5	150945518	C	T	FAT2
5	150946436	A	G	FAT2
5	150946773	G	A	FAT2
5	150948147	T	G	FAT2

Chr	Position	ref	alt	Gene
5	155935708	G	A	SGCD
5	156376703	A	G	TIMD4
5	156378666	G	A	TIMD4
5	156479426	T	C	HAVCR1
5	156479443	CT TG	C	HAVCR1
5	156479488	G	A	HAVCR1
5	156479509	A	G	HAVCR1
5	156531736	C	A	HAVCR2
5	156887270	C	T	NIPAL4
5	156918850	C	T	ADAM19
5	156936364	T	C	ADAM19
5	161116672	C	T	GABRA6
5	161529571	A	G	GABRG2
5	161529631	G	T	GABRG2
5	167642193	A	G	TENM2
5	169805866	G	A	KCNMB1
5	169810796	C	T	KCNMB1
5	175995711	C	A	CDHR2
5	176002734	G	A	CDHR2
5	176004476	T	C	CDHR2
5	176011579	T	C	CDHR2
5	176017455	C	T	CDHR2
5	176517797	C	T	FGFR4
5	177683377	T	C	COL23A1
5	177683905	G	A	COL23A1
5	177987740	C	T	COL23A1
5	178409927	G	A	GRM6
5	178421594	G	T	GRM6
5	178421770	T	G	GRM6
5	180049808	T	C	FLT4
5	180057293	T	C	FLT4
5	180058762	G	T	FLT4
5	180166256	C	T	OR2Y1
5	180338368	A	G	BTNL8
5	180374523	G	A	BTNL8
5	180420155	T	C	BTNL3
5	180424423	G	C	BTNL3
5	180477168	T	G	BTNL9
5	180551775	T	C	OR2V1
5	21765178	A	G	CDH12
5	21817206	T	C	CDH12
5	22078584	C	T	CDH12
5	35084647	T	C	PRLR

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
5	35861068	T	C	IL7R
5	35871190	G	A	IL7R
5	35965970	A	C	UGT3A1
5	36677083	G	C	SLC1A3
5	38486065	C	T	LIFR
5	38496637	C	T	LIFR
5	38527308	G	A	LIFR
5	38884071	T	G	OSMR
5	38921788	G	A	OSMR
5	52201722	C	T	ITGA1
5	52214581	G	A	ITGA1
5	52229745	T	G	ITGA1
5	52240810	A	G	ITGA1
5	52351838	G	A	ITGA2
5	52358757	G	A	ITGA2
5	55168132	A	G	IL31RA
5	55250727	A	G	IL6ST
5	55251931	G	C	IL6ST
5	55264153	C	G	IL6ST
5	66479383	T	C	CD180
5	66480374	G	C	CD180
5	66481777	G	C	CD180
5	75591710	C	G	SV2C
5	75594743	G	A	SV2C
5	75913784	T	C	F2RL2
5	89914925	T	G	GPR98
5	89920969	C	A	GPR98
5	89925169	T	C	GPR98
5	89949754	G	A	GPR98
5	89979518	C	T	GPR98
5	89979589	G	A	GPR98
5	89979698	C	T	GPR98
5	89979750	G	T	GPR98
5	89985882	A	G	GPR98
5	89988504	A	G	GPR98
5	89989802	A	G	GPR98
5	89990324	A	G	GPR98
5	90006849	G	A	GPR98
5	90012379	G	A	GPR98
5	90016871	G	A	GPR98
5	90021035	T	C	GPR98
5	90024735	G	A	GPR98
5	90052289	G	A	GPR98
5	90085654	T	C	GPR98

Chr	Position	ref	alt	Gene
5	90107108	A	G	GPR98
5	90119324	G	A	GPR98
5	90151589	G	A	GPR98
5	9050549	T	C	SEMA5A
5	96322360	G	A	LNPEP
5	96350710	G	A	LNPEP
5	96363459	A	G	LNPEP
5	99871518	A	G	FAM174A
6	10647272	C	T	GCNT6
6	117130544	A	C	GPRC6A
6	117130704	G	A	GPRC6A
6	117150008	G	A	GPRC6A
6	117650532	C	G	ROS1
6	117665231	T	C	ROS1
6	117683821	G	A	ROS1
6	117710661	T	C	ROS1
6	117724379	C	T	ROS1
6	117725448	T	G	ROS1
6	117860508	A	G	DCBLD1
6	132047245	G	A	ENPP3
6	132061420	G	A	ENPP3
6	132172368	A	C	ENPP1
6	132206079	C	T	ENPP1
6	132939217	C	T	TAAR2
6	137338202	T	C	IL20RA
6	14131854	A	G	CD83
6	142688969	A	G	GPR126
6	142689042	T	A	GPR126
6	142691549	A	C	GPR126
6	150158558	C	T	LRP11
6	150164259	A	C	LRP11
6	150174278	A	C	LRP11
6	150184601	G	A	LRP11
6	150184684	AG CA CG GC TG CC GG GG GC GC GG	A	LRP11

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
		GG CG CC GG		
6	150184882	G	C	LRP11
6	150210681	G	A	RAET1E
6	150210685	C	T	RAET1E
6	150210723	C	T	RAET1E
6	150211071	C	T	RAET1E
6	150211123	A	T	RAET1E
6	150239484	C	T	RAET1G
6	150240829	G	C	RAET1G
6	154360569	C	T	OPRM1
6	154360797	A	G	OPRM1
6	160543148	C	T	SLC22A1
6	166307718	T	C	SDIM1
6	166307982	C	T	SDIM1
6	170593037	C	T	DLL1
6	24564540	A	G	KIAA0319
6	24564544	T	C	KIAA0319
6	24576631	C	T	KIAA0319
6	24596433	A	C	KIAA0319
6	24596478	T	G	KIAA0319
6	26091179	C	G	HFE
6	26093141	G	A	HFE
6	26370630	T	G	BTN3A2
6	26370657	A	G	BTN3A2
6	26370660	G	A	BTN3A2
6	26370748	G	A	BTN3A2
6	26388405	G	A	BTN2A2
6	26463574	G	T	BTN2A1
6	26463575	G	T	BTN2A1
6	26463660	G	A	BTN2A1
6	26505362	G	A	BTN1A1
6	27879551	C	G	OR2B2
6	27925827	A	G	OR2B6
6	29012712	T	C	OR2W1
6	29068759	C	A	OR2J1
6	29069264	T	C	OR2J1
6	29069299	C	T	OR2J1
6	29274486	T	C	OR14J1
6	29323905	A	C	OR5V1
6	29342775	G	A	OR12D3
6	29364508	T	C	OR12D2

Chr	Position	ref	alt	Gene
6	29364951	G	A	OR12D2
6	29407800	C	A	OR10C1
6	29408275	C	A	OR10C1
6	29408313	C	A	OR10C1
6	29455465	G	A	MAS1L
6	29600104	G	A	GABBR1
6	29693011	C	T	HLA-F
6	29795913	A	T	HLA-G
6	29796376	C	A	HLA-G
6	29910557	T	A	HLA-A
6	29910566	G	A	HLA-A
6	29910660	A	G	HLA-A
6	29910688	A	G	HLA-A
6	29910693	A	G	HLA-A
6	29910698	G	A	HLA-A
6	29910721	G	C	HLA-A
6	29910750	C	T	HLA-A
6	29910761	G	A	HLA-A
6	29910767	G	C	HLA-A
6	29910801	C	A	HLA-A
6	29911064	A	G	HLA-A
6	29911086	T	C	HLA-A
6	29911092	G	T	HLA-A
6	29911098	T	C	HLA-A
6	29911198	T	C	HLA-A
6	29911207	G	A	HLA-A
6	29911218	G	A	HLA-A
6	29911222	C	T	HLA-A
6	29911271	G	C	HLA-A
6	29911296	G	A	HLA-A
6	29911306	C	T	HLA-A
6	29911970	G	A	HLA-A
6	29912028	AG	A	HLA-A
6	29912030	G	C	HLA-A
6	29912042	G	A	HLA-A
6	29912085	C	T	HLA-A
6	29912153	A	G	HLA-A
6	29912297	A	G	HLA-A
6	30458064	G	A	HLA-E
6	30459165	C	T	HLA-E
6	30916705	A	G	DPCR1
6	30917212	T	C	DPCR1
6	30917482	C	T	DPCR1
6	30954245	T	C	MUC21

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
6	30954356	C	T	MUC21
6	30954389	GG GC TA GC AC AG CC AC CA AC TC TG AC TC CA GC AC AA CC	G	MUC21
6	30954559	A	C	MUC21
6	30954560	C	T	MUC21
6	30954684	G	C	MUC21
6	30954909	C	G	MUC21
6	30955010	C	T	MUC21
6	30955247	C	T	MUC21
6	30955250	A	G	MUC21
6	30993377	T	A	MUC22
6	30993440	G	A	MUC22
6	30993494	G	C	MUC22
6	30993533	A	G	MUC22
6	30993590	A	G	MUC22
6	30993719	A	G	MUC22
6	30993776	A	T	MUC22
6	30993813	G	A	MUC22
6	30993866	A	T	MUC22
6	30993880	G	C	MUC22
6	30994022	AC CA CA GG CT	A	MUC22
6	30994025	A	G	MUC22
6	30994034	GA GA	G	MUC22

Chr	Position	ref	alt	Gene
		CC AC TA CA AT CC TG AT TA		
6	30994059	C	G	MUC22
6	30994248	C	G	MUC22
6	30994253	G	A	MUC22
6	30994259	A	T	MUC22
6	30994283	G	A	MUC22
6	30994412	G	A	MUC22
6	30994431	C	T	MUC22
6	30994470	C	G	MUC22
6	30994532	G	T	MUC22
6	30994617	C	G	MUC22
6	30994635	C	T	MUC22
6	30994730	G	A	MUC22
6	30995114	AC	A	MUC22
6	30995178	C	T	MUC22
6	30995187	T	C	MUC22
6	30995297	A	G	MUC22
6	30995532	T	C	MUC22
6	30995786	A	G	MUC22
6	30995894	A	T	MUC22
6	30996132	C	T	MUC22
6	30996154	TG AG AC CA CC AC AG CC TC TA CT GA AG GC TC C	T	MUC22

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
6	30996314	A	G	MUC22
6	30996354	C	T	MUC22
6	30996487	GACC	G	MUC22
6	30996509	G	A	MUC22
6	30996699	A	C	MUC22
6	30996959	G	A	MUC22
6	30996981	T	TCA	MUC22
6	30996984	C	A	MUC22
6	30997334	A	G	MUC22
6	30997352	A	G	MUC22
6	30997517	A	T	MUC22
6	30997692	G	C	MUC22
6	30997824	T	C	MUC22
6	30997877	G	C	MUC22
6	30999997	T	C	MUC22
6	31000005	T	A	MUC22
6	31237862	T	C	HLA-C
6	31238002	G	A	HLA-C
6	31238027	C	A	HLA-C
6	31238053	G	C	HLA-C
6	31238068	C	T	HLA-C
6	31238155	G	A	HLA-C
6	31238232	G	A	HLA-C
6	31238868	C	T	HLA-C
6	31238880	C	T	HLA-C
6	31238886	A	G	HLA-C
6	31238925	C	T	HLA-C
6	31238970	T	A	HLA-C
6	31239006	G	T	HLA-C
6	31239060	A	G	HLA-C
6	31239116	G	A	HLA-C
6	31239407	G	T	HLA-C
6	31239417	C	T	HLA-C
6	31239430	C	T	HLA-C
6	31239449	C	G	HLA-C
6	31239501	G	T	HLA-C
6	31239543	C	T	HLA-C
6	31239585	C	T	HLA-C
6	31239607	G	A	HLA-C
6	31239616	C	A	HLA-C
6	31239630	C	T	HLA-C
6	31322980	C	T	HLA-B

Chr	Position	ref	alt	Gene
6	31323115	T	C	HLA-B
6	31323202	T	C	HLA-B
6	31323262	G	A	HLA-B
6	31323321	G	A	HLA-B
6	31323337	T	C	HLA-B
6	31323953	C	G	HLA-B
6	31323958	T	G	HLA-B
6	31323991	C	G	HLA-B
6	31324022	TCC	T	HLA-B
6	31324064	T	A	HLA-B
6	31324100	G	T	HLA-B
6	31324210	G	A	HLA-B
6	31324492	GC	G	HLA-B
6	31324506	C	T	HLA-B
6	31324524	GCC	G	HLA-B
6	31324542	T	C	HLA-B
6	31324549	T	C	HLA-B
6	31324552	G	C	HLA-B
6	31324580	T	C	HLA-B
6	31324582	T	C	HLA-B
6	31324599	T	G	HLA-B
6	31324615	C	T	HLA-B
6	31324641	T	A	HLA-B
6	31324647	T	C	HLA-B
6	31324674	C	T	HLA-B
6	31324702	C	T	HLA-B
6	31378388	G	A	MICA
6	31378864	A	G	MICA
6	31378932	G	A	MICA
6	31378956	C	G	MICA
6	31378977	G	A	MICA
6	31379043	A	G	MICA
6	31379109	G	A	MICA
6	31379115	G	A	MICA
6	31379134	C	G	MICA
6	31379795	A	G	MICA
6	31379807	C	T	MICA
6	31379817	T	C	MICA
6	31379823	C	G	MICA
6	31379931	G	A	MICA
6	31473546	A	G	MICB
6	31473561	A	G	MICB

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
6	31473957	C	G	MICB
6	31474000	G	A	MICB
6	31474820	C	T	MICB
6	31475056	G	A	MICB
6	31836976	G	A	SLC44A4
6	31839309	C	T	SLC44A4
6	32178570	C	T	NOTCH4
6	32185796	C	T	NOTCH4
6	32188383	T	C	NOTCH4
6	32188640	T	C	NOTCH4
6	32188823	G	A	NOTCH4
6	32188943	G	A	NOTCH4
6	32190390	T	G	NOTCH4
6	32362703	G	T	BTNL2
6	32362741	C	T	BTNL2
6	32362745	G	A	BTNL2
6	32363816	T	C	BTNL2
6	32363888	C	T	BTNL2
6	32364011	T	C	BTNL2
6	32364046	T	A	BTNL2
6	32370816	G	A	BTNL2
6	32370835	T	C	BTNL2
6	32370879	C	T	BTNL2
6	32370927	G	A	BTNL2
6	32370969	TG	T	BTNL2
6	32370975	C	T	BTNL2
6	32370993	C	T	BTNL2
6	32372863	A	T	BTNL2
6	32487165	G	C	HLA-DRB5
6	32487170	G	A	HLA-DRB5
6	32487215	C	T	HLA-DRB5
6	32487242	G	A	HLA-DRB5
6	32487294	C	T	HLA-DRB5
6	32487309	T	C	HLA-DRB5
6	32487353	T	C	HLA-DRB5
6	32487398	C	T	HLA-DRB5
6	32487402	C	A	HLA-DRB5
6	32489708	C	A	HLA-DRB5
6	32489711	A	G	HLA-DRB5
6	32489732	T	A	HLA-DRB5
6	32489733	A	C	HLA-DRB5
6	32489735	G	T	HLA-DRB5
6	32489786	T	G	HLA-DRB5
6	32489792	G	T	HLA-DRB5

Chr	Position	ref	alt	Gene
6	32489796	C	T	HLA-DRB5
6	32489813	G	C	HLA-DRB5
6	32489825	T	A	HLA-DRB5
6	32489888	A	T	HLA-DRB5
6	32489919	G	A	HLA-DRB5
6	32489949	G	A	HLA-DRB5
6	32497914	C	T	HLA-DRB5
6	32548628	G	A	HLA-DRB1
6	32548632	T	A	HLA-DRB1
6	32549340	C	G	HLA-DRB1
6	32549357	G	A	HLA-DRB1
6	32549424	C	T	HLA-DRB1
6	32549475	T	C	HLA-DRB1
6	32549481	C	T	HLA-DRB1
6	32549520	T	C	HLA-DRB1
6	32549525	C	G	HLA-DRB1
6	32549531	T	C	HLA-DRB1
6	32549540	C	T	HLA-DRB1
6	32549579	TG	T	HLA-DRB1
6	32549588	G	A	HLA-DRB1
6	32549589	A	C	HLA-DRB1
6	32551886	C	T	HLA-DRB1
6	32551915	A	G	HLA-DRB1
6	32551939	G	T	HLA-DRB1
6	32551990	T	G	HLA-DRB1
6	32551996	G	T	HLA-DRB1
6	32552000	C	T	HLA-DRB1
6	32552029	A	T	HLA-DRB1
6	32552048	C	T	HLA-DRB1
6	32552075	A	G	HLA-DRB1
6	32552092	A	T	HLA-DRB1
6	32552123	G	A	HLA-DRB1
6	32552143	C	T	HLA-DRB1
6	32552146	AG	A	HLA-DRB1
6	32605309	A	G	HLA-DQA1
6	32609105	G	A	HLA-DQA1
6	32609126	T	C	HLA-DQA1
6	32609147	A	T	HLA-DQA1
6	32609150	C	G	HLA-DQA1
6	32609173	C	G	HLA-DQA1
6	32609192	A	G	HLA-DQA1
6	32609195	G	A	HLA-DQA1
6	32609207	C	T	HLA-DQA1
6	32609213	G	A	HLA-DQA1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
6	32609216	G	T	HLA-DQA1
6	32609220	TGA	T	HLA-DQA1
6	32609221	G	C	HLA-DQA1
6	32609222	A	T	HLA-DQA1
6	32609224	T	TTC	HLA-DQA1
6	32609229	C	A	HLA-DQA1
6	32609231	A	G	HLA-DQA1
6	32609238	AGGT	A	HLA-DQA1
6	32609239	G	A	HLA-DQA1
6	32609254	G	T	HLA-DQA1
6	32609255	G	T	HLA-DQA1
6	32609264	G	C	HLA-DQA1
6	32609276	TGG	T	HLA-DQA1
6	32609806	C	T	HLA-DQA1
6	32609874	T	G	HLA-DQA1
6	32609952	T	C	HLA-DQA1
6	32609964	G	T	HLA-DQA1
6	32609965	C	A	HLA-DQA1
6	32609969	T	G	HLA-DQA1
6	32609974	T	G	HLA-DQA1
6	32610401	G	A	HLA-DQA1
6	32629755	G	A	HLA-DQB1
6	32629764	C	T	HLA-DQB1
6	32629891	C	T	HLA-DQB1
6	32629905	T	C	HLA-DQB1
6	32629920	C	T	HLA-DQB1
6	32629931	C	G	HLA-DQB1
6	32629935	C	G	HLA-DQB1
6	32629936	C	T	HLA-DQB1
6	32629963	C	T	HLA-DQB1
6	32632593	C	T	HLA-DQB1
6	32632599	A	G	HLA-DQB1
6	32632605	C	A	HLA-DQB1
6	32632608	C	G	HLA-DQB1
6	32632650	C	T	HLA-DQB1
6	32632659	C	T	HLA-DQB1
6	32632660	T	G	HLA-DQB1
6	32632700	T	A	HLA-DQB1
6	32632703	G	A	HLA-DQB1
6	32632721	A	T	HLA-DQB1

Chr	Position	ref	alt	Gene
6	32632724	C	T	HLA-DQB1
6	32632745	G	A	HLA-DQB1
6	32632748	T	A	HLA-DQB1
6	32632769	T	C	HLA-DQB1
6	32632775	G	C	HLA-DQB1
6	32632790	C	A	HLA-DQB1
6	32632818	T	G	HLA-DQB1
6	32632832	A	T	HLA-DQB1
6	32634282	A	G	HLA-DQB1
6	32713784	C	A	HLA-DQA2
6	32726753	G	A	HLA-DQB2
6	32726788	T	C	HLA-DQB2
6	32726803	C	T	HLA-DQB2
6	33036853	A	C	HLA-DPA1
6	33036951	A	G	HLA-DPA1
6	33036999	T	C	HLA-DPA1
6	33037424	T	C	HLA-DPA1
6	33037474	A	G	HLA-DPA1
6	33037522	T	C	HLA-DPA1
6	33037579	A	T	HLA-DPA1
6	33037580	T	G	HLA-DPA1
6	33037639	G	A	HLA-DPA1
6	33037640	C	T	HLA-DPA1
6	33053577	G	A	HLA-DPB1
6	33659472	C	G	ITPR3
6	39033595	G	A	GLP1R
6	40400288	C	T	LRFN2
6	41119109	CTCT	C	TREML1
6	41162518	T	C	TREML2
6	41162562	G	C	TREML2
6	41166021	C	T	TREML2
6	41166149	A	G	TREML2
6	41166154	G	C	TREML2
6	41250466	T	A	TREM1
6	41303995	A	G	NCR2
6	41309552	T	C	NCR2
6	42891022	G	A	PTCRA
6	46108083	A	G	ENPP4
6	46801071	G	T	MEP1A
6	46801092	A	G	MEP1A
6	46803018	A	T	MEP1A
6	46803102	A	G	MEP1A
6	46827073	A	G	GPR116

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
6	46827239	C	T	GPR116
6	46834685	G	A	GPR116
6	46977458	T	C	GPR110
6	46977783	C	T	GPR110
6	47624239	A	G	GPR111
6	47624299	A	G	GPR111
6	47626833	C	A	GPR111
6	47646748	T	C	GPR111
6	47646842	A	G	GPR111
6	47649265	T	A	GPR111
6	47649574	A	G	GPR111
6	51497519	C	T	PKHD1
6	51612837	C	T	PKHD1
6	51777251	G	A	PKHD1
6	51875250	A	C	PKHD1
6	51890823	G	A	PKHD1
6	51910905	T	C	PKHD1
6	51914956	G	A	PKHD1
6	51920465	A	C	PKHD1
6	55196587	C	T	GFRAL
6	69349073	A	G	BAI3
6	69666684	A	G	BAI3
6	70407465	A	T	LMBRD1
6	87994504	C	T	GJB7
6	89926962	T	C	GABRR1
6	89926966	T	C	GABRR1
6	94120219	G	A	EPHA7
6	94120639	T	C	EPHA7
6	97247547	C	T	GPR63
7	100218631	C	T	TFR2
7	100230864	G	C	TFR2
7	100350763	T	G	ZAN
7	100353013	TA CA	T	ZAN
7	100361675	G	A	ZAN
7	100361819	C	T	ZAN
7	100366284	T	C	ZAN
7	100367573	T	C	ZAN
7	100371114	C	T	ZAN
7	100371358	G	A	ZAN
7	100371417	G	A	ZAN
7	100371474	G	A	ZAN
7	100373077	C	G	ZAN
7	100373367	G	C	ZAN

Chr	Position	ref	alt	Gene
7	100374087	A	G	ZAN
7	100377082	T	C	ZAN
7	100377138	A	AG GG C	ZAN
7	100377364	G	A	ZAN
7	100385532	A	C	ZAN
7	100385578	G	C	ZAN
7	100385714	G	A	ZAN
7	100386900	T	C	ZAN
7	100389590	C	T	ZAN
7	100395003	G	C	ZAN
7	100612952	G	A	MUC12
7	100612955	A	AC TG	MUC12
7	100612961	G	C	MUC12
7	100634356	G	A	MUC12
7	100634358	C	T	MUC12
7	100634821	A	G	MUC12
7	100634838	C	A	MUC12
7	100634877	C	T	MUC12
7	100634892	G	A	MUC12
7	100634933	G	C	MUC12
7	100634964	C	A	MUC12
7	100635205	C	T	MUC12
7	100635375	A	C	MUC12
7	100635535	C	T	MUC12
7	100635661	T	C	MUC12
7	100635679	C	G	MUC12
7	100635767	G	C	MUC12
7	100636147	C	T	MUC12
7	100636383	G	A	MUC12
7	100636408	G	A	MUC12
7	100636446	G	A	MUC12
7	100636447	A	G	MUC12
7	100636461	C	A	MUC12
7	100636551	C	T	MUC12
7	100636584	T	C	MUC12
7	100637008	C	A	MUC12
7	100637035	A	C	MUC12
7	100637193	C	A	MUC12
7	100637547	G	A	MUC12
7	100637556	G	A	MUC12
7	100637605	A	C	MUC12

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
7	100637839	C	G	MUC12
7	100637887	A	G	MUC12
7	100638135	G	A	MUC12
7	100638279	T	C	MUC12
7	100638444	G	A	MUC12
7	100638484	A	T	MUC12
7	100638828	C	T	MUC12
7	100638856	A	G	MUC12
7	100639012	C	T	MUC12
7	100639054	G	A	MUC12
7	100639066	C	T	MUC12
7	100639147	A	G	MUC12
7	100639153	C	T	MUC12
7	100639177	C	T	MUC12
7	100639203	A	C	MUC12
7	100639632	G	A	MUC12
7	100639708	C	T	MUC12
7	100641130	C	T	MUC12
7	100641631	C	T	MUC12
7	100641744	C	T	MUC12
7	100641745	G	A	MUC12
7	100641798	C	G	MUC12
7	100641913	G	A	MUC12
7	100641922	C	G	MUC12
7	100642455	G	A	MUC12
7	100642924	G	A	MUC12
7	100642986	G	A	MUC12
7	100643073	C	A	MUC12
7	100643080	C	T	MUC12
7	100643088	A	G	MUC12
7	100643104	C	T	MUC12
7	100643155	A	T	MUC12
7	100643490	G	T	MUC12
7	100643638	C	G	MUC12
7	100643832	C	G	MUC12
7	100644027	C	A	MUC12
7	100644059	G	C	MUC12
7	100644082	G	A	MUC12
7	100644089	C	A	MUC12
7	100644090	G	A	MUC12
7	100644167	T	G	MUC12
7	100644184	C	T	MUC12
7	100644303	G	A	MUC12
7	100644660	G	A	MUC12

Chr	Position	ref	alt	Gene
7	100644772	C	T	MUC12
7	100644793	C	T	MUC12
7	100645453	C	T	MUC12
7	100645525	A	C	MUC12
7	100645569	T	A	MUC12
7	100645825	C	A	MUC12
7	100646199	T	C	MUC12
7	100646322	C	A	MUC12
7	100646364	G	A	MUC12
7	100646676	A	G	MUC12
7	100646712	A	C	MUC12
7	100647276	C	A	MUC12
7	100647338	C	A	MUC12
7	100647339	G	A	MUC12
7	100647553	G	A	MUC12
7	100647565	C	T	MUC12
7	100647646	A	G	MUC12
7	100647652	C	T	MUC12
7	100647702	A	C	MUC12
7	100647819	C	T	MUC12
7	100648029	G	A	MUC12
7	100648117	C	G	MUC12
7	100648476	T	C	MUC12
7	100675128	G	A	MUC17
7	100675376	A	C	MUC17
7	100675512	G	A	MUC17
7	100675976	G	A	MUC17
7	100676409	C	T	MUC17
7	100677279	G	C	MUC17
7	100677285	G	A	MUC17
7	100677333	C	G	MUC17
7	100677405	G	A	MUC17
7	100677429	G	A	MUC17
7	100677455	G	A	MUC17
7	100677516	A	G	MUC17
7	100677523	A	C	MUC17
7	100677572	A	G	MUC17
7	100677630	C	T	MUC17
7	100677642	C	T	MUC17
7	100677645	C	A	MUC17
7	100677704	C	T	MUC17
7	100677710	A	G	MUC17
7	100677714	C	T	MUC17
7	100677816	G	A	MUC17

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
7	100677944	T	C	MUC17
7	100677974	A	G	MUC17
7	100677995	A	G	MUC17
7	100678013	G	A	MUC17
7	100678029	G	C	MUC17
7	100678032	T	C	MUC17
7	100678173	C	T	MUC17
7	100678370	C	T	MUC17
7	100678421	T	A	MUC17
7	100678433	A	T	MUC17
7	100678434	C	A	MUC17
7	100678464	C	T	MUC17
7	100678481	A	T	MUC17
7	100678527	G	T	MUC17
7	100678568	A	T	MUC17
7	100678610	A	T	MUC17
7	100678616	G	A	MUC17
7	100678622	G	A	MUC17
7	100678740	T	C	MUC17
7	100679136	T	C	MUC17
7	100679316	T	A	MUC17
7	100679318	A	C	MUC17
7	100679366	A	G	MUC17
7	100679388	A	C	MUC17
7	100679390	A	G	MUC17
7	100679550	G	A	MUC17
7	100679754	C	G	MUC17
7	100679760	G	A	MUC17
7	100680132	G	A	MUC17
7	100680137	C	T	MUC17
7	100680256	A	C	MUC17
7	100680268	A	G	MUC17
7	100680269	G	C	MUC17
7	100680286	T	G	MUC17
7	100680521	G	A	MUC17
7	100680761	G	A	MUC17
7	100680939	C	A	MUC17
7	100680983	G	A	MUC17
7	100681172	A	G	MUC17
7	100681751	A	G	MUC17
7	100683036	T	C	MUC17
7	100683050	T	G	MUC17
7	100683053	A	G	MUC17
7	100683864	C	T	MUC17

Chr	Position	ref	alt	Gene
7	100684593	G	A	MUC17
7	100686491	G	C	MUC17
7	100686777	C	T	MUC17
7	100695138	G	A	MUC17
7	105615406	G	A	CDHR3
7	105615426	G	C	CDHR3
7	105658451	G	A	CDHR3
7	110762920	G	A	LRRN3
7	11500372	C	T	THSD7A
7	11581121	T	C	THSD7A
7	116340262	A	G	MET
7	11676065	G	C	THSD7A
7	11871406	G	C	THSD7A
7	121653250	G	A	PTPRZ1
7	121653398	G	A	PTPRZ1
7	122635173	A	C	TAS2R16
7	123514896	G	T	HYAL4
7	126173902	G	C	GRM8
7	126542667	A	T	GRM8
7	128843396	G	A	SMO
7	131191476	C	T	PODXL
7	131193739	C	T	PODXL
7	131194255	G	C	PODXL
7	131195712	G	A	PODXL
7	131195959	C	T	PODXL
7	132193371	G	A	PLXNA4
7	138486096	G	C	TMEM213
7	141478308	T	C	TAS2R4
7	141478800	G	A	TAS2R4
7	142563253	T	G	EPHB6
7	142749439	T	C	OR6V1
7	142749919	G	A	OR6V1
7	142749928	T	C	OR6V1
7	142750243	G	A	OR6V1
7	142919731	C	A	TAS2R40
7	143097100	A	G	EPHA1
7	143632618	C	T	OR2F2
7	143632833	A	G	OR2F2
7	143771557	T	C	OR2A25
7	143792991	G	A	OR2A12
7	143806688	C	A	OR2A2
7	143826697	C	G	OR2A14
7	143826729	AC TT	A	OR2A14

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
7	143929434	G	C	OR2A42
7	143956181	A	C	OR2A7
7	147183121	A	C	CNTNAP2
7	147674989	A	C	CNTNAP2
7	147926842	A	C	CNTNAP2
7	154684153	T	C	DPP6
7	157931091	C	T	PTPRN2
7	157931144	C	T	PTPRN2
7	157931151	C	G	PTPRN2
7	157959794	T	C	PTPRN2
7	157959895	C	T	PTPRN2
7	157959911	A	G	PTPRN2
7	157959965	C	T	PTPRN2
7	157985149	C	G	PTPRN2
7	23300255	C	T	GPNMB
7	23300345	C	T	GPNMB
7	2692639	A	G	TTYH3
7	30915263	A	G	AQP1
7	31008686	G	A	GHRHR
7	31009576	G	T	GHRHR
7	34818113	A	T	NPSR1
7	44578836	T	C	NPC1L1
7	45216926	G	A	RAMP3
7	45217015	T	C	RAMP3
7	47840310	C	G	PKD1L1
7	47840387	C	T	PKD1L1
7	47847865	G	A	PKD1L1
7	47851623	C	T	PKD1L1
7	47852837	C	T	PKD1L1
7	47886583	T	C	PKD1L1
7	47913579	T	C	PKD1L1
7	47925525	G	T	PKD1L1
7	47927650	G	A	PKD1L1
7	47927744	C	T	PKD1L1
7	47930180	T	C	PKD1L1
7	47933494	C	T	PKD1L1
7	47968927	C	A	PKD1L1
7	5327517	A	C	SLC29A4
7	55229255	G	A	EGFR
7	76054372	G	A	ZP3
7	76069811	T	C	ZP3
7	76069881	A	G	ZP3
7	76069886	A	G	ZP3
7	76069902	G	C	ZP3

Chr	Position	ref	alt	Gene
7	76071228	A	G	ZP3
7	80300449	T	G	CD36
7	86539251	A	C	KIAA1324L
7	86539302	G	C	KIAA1324L
7	92098776	C	T	ERVW-1
7	92099196	A	G	ERVW-1
7	99473858	G	C	OR2AE1
7	99526754	G	A	GJC3
7	99971849	A	G	PILRA
8	10390452	C	T	PRSS55
8	10396056	G	C	PRSS55
8	10411495	T	C	PRSS55
8	10411512	TG	T	PRSS55
8	104312432	A	G	FZD6
8	104336788	C	G	FZD6
8	105361354	G	C	DCSTAMP
8	105367121	A	G	DCSTAMP
8	110397780	A	G	PKHD1L1
8	110406671	T	A	PKHD1L1
8	110413762	T	C	PKHD1L1
8	110437443	T	C	PKHD1L1
8	110441582	A	G	PKHD1L1
8	110448635	A	G	PKHD1L1
8	110455184	C	T	PKHD1L1
8	110455187	A	T	PKHD1L1
8	110455321	C	A	PKHD1L1
8	110457011	A	G	PKHD1L1
8	110460488	G	T	PKHD1L1
8	110476776	G	A	PKHD1L1
8	110492279	G	A	PKHD1L1
8	110495303	T	C	PKHD1L1
8	110504218	A	G	PKHD1L1
8	110510993	C	A	PKHD1L1
8	110523026	T	G	PKHD1L1
8	110527429	C	T	PKHD1L1
8	113241088	T	G	CSMD3
8	113299353	A	G	CSMD3
8	114186003	T	C	CSMD3
8	116725	T	G	OR4F21
8	145640235	C	T	SLC39A4
8	145640411	A	G	SLC39A4
8	145641328	C	T	SLC39A4
8	145641417	G	A	SLC39A4
8	145642002	C	T	SLC39A4

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
8	22262321	T	C	SLC39A14
8	22886020	A	G	TNFRSF10B
8	22900701	G	A	TNFRSF10B
8	23058220	T	G	TNFRSF10A
8	23059324	C	G	TNFRSF10A
8	23060256	T	C	TNFRSF10A
8	23082369	C	G	TNFRSF10A
8	23082477	G	A	TNFRSF10A
8	24178738	G	T	ADAM28
8	24199218	C	A	ADAM28
8	24339679	G	A	ADAM7
8	24356818	A	C	ADAM7
8	27324822	T	C	CHRNA2
8	27516955	G	A	SCARA3
8	27528446	T	C	SCARA3
8	27729527	C	T	SCARA5
8	27767231	C	G	SCARA5
8	27779093	G	A	SCARA5
8	27779612	G	A	SCARA5
8	2909992	G	A	CSMD1
8	3000108	C	G	CSMD1
8	3076959	T	C	CSMD1
8	3245054	C	T	CSMD1
8	32453358	G	A	NRG1
8	37654907	A	G	GPR124
8	37690619	A	C	GPR124
8	38879190	C	T	ADAM9
8	39080632	C	G	ADAM32
8	39112033	T	C	ADAM32
8	39564367	A	G	ADAM18
8	70594420	C	A	SLCO5A1
8	82607068	C	G	SLC10A5
8	95143186	C	G	CDH17
8	95188850	T	C	CDH17
8	97614625	G	A	SDC2
8	97614661	T	A	SDC2
9	104385711	C	T	GRIN3A
9	104433235	C	T	GRIN3A
9	104433255	C	T	GRIN3A
9	107266596	T	C	OR13F1
9	107267352	T	C	OR13F1
9	107299001	C	A	OR13C3
9	107331504	C	A	OR13C8
9	107360910	G	A	OR13C5

Chr	Position	ref	alt	Gene
9	107361111	T	C	OR13C5
9	107361129	C	T	OR13C5
9	107361460	G	A	OR13C5
9	107361642	G	A	OR13C5
9	107367392	TGTTA	T	OR13C2
9	107367664	AGC	A	OR13C2
9	107367841	A	T	OR13C2
9	107379895	G	T	OR13C9
9	107380215	T	A	OR13C9
9	107456763	T	C	OR13D1
9	107562804	T	C	ABCA1
9	107620835	G	A	ABCA1
9	107620867	C	T	ABCA1
9	113538122	G	A	MUSK
9	116018501	C	G	SLC31A1
9	120475302	A	G	TLR4
9	120475602	C	T	TLR4
9	123476303	A	C	MEGF9
9	125273574	G	A	OR1J2
9	125289521	G	A	OR1N1
9	125315557	T	C	OR1N2
9	125316028	T	C	OR1N2
9	125330714	G	A	OR1L8
9	125377087	A	G	OR1Q1
9	125377505	A	G	OR1Q1
9	125391241	G	A	OR1B1
9	125424189	C	G	OR1L1
9	125437482	G	A	OR1L3
9	125512193	C	A	OR1L6
9	125512245	C	T	OR1L6
9	125512770	T	C	OR1L6
9	125562651	G	A	OR1K1
9	125563212	T	C	OR1K1
9	127215344	A	G	GPR144
9	127215772	C	G	GPR144
9	127215778	C	T	GPR144
9	127220392	G	C	GPR144
9	127220938	G	C	GPR144
9	127231554	G	A	GPR144
9	127231579	A	G	GPR144
9	139399320	C	T	NOTCH1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
9	139413097	T	G	NOTCH1
9	139417464	T	G	NOTCH1
9	139748483	G	A	MAMDC4
9	139751164	G	A	MAMDC4
9	139751649	G	A	MAMDC4
9	140332478	A	G	ENTPD8
9	24543732	C	T	IZUMO3
9	24545451	G	A	IZUMO3
9	2635545	G	A	VLDLR
9	27168571	T	C	TEK
9	27190655	G	A	TEK
9	27203078	G	A	TEK
9	33385698	A	G	AQP7
9	33385733	C	T	AQP7
9	33387044	T	A	AQP7
9	33387047	T	G	AQP7
9	33387060	C	G	AQP7
9	34371788	A	T	KIAA1161
9	34372347	G	T	KIAA1161
9	35612978	G	A	CD72
9	35674347	A	T	CA9
9	35679251	A	G	CA9
9	35957615	T	C	OR2S2
9	35957831	G	A	OR2S2
9	35958047	T	C	OR2S2
9	39085808	T	C	CNTNAP3
9	39088517	C	A	CNTNAP3
9	39100130	G	C	CNTNAP3
9	39102655	A	G	CNTNAP3
9	39103743	C	T	CNTNAP3
9	39118196	C	A	CNTNAP3
9	39133031	C	A	CNTNAP3
9	43800940	G	A	CNTNAP3B
9	43822668	C	T	CNTNAP3B
9	43822704	G	A	CNTNAP3B
9	43822762	C	T	CNTNAP3B
9	43844281	G	C	CNTNAP3B
9	43849812	T	G	CNTNAP3B
9	43853583	CT	C	CNTNAP3B
9	43861081	T	G	CNTNAP3B
9	43891504	A	T	CNTNAP3B
9	43891683	G	A	CNTNAP3B
9	5431913	A	G	PLGRKT
9	72459437	C	T	C9orf135

Chr	Position	ref	alt	Gene
9	74305098	C	T	TMEM2
9	74319677	T	C	TMEM2
9	74332970	G	A	TMEM2
9	74349846	A	T	TMEM2
9	74360096	C	T	TMEM2
9	74360234	C	T	TMEM2
9	78853916	G	C	PCSK5
9	78854033	T	A	PCSK5
9	78910241	A	C	PCSK5
9	78910273	G	A	PCSK5
9	78923638	C	T	PCSK5
9	78936358	G	A	PCSK5
9	78936492	A	G	PCSK5
9	78937974	C	T	PCSK5
9	78938043	G	A	PCSK5
9	78938186	G	C	PCSK5
9	78969059	C	A	PCSK5
9	8485787	C	G	PTPRD
9	8518052	G	C	PTPRD
9	91994101	G	A	SEMA4D
9	91994433	C	T	SEMA4D
9	92003679	C	T	SEMA4D
9	94493330	G	C	ROR2
9	94495608	T	C	ROR2
10	101560169	G	A	ABCC2
10	102056790	G	A	PKD2L1
10	102057262	C	T	PKD2L1
10	102057362	A	C	PKD2L1
10	104678350	G	A	CNNM2
10	105810400	T	C	COL17A1
10	115804036	A	G	ADRB1
10	117075090	T	G	ATRNL1
10	120353843	G	A	PRLHR
10	123310871	A	G	FGFR2
10	135084781	G	A	ADAM8
10	135086766	G	A	ADAM8
10	15573050	A	G	ITGA8
10	15649698	T	G	ITGA8
10	15649710	G	A	ITGA8
10	15719621	C	G	ITGA8
10	18116531	C	T	MRC1
10	18138639	A	G	MRC1
10	18183217	G	A	MRC1
10	18242311	A	G	SLC39A12

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
10	20506418	G	A	PLXDC2
10	25464527	C	A	GPR158
10	33199278	T	G	ITGB1
10	33475282	C	T	NRP1
10	35929254	GC CC GC GC CC GC GC CC GC CG CG CC CG CG CC CG CC GC CG CG CC GC CC GC GC CC CC AG CG CC C	G	FZD8
10	35929308	AG CG CC CC CC	A	FZD8
10	47658590	G	C	ANTXRL
10	47658597	T	G	ANTXRL
10	47669277	C	G	ANTXRL
10	47674009	G	A	ANTXRL
10	50315893	A	G	VSTM4

Chr	Position	ref	alt	Gene
10	55755491	C	T	PCDH15
10	55892642	T	C	PCDH15
10	55955444	T	G	PCDH15
10	55955610	C	T	PCDH15
10	6002368	T	G	IL15RA
10	6063567	C	T	IL2RA
10	71176012	A	G	TACR2
10	71176066	T	C	TACR2
10	71265955	A	G	TSPAN15
10	71582188	C	T	COL13A1
10	71690231	G	A	COL13A1
10	72015429	C	A	NPFFR1
10	73046617	A	G	UNC5B
10	73103969	T	C	SLC29A3
10	73111408	C	T	SLC29A3
10	73199595	C	T	CDH23
10	73434888	G	C	CDH23
10	73434906	G	A	CDH23
10	73515221	T	C	C10orf54
10	73520632	A	C	C10orf54
10	73544086	G	A	CDH23
10	73550117	C	G	CDH23
10	73558128	G	A	CDH23
10	73558886	G	A	CDH23
10	73558952	C	T	CDH23
10	73571765	T	C	CDH23
10	85960404	G	C	CDHR1
10	85962824	C	T	CDHR1
10	86017740	C	T	RGR
10	87489317	T	C	GRID1
10	88414569	C	T	OPN4
10	97453464	T	G	TCTN3
10	97607266	G	A	ENTPD1
11	102272839	C	A	TMEM123
11	102272884	C	T	TMEM123
11	112832299	C	T	NCAM1
11	112832308	A	AG	NCAM1
11	113561054	A	G	TMPRSS5
11	113567640	TG	T	TMPRSS5
11	113803028	A	C	HTR3B
11	113803666	G	A	HTR3B
11	117403235	G	T	DSCAML1
11	117864063	A	G	IL10RA
11	117864846	A	G	IL10RA

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
11	117978578	G	A	TMPRSS4
11	117982464	A	G	TMPRSS4
11	117988105	C	T	TMPRSS4
11	118074337	A	G	AMICA1
11	118081345	A	T	AMICA1
11	119181832	C	G	MCAM
11	119185677	T	C	MCAM
11	119215586	C	T	MFRP
11	119216504	C	T	MFRP
11	119216555	T	C	MFRP
11	120811161	G	A	GRIK4
11	121393684	G	A	SORL1
11	121437819	C	G	SORL1
11	121491782	G	A	SORL1
11	122722440	C	A	CRTAM
11	122726430	A	G	CRTAM
11	122955402	C	T	CLMP
11	123624425	T	C	OR6X1
11	123624658	G	T	OR6X1
11	123814478	A	G	OR6T1
11	123847558	C	T	OR10S1
11	123848121	T	C	OR10S1
11	123886307	C	T	OR10G4
11	123886550	C	T	OR10G4
11	123886822	C	T	OR10G4
11	123886865	T	A	OR10G4
11	123893745	C	T	OR10G9
11	123893988	C	T	OR10G9
11	123894234	A	G	OR10G9
11	123894246	A	G	OR10G9
11	123900385	C	A	OR10G8
11	123900916	C	T	OR10G8
11	123909650	C	G	OR10G7
11	123909653	G	T	OR10G7
11	123909656	G	A	OR10G7
11	123909671	G	A	OR10G7
11	123909695	G	C	OR10G7
11	124056566	G	A	OR10D3
11	124180082	A	G	OR8D1
11	124189306	G	A	OR8D2
11	124190036	G	A	OR8D2
11	124252750	G	A	OR8B2
11	124253170	G	A	OR8B2
11	124266697	A	AG	OR8B3

Chr	Position	ref	alt	Gene
11	124267177	T	C	OR8B3
11	124294236	A	G	OR8B4
11	124294703	T	C	OR8B4
11	124413261	A	T	OR8B12
11	124440617	C	T	OR8A1
11	124742365	G	A	ROBO3
11	124746180	C	T	ROBO3
11	125864206	T	C	CDON
11	125871715	G	A	CDON
11	125885283	G	C	CDON
11	125889526	C	T	CDON
11	125891269	C	T	CDON
11	125891295	T	C	CDON
11	126343227	T	C	KIRREL3
11	130010313	G	A	APLP2
11	130058437	G	A	ST14
11	130060569	G	A	ST14
11	130064567	A	G	ST14
11	1769226	G	T	IFITM10
11	18158993	T	C	MRGPRX3
11	18159254	A	G	MRGPRX3
11	18159455	T	C	MRGPRX3
11	18194827	C	G	MRGPRX4
11	18194878	T	G	MRGPRX4
11	18195051	T	C	MRGPRX4
11	18195537	A	G	MRGPRX4
11	1855821	G	C	SYT8
11	1856344	C	A	SYT8
11	19077904	T	G	MRGPRX2
11	2432666	C	T	TRPM5
11	26586801	G	A	MUC15
11	26660708	T	G	ANO3
11	26743240	C	T	SLC5A12
11	27390220	T	C	LGR4
11	27393886	G	A	LGR4
11	3239559	G	A	MRGPRG
11	3239818	C	T	MRGPRG
11	3249984	C	T	MRGPRE
11	35226155	A	G	CD44
11	35229673	T	C	CD44
11	408174	G	T	SIGIRR
11	44940828	G	A	TSPAN18
11	4608116	T	C	OR52I2
11	4615767	A	G	OR52I1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
11	4615771	A	G	OR52I1
11	4615837	G	A	OR52I1
11	4615857	G	A	OR52I1
11	4616062	C	T	OR52I1
11	4674282	A	G	OR51E1
11	46886077	A	T	LRP4
11	46897446	G	A	LRP4
11	46898771	T	C	LRP4
11	46916179	T	G	LRP4
11	46917868	A	G	LRP4
11	48145375	A	C	PTPRJ
11	48146522	G	A	PTPRJ
11	48146622	G	A	PTPRJ
11	48149352	G	A	PTPRJ
11	48166267	G	C	PTPRJ
11	48285906	C	T	OR4X1
11	48285981	CC TT	C	OR4X1
11	48346523	C	T	OR4C3
11	48346541	A	T	OR4C3
11	48346547	C	T	OR4C3
11	48346551	C	A	OR4C3
11	48346962	A	G	OR4C3
11	48346996	G	A	OR4C3
11	48347014	G	A	OR4C3
11	48347067	C	T	OR4C3
11	48347267	T	C	OR4C3
11	48387186	A	G	OR4C5
11	48387201	G	A	OR4C5
11	48387424	G	T	OR4C5
11	48387668	C	G	OR4C5
11	48387683	GT	G	OR4C5
11	48387689	T	C	OR4C5
11	48387705	C	T	OR4C5
11	48387890	T	C	OR4C5
11	48387894	C	T	OR4C5
11	48387900	G	A	OR4C5
11	48387917	AT	A	OR4C5
11	48387930	T	C	OR4C5
11	48387945	C	CC A	OR4C5
11	48387960	T	C	OR4C5
11	48387978	T	A	OR4C5
11	48387983	T	C	OR4C5

Chr	Position	ref	alt	Gene
11	48510919	C	A	OR4A47
11	49186274	G	A	FOLH1
11	49204779	C	T	FOLH1
11	49208267	G	A	FOLH1
11	49227620	A	G	FOLH1
11	49973979	C	T	OR4C13
11	5020799	C	T	OR51L1
11	5068297	G	A	OR52J3
11	5080068	G	A	OR52E2
11	5080337	T	C	OR52E2
11	5080359	G	A	OR52E2
11	5080844	T	C	OR52E2
11	5091576	A	AC	OR52E1
11	51411914	T	C	OR4A5
11	51412327	T	A	OR4A5
11	51412353	A	T	OR4A5
11	51412379	T	C	OR4A5
11	51435852	G	T	OR4A8P
11	51435866	A	G	OR4A8P
11	51515834	C	A	OR4C46
11	5172786	A	G	OR52A1
11	5172795	A	AC	OR52A1
11	55135435	C	T	OR4A15
11	55136219	C	T	OR4A15
11	55321823	T	A	OR4C15
11	55339652	C	T	OR4C16
11	55340170	A	C	OR4C16
11	55340379	T	C	OR4C16
11	55371381	G	A	OR4C11
11	55371807	A	T	OR4C11
11	55371828	G	T	OR4C11
11	55418620	G	A	OR4S2
11	55563602	G	A	OR5D14
11	55587914	A	G	OR5D18
11	55594936	T	G	OR5L2
11	55606472	T	C	OR5D16
11	55681566	G	A	OR5W2
11	55703861	T	C	OR5I1
11	55761528	T	C	OR5F1
11	55861277	GT	G	OR8I2
11	55861297	A	G	OR8I2
11	55861306	CA	C	OR8I2
11	55873013	A	T	OR8H2
11	55873026	G	T	OR8H2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
11	55873293	T	C	OR8H2
11	55889895	C	T	OR8H3
11	55890356	G	T	OR8H3
11	55904382	A	C	OR8J3
11	55927763	C	T	OR8K5
11	55978721	G	A	OR8J2
11	55979047	T	G	OR8J2
11	55979246	G	A	OR8J2
11	55999737	G	C	OR5T2
11	56000471	G	A	OR5T2
11	56000600	G	T	OR5T2
11	56020049	T	C	OR5T3
11	56058474	T	A	OR8H1
11	56058535	C	T	OR8H1
11	56086299	G	A	OR8K3
11	56113516	T	C	OR8K1
11	56113575	A	G	OR8K1
11	56113593	C	A	OR8K1
11	56113764	G	A	OR8K1
11	56127971	T	G	OR8J1
11	56143103	G	A	OR8U1
11	56143108	C	G	OR8U1
11	56143113	A	G	OR8U1
11	56143125	C	T	OR8U1
11	56143158	A	G	OR8U1
11	56143370	G	A	OR8U1
11	56143371	T	G	OR8U1
11	56143394	A	G	OR8U1
11	56143592	C	T	OR8U1
11	56185224	A	G	OR5R1
11	56185689	A	G	OR5R1
11	56237966	T	C	OR5M3
11	56310222	C	T	OR5M11
11	56431216	C	T	OR5AR1
11	56431667	G	A	OR5AR1
11	56468155	T	G	OR9G1
11	56468368	C	T	OR9G1
11	56468416	G	A	OR9G1
11	56468449	A	T	OR9G1
11	56511160	A	G	OR9G4
11	56756398	G	T	OR5AK2
11	56756399	G	T	OR5AK2
11	56756664	G	A	OR5AK2
11	56756915	A	G	OR5AK2

Chr	Position	ref	alt	Gene
11	57114109	G	A	P2RX3
11	5758051	G	A	OR56B1
11	5758270	G	A	OR56B1
11	5776479	A	C	OR52N4
11	5776779	T	C	OR52N4
11	57958497	T	C	OR9Q2
11	57982229	A	G	OR1S1
11	57982763	A	G	OR1S1
11	57982832	T	A	OR1S1
11	57995777	G	A	OR10Q1
11	5809241	T	C	OR52N1
11	5809548	G	A	OR52N1
11	5809746	C	T	OR52N1
11	5809806	GA	G	OR52N1
11	5809811	G	T	OR52N1
11	58125774	A	G	OR5B17
11	58126305	A	T	OR5B17
11	58170342	C	T	OR5B3
11	58170374	T	C	OR5B3
11	5842356	A	G	OR52N2
11	5842384	C	G	OR52N2
11	5862845	A	G	OR52E6
11	58978684	G	A	MPEG1
11	58978940	C	T	MPEG1
11	58979393	G	A	MPEG1
11	5906048	G	A	OR52E4
11	59189912	G	A	OR5A2
11	59211188	G	A	OR5A1
11	59224464	G	C	OR4D6
11	59224720	A	G	OR4D6
11	59225221	T	C	OR4D6
11	59282960	C	G	OR4D9
11	60070176	A	G	MS4A4A
11	6007613	T	G	OR52L1
11	60637164	C	T	ZP1
11	60775227	C	T	CD6
11	60776186	C	T	CD6
11	60776209	C	T	CD6
11	60776306	C	T	CD6
11	60777073	G	A	CD6
11	60886913	C	T	CD5
11	6129837	C	T	OR56B4
11	6221249	A	G	OR52W1
11	62656046	A	C	SLC3A2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
11	6291120	G	A	CCKBR
11	63057925	G	A	SLC22A10
11	64480930	A	T	NRXN2
11	64820695	T	G	NAALADL1
11	66083129	T	C	CD248
11	6646584	C	T	DCHS1
11	6647461	A	T	DCHS1
11	6648388	G	A	DCHS1
11	6648424	G	A	DCHS1
11	6790106	T	C	OR2AG2
11	68030173	C	A	C11orf24
11	6806827	G	T	OR2AG1
11	68115489	A	G	LRP5
11	6816875	G	A	OR6A2
11	68174189	G	A	LRP5
11	68201295	C	T	LRP5
11	6867167	C	A	OR10A5
11	6867199	C	T	OR10A5
11	68777323	T	C	MRGPRF
11	6891758	A	C	OR10A2
11	6912921	AT T	A	OR2D2
11	6913243	T	C	OR2D2
11	6913244	A	G	OR2D2
11	6913482	G	A	OR2D2
11	74907582	C	T	SLCO2B1
11	75152243	C	T	GDPD5
11	76371418	C	T	LRRC32
11	76371705	C	G	LRRC32
11	76954788	T	TA	GDPD4
11	76954812	T	C	GDPD4
11	76954833	G	A	GDPD4
11	76979550	T	C	GDPD4
11	7818471	C	T	OR5P2
11	78525384	T	G	TENM4
11	78565314	C	G	TENM4
11	7949707	A	G	OR10A6
11	89880604	G	A	NAALAD2
11	89883708	A	G	NAALAD2
11	89883726	C	T	NAALAD2
11	89902154	A	G	NAALAD2
11	92086513	C	T	FAT3
11	92086662	A	G	FAT3
11	92531356	A	G	FAT3

Chr	Position	ref	alt	Gene
11	92533057	A	G	FAT3
11	92534442	A	G	FAT3
11	92702962	G	A	MTNR1B
11	93797619	A	G	HEPHL1
12	10041364	G	A	KLRF2
12	10046052	C	A	KLRF2
12	10069302	C	T	CLEC2A
12	101336201	G	C	ANO4
12	10137557	A	C	CLEC12A
12	101560328	C	A	SLC5A8
12	10279261	G	T	CLEC7A
12	10313448	C	G	OLR1
12	104062457	T	G	STAB2
12	104069795	G	A	STAB2
12	104124018	A	C	STAB2
12	104139034	C	A	STAB2
12	104153004	C	G	STAB2
12	10560957	T	C	KLRC4
12	10571091	T	G	KLRC3
12	10571658	C	T	KLRC3
12	10587111	A	G	KLRC2
12	108985945	A	G	TMEM119
12	108985976	G	A	TMEM119
12	108986017	G	A	TMEM119
12	109017264	T	C	SELPLG
12	109017266	G	A	SELPLG
12	109017411	GA GT GG TC TG TG CC TC CA TG GC TG CT GG TG GA GT GG	G	SELPLG

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
		TC TG TG CT TC CA TG GC TG CT GG TG C		
12	109017650	GG AG TG GT CT GT GC CT CC GT GG GC AC TG GT T	G	SELPLG
12	109017898	C	T	SELPLG
12	10954383	T	A	TAS2R7
12	10954632	C	A	TAS2R7
12	10954916	A	G	TAS2R7
12	10978402	G	A	TAS2R10
12	11061122	T	C	TAS2R13
12	11149711	C	A	TAS2R20
12	11149720	A	G	TAS2R20
12	11150033	G	T	TAS2R20
12	11150240	T	C	TAS2R20
12	11183427	T	G	TAS2R31
12	11183484	C	G	TAS2R31
12	11183642	A	G	TAS2R31
12	11183676	C	T	TAS2R31
12	11183697	C	A	TAS2R31
12	11214437	T	C	TAS2R46

Chr	Position	ref	alt	Gene
12	11244299	A	G	TAS2R43
12	11244303	T	C	TAS2R43
12	11244309	A	G	TAS2R43
12	11244378	C	G	TAS2R43
12	11244603	T	A	TAS2R43
12	11286088	A	C	TAS2R30
12	11338750	T	C	TAS2R42
12	11339020	T	A	TAS2R42
12	113753203	G	A	SLC8B1
12	113758166	G	A	SLC8B1
12	118511726	C	T	VSIG10
12	121592689	T	C	P2RX7
12	121600253	T	C	P2RX7
12	121666646	A	G	P2RX4
12	122079295	A	G	ORAI1
12	12301898	C	T	LRP6
12	12483134	T	A	MANSC1
12	12483764	C	T	MANSC1
12	12496086	C	T	MANSC1
12	125834697	A	T	TMEM132B
12	126137060	C	T	TMEM132B
12	126138520	G	A	TMEM132B
12	128899673	A	G	TMEM132C
12	128899675	G	A	TMEM132C
12	128899748	G	A	TMEM132C
12	128899885	G	A	TMEM132C
12	128900005	G	A	TMEM132C
12	129153986	G	A	TMEM132C
12	129189702	C	G	TMEM132C
12	129189941	G	A	TMEM132C
12	129559086	C	A	TMEM132D
12	129559421	C	T	TMEM132D
12	130185054	A	T	TMEM132D
12	13103266	G	T	GPRC5D
12	131456080	T	G	GPR133
12	131498802	T	G	GPR133
12	1910786	C	T	CACNA2D4
12	21207389	T	C	SLCO1B7
12	21329738	A	G	SLCO1B1
12	21329813	C	A	SLCO1B1
12	26383834	G	A	SSPN
12	27916224	A	G	MANSC4
12	47186770	C	T	SLC38A4
12	48596484	A	T	OR10AD1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
12	48596546	C	T	OR10AD1
12	48597053	A	G	OR10AD1
12	48919659	T	C	OR8S1
12	51236931	G	A	TMPRSS12
12	51237816	G	A	TMPRSS12
12	53591647	T	A	ITGB7
12	55523586	AT	A	OR9K2
12	55523685	C	T	OR9K2
12	55523860	A	C	OR9K2
12	55524172	G	A	OR9K2
12	55641075	A	G	OR6C74
12	55641328	G	A	OR6C74
12	55688448	G	A	OR6C6
12	55688454	G	C	OR6C6
12	55714876	C	G	OR6C1
12	55725746	A	T	OR6C3
12	55820104	G	A	OR6C76
12	55846538	C	G	OR6C2
12	55945258	T	C	OR6C4
12	55968994	G	C	OR2AP1
12	56030938	C	T	OR10P1
12	56089357	C	T	ITGA7
12	56090126	C	T	ITGA7
12	57578673	G	A	LRP1
12	63544601	C	T	AVPR1A
12	6443001	G	A	TNFRSF1A
12	6464581	C	T	SCNN1A
12	6554628	G	A	CD27
12	6925294	T	C	CD4
12	6925407	C	T	CD4
12	70963619	G	A	PTPRB
12	70970293	T	C	PTPRB
12	70983895	A	C	PTPRB
12	71002893	C	T	PTPRB
12	71016340	C	T	PTPRB
12	71029733	C	T	PTPRB
12	71533622	C	T	TSPAN8
12	7288432	A	G	CLSTN3
12	7527124	A	G	CD163L1
12	7548996	C	G	CD163L1
12	7549009	C	T	CD163L1
12	7586017	C	T	CD163L1
12	7586260	T	C	CD163L1
12	7637769	G	A	CD163

Chr	Position	ref	alt	Gene
12	7640079	A	T	CD163
12	7649484	T	C	CD163
12	7890040	G	T	CLEC4C
12	7894056	G	A	CLEC4C
12	80818876	A	G	PTPRQ
12	80828843	A	G	PTPRQ
12	80855803	G	A	PTPRQ
12	80855825	C	T	PTPRQ
12	80878310	C	G	PTPRQ
12	80878317	G	A	PTPRQ
12	80889829	T	A	PTPRQ
12	80899901	C	A	PTPRQ
12	80933609	A	G	PTPRQ
12	80935345	T	C	PTPRQ
12	80935411	G	C	PTPRQ
12	81007527	T	C	PTPRQ
12	85266484	G	A	SLC6A15
12	9750669	A	G	KLRB1
12	9885578	G	A	CLECL1
12	9985915	G	T	KLRF1
13	114303822	G	A	ATP4B
13	23824818	G	A	SGCG
13	24167505	T	A	TNFRSF19
13	26624789	C	T	SHISA2
13	26624935	C	T	SHISA2
13	28624294	G	A	FLT3
13	33590718	G	A	KL
13	33628138	T	G	KL
13	33628193	G	C	KL
13	33629393	C	T	KL
13	39263646	G	A	FREM2
13	39264083	C	G	FREM2
13	39264597	T	A	FREM2
13	39264690	T	C	FREM2
13	39343822	C	T	FREM2
13	39424253	C	G	FREM2
13	39430314	C	T	FREM2
13	39452993	C	T	FREM2
13	52523808	C	T	ATP7B
13	53420344	A	G	PCDH8
13	53421473	T	C	PCDH8
13	86369403	T	C	SLITRK6
13	99356611	C	T	SLC15A1
13	99358401	C	G	SLC15A1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
13	99376181	C	T	SLC15A1
14	101195359	A	T	DLK1
14	101200860	G	A	DLK1
14	105609836	G	A	JAG2
14	105614675	T	G	JAG2
14	105614734	C	T	JAG2
14	105617042	C	T	JAG2
14	19377881	G	A	OR11H12
14	19377907	A	C	OR11H12
14	19378189	G	T	OR11H12
14	20295627	C	G	OR4N2
14	20389039	A	G	OR4K5
14	20389541	T	C	OR4K5
14	20389543	T	C	OR4K5
14	20404076	A	T	OR4K1
14	20404091	G	A	OR4K1
14	20404395	G	A	OR4K1
14	20404614	C	A	OR4K1
14	20444012	A	T	OR4K15
14	20444026	T	G	OR4K15
14	20470852	CT	C	OR4Q2
14	20528250	G	T	OR4L1
14	20528448	TC AT AG AT TT GC TC AC TG AC	T	OR4L1
14	20528682	T	C	OR4L1
14	20612465	A	G	OR4N5
14	20612672	T	C	OR4N5
14	20665538	C	T	OR11G2
14	20665627	A	G	OR11G2
14	20665640	G	A	OR11G2
14	20665840	G	A	OR11G2
14	20691888	C	A	OR11H6
14	20691962	C	G	OR11H6
14	20692188	T	C	OR11H6
14	20692453	G	T	OR11H6
14	23312594	G	A	MMP14

Chr	Position	ref	alt	Gene
14	24780016	G	A	LTB4R2
14	38724185	C	T	CLEC14A
14	53019944	A	G	GPR137C
14	53019964	CG CT GC AG CC TC AG GC GC CG CG	C	GPR137C
14	58563694	G	C	C14orf37
14	58605043	G	A	C14orf37
14	58605072	C	G	C14orf37
14	59931931	T	G	GPR135
14	70924955	A	C	ADAM21
14	70925501	G	C	ADAM21
14	74876355	G	C	SYNDIG1L
14	86089315	G	A	FLRT2
14	92958522	A	C	SLC24A4
14	96703484	C	T	BDKRB2
15	100269635	G	C	LYSMD4
15	100269680	G	C	LYSMD4
15	100269737	G	A	LYSMD4
15	102462478	TT AT C	T	OR4F4
15	22368855	T	C	OR4M2
15	22368862	G	A	OR4M2
15	22369132	G	A	OR4M2
15	22382728	C	T	OR4N4
15	22383249	G	A	OR4N4
15	28260053	G	A	OCA2
15	31369123	A	G	TRPM1
15	31453147	G	A	TRPM1
15	32446127	G	A	CHRNA7
15	41803754	G	A	LTK
15	41860490	T	A	TYRO3
15	45392075	G	A	DUOX2
15	45399075	G	A	DUOX2
15	45403967	A	G	DUOX2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
15	45404066	G	A	DUOX2
15	45427488	C	T	DUOX1
15	48056219	A	G	SEMA6D
15	48058071	G	A	SEMA6D
15	51676020	T	G	GLDN
15	51696856	G	A	GLDN
15	55929515	C	G	PRTG
15	55972797	T	C	PRTG
15	65688138	A	G	IGDCC4
15	65689267	T	C	IGDCC4
15	65916527	A	T	SLC24A1
15	65917355	T	G	SLC24A1
15	66411441	C	T	MEGF11
15	66850362	C	T	LCTL
15	68605169	G	A	ITGA11
15	68609647	G	T	ITGA11
15	68628049	T	G	ITGA11
15	68628163	C	T	ITGA11
15	73994806	C	T	CD276
15	73995173	C	T	CD276
15	73996066	G	A	CD276
15	73996101	G	A	CD276
15	75310766	A	G	SCAMP5
15	75969335	C	T	CSPG4
15	75970070	C	T	CSPG4
15	75981550	C	T	CSPG4
15	75981551	G	A	CSPG4
15	75981803	G	A	CSPG4
15	75981816	C	T	CSPG4
15	75981841	AC CT CC AG CA CC AG	A	CSPG4
15	75981860	C	T	CSPG4
15	75982058	T	A	CSPG4
15	75982072	G	A	CSPG4
15	75982085	C	T	CSPG4
15	75982271	C	T	CSPG4
15	75982492	G	A	CSPG4
15	75982513	A	G	CSPG4
15	75982581	G	C	CSPG4

Chr	Position	ref	alt	Gene
15	75982670	G	A	CSPG4
15	75985464	C	T	CSPG4
15	75985469	A	C	CSPG4
15	76254311	C	T	NRG4
15	78922229	T	C	CHRNB4
15	85448875	C	A	SLC28A1
15	85478696	C	T	SLC28A1
15	85478729	G	A	SLC28A1
15	90039643	T	C	RHCG
15	90335788	C	T	ANPEP
15	90344352	T	C	ANPEP
15	90347814	G	A	ANPEP
15	90349558	C	T	ANPEP
15	90764219	G	A	SEMA4B
15	90768942	C	T	SEMA4B
15	93198678	TT GG AG C	T	FAM174B
15	93198784	A	G	FAM174B
16	1250389	A	G	CACNA1H
16	14956982	G	A	NOMO1
16	14960480	A	G	NOMO1
16	14988868	A	G	NOMO1
16	1584438	G	C	TMEM204
16	1584446	G	C	TMEM204
16	16358944	G	A	NOMO3
16	16367613	G	T	NOMO3
16	19041595	G	A	TMC7
16	19471571	G	A	TMC5
16	19475099	C	A	TMC5
16	2140554	G	A	PKD1
16	2152387	A	G	PKD1
16	2152865	C	G	PKD1
16	2153414	A	T	PKD1
16	2155426	T	C	PKD1
16	2158672	G	A	PKD1
16	2158680	C	T	PKD1
16	2159341	C	T	PKD1
16	2160973	A	G	PKD1
16	2161666	G	A	PKD1
16	2161744	G	A	PKD1
16	2162361	A	G	PKD1
16	2164211	G	A	PKD1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
16	2164808	C	T	PKD1
16	2165395	G	A	PKD1
16	2166538	G	A	PKD1
16	23388540	G	T	SCNN1B
16	24902299	C	G	SLC5A11
16	27356203	A	G	IL4R
16	28944396	C	G	CD19
16	29675114	C	T	SPN
16	29675326	A	G	SPN
16	29899123	G	T	SEZ6L2
16	29979430	T	C	TMEM219
16	30124072	C	G	GDPD3
16	30492823	C	T	ITGAL
16	30516565	C	A	ITGAL
16	30518041	G	C	ITGAL
16	309998	C	T	ITFG3
16	31276811	G	A	ITGAM
16	31336888	C	T	ITGAM
16	31367318	T	C	ITGAX
16	31371674	G	A	ITGAX
16	31374535	C	G	ITGAX
16	31391120	T	C	ITGAX
16	318822	G	T	ITFG3
16	3405986	G	A	OR2C1
16	426329	A	C	TMEM8A
16	426432	T	C	TMEM8A
16	427479	T	C	TMEM8A
16	57689805	A	C	GPR56
16	57710156	C	T	GPR97
16	57718325	G	A	GPR97
16	57935442	G	A	CNGB1
16	57935470	C	T	CNGB1
16	57937788	T	C	CNGB1
16	58079165	G	A	MMP15
16	65022234	C	T	CDH11
16	65025718	G	A	CDH11
16	66432381	T	C	CDH5
16	66432423	T	C	CDH5
16	66944180	C	T	CDH16
16	66945947	C	T	CDH16
16	66948130	G	A	CDH16
16	68325203	T	A	SLC7A6
16	68719113	G	A	CDH3
16	68721533	G	C	CDH3

Chr	Position	ref	alt	Gene
16	76482747	C	G	CNTNAP4
16	76501304	G	C	CNTNAP4
16	76532583	A	G	CNTNAP4
16	76587202	T	G	CNTNAP4
16	81180988	C	G	PKD1L2
16	81180995	T	C	PKD1L2
16	81181097	G	T	PKD1L2
16	81181783	T	C	PKD1L2
16	81181821	T	C	PKD1L2
16	81181869	T	C	PKD1L2
16	81183325	T	A	PKD1L2
16	81185412	C	T	PKD1L2
16	81190598	T	C	PKD1L2
16	81190613	A	G	PKD1L2
16	81190669	T	C	PKD1L2
16	81193358	C	G	PKD1L2
16	81194335	T	C	PKD1L2
16	81199520	T	C	PKD1L2
16	81199538	T	C	PKD1L2
16	81199544	G	A	PKD1L2
16	81208203	G	T	PKD1L2
16	81208348	G	T	PKD1L2
16	81208473	G	T	PKD1L2
16	81208515	G	A	PKD1L2
16	81241100	G	C	PKD1L2
16	81242102	G	A	PKD1L2
16	81242194	T	C	PKD1L2
16	81242198	G	A	PKD1L2
16	81249892	T	C	PKD1L2
16	81249927	C	T	PKD1L2
16	81249954	T	A	PKD1L2
16	81253759	A	G	PKD1L2
16	81253917	A	G	PKD1L2
16	824270	C	A	MSLNL
16	824473	G	A	MSLNL
16	824927	T	C	MSLNL
16	825234	C	T	MSLNL
16	825628	G	A	MSLNL
16	828760	G	C	MSLNL
16	84256103	C	T	KCNG4
16	84256410	C	T	KCNG4
16	84256422	C	T	KCNG4
16	84256619	C	T	KCNG4
16	84474484	G	A	ATP2C2

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
16	84476200	A	T	ATP2C2
16	84485677	T	A	ATP2C2
16	84495704	G	C	ATP2C2
16	89262657	G	A	SLC22A31
17	1183338	C	T	TUSC5
17	1183354	T	C	TUSC5
17	1183464	A	G	TUSC5
17	21319007	G	A	KCNJ12
17	21319121	C	T	KCNJ12
17	27284194	T	C	SEZ6
17	27284443	A	G	SEZ6
17	27286851	T	C	SEZ6
17	27287531	C	T	SEZ6
17	28706114	C	T	CPD
17	28749880	A	G	CPD
17	2996217	C	T	OR1D2
17	3030582	C	G	OR1G1
17	30372739	C	T	LRRC37B
17	3101590	C	T	OR1A2
17	31323899	G	A	SPACA3
17	3181644	G	C	OR3A2
17	3181662	G	C	OR3A2
17	3182172	C	T	OR3A2
17	3195603	T	A	OR3A1
17	32956086	A	C	TMEM132E
17	32957114	G	A	TMEM132E
17	32961994	T	G	TMEM132E
17	3431403	C	G	TRPV3
17	3635740	C	G	ITGAE
17	3657159	C	T	ITGAE
17	3657175	T	C	ITGAE
17	40841001	G	C	CNTNAP1
17	41003859	C	T	AOC3
17	41004637	G	A	AOC3
17	41004681	C	T	AOC3
17	41008373	G	A	AOC3
17	41931375	A	G	CD300LG
17	42437000	G	A	FAM171A2
17	42453065	A	C	ITGA2B
17	44372608	G	A	LRRC37A
17	44405764	A	C	LRRC37A
17	44407828	C	T	LRRC37A
17	44408406	A	G	LRRC37A
17	44409165	G	A	LRRC37A

Chr	Position	ref	alt	Gene
17	44409255	A	G	LRRC37A
17	44590284	G	A	LRRC37A2
17	44623627	A	C	LRRC37A2
17	44625834	C	G	LRRC37A2
17	44625866	A	C	LRRC37A2
17	44625928	C	A	LRRC37A2
17	44626347	T	C	LRRC37A2
17	44626866	T	C	LRRC37A2
17	44627027	G	A	LRRC37A2
17	44627117	A	G	LRRC37A2
17	45360730	T	C	ITGB3
17	4638563	G	A	CXCL16
17	4638737	A	G	CXCL16
17	47587819	C	T	NGFR
17	48149503	A	T	ITGA3
17	48155425	G	A	ITGA3
17	4836381	C	T	GP1BA
17	4837202	AC CA CC CC AG AG CC CA C	A	GP1BA
17	48712329	G	A	ABCC3
17	48712336	G	C	ABCC3
17	56233034	C	A	OR4D1
17	56247575	C	T	OR4D2
17	56492800	T	C	RNF43
17	60741917	G	A	MRC2
17	60744158	G	T	MRC2
17	60758296	A	C	MRC2
17	60767015	G	A	MRC2
17	61584720	A	T	ACE
17	62020348	T	C	SCN4A
17	62856153	G	A	LRRC37A3
17	62856621	T	C	LRRC37A3
17	62890818	C	T	LRRC37A3
17	62890867	C	T	LRRC37A3
17	62891154	G	A	LRRC37A3
17	62892071	G	C	LRRC37A3
17	62892159	G	T	LRRC37A3

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
17	62892181	G	C	LRRC37A3
17	62892442	G	C	LRRC37A3
17	6980273	C	T	CLEC10A
17	7012079	C	T	ASGR2
17	71238433	G	A	C17orf80
17	71380062	G	A	SDK2
17	71380087	G	C	SDK2
17	71384080	G	C	SDK2
17	71426656	G	T	SDK2
17	71434193	C	T	SDK2
17	71452153	A	G	SDK2
17	72233559	G	A	TTYH2
17	72240168	G	A	TTYH2
17	72240177	T	G	TTYH2
17	72363857	GC	G	GPR142
17	72469966	G	A	CD300A
17	72540936	G	A	CD300C
17	72610089	C	T	CD300E
17	72610164	T	C	CD300E
17	7339627	C	T	TMEM102
17	7348625	A	G	CHRNB1
17	7462969	A	G	TNFSF12
17	78215587	C	T	SLC26A11
17	7906519	G	T	GUCY2D
17	79768748	A	C	GCGR
17	80282566	C	T	SECTM1
17	9729445	A	T	GLP2R
18	10471732	G	A	APCDD1
18	10485612	A	C	APCDD1
18	28720072	G	A	DSC1
18	28720147	C	T	DSC1
18	28919779	A	C	DSG1
18	28919794	C	A	DSG1
18	28919884	A	C	DSG1
18	28919911	A	G	DSG1
18	28919913	G	A	DSG1
18	29046606	G	A	DSG3
18	29790520	G	A	MEP1B
18	29795101	T	C	MEP1B
18	334742	C	T	COLEC12
18	334994	A	G	COLEC12
18	335099	T	C	COLEC12
18	43417684	G	A	SIGLEC15
18	43417698	C	G	SIGLEC15

Chr	Position	ref	alt	Gene
18	43417812	C	A	SIGLEC15
18	50432602	C	G	DCC
18	50936935	A	G	DCC
18	52265308	A	C	DYNAP
18	59174759	C	A	CDH20
18	60021761	C	T	TNFRSF11A
18	60027241	C	T	TNFRSF11A
18	60028921	G	A	TNFRSF11A
18	63530016	A	G	CDH7
18	64176319	T	G	CDH19
18	64211251	C	T	CDH19
18	64235877	G	A	CDH19
18	7774190	T	A	PTPRM
19	1000665	G	A	GRIN3B
19	1000717	C	A	GRIN3B
19	1000785	C	T	GRIN3B
19	1003162	C	T	GRIN3B
19	1003172	C	T	GRIN3B
19	1003221	C	A	GRIN3B
19	1003657	G	A	GRIN3B
19	1044712	A	G	ABCA7
19	10449358	T	C	ICAM3
19	1055191	G	A	ABCA7
19	10742170	A	G	SLC44A2
19	10961024	C	G	C19orf38
19	11222300	G	A	LDLR
19	11233909	A	C	LDLR
19	14153293	T	C	IL27RA
19	14269279	T	G	LPHN1
19	14499616	C	T	CD97
19	14507213	A	C	CD97
19	14507228	A	C	CD97
19	14758168	G	A	EMR3
19	14769339	C	G	EMR3
19	14862458	G	A	EMR2
19	14875388	G	A	EMR2
19	14876195	G	T	EMR2
19	14877799	G	C	EMR2
19	14910438	C	T	OR7C1
19	14910654	T	C	OR7C1
19	14951879	C	T	OR7A10
19	14951882	T	A	OR7A10
19	14951884	T	G	OR7A10
19	14952143	G	C	OR7A10

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
19	15197898	G	A	OR1I1
19	15198363	A	C	OR1I1
19	15290007	C	T	NOTCH3
19	15839365	C	T	OR10H2
19	15852495	G	T	OR10H3
19	15918349	C	T	OR10H1
19	15918802	C	G	OR10H1
19	16060110	G	T	OR10H4
19	17471634	G	A	PLVAP
19	17994836	G	A	SLC5A5
19	18174731	C	T	IL12RB1
19	18180451	A	G	IL12RB1
19	18186618	T	C	IL12RB1
19	18188408	C	T	IL12RB1
19	18193060	G	A	IL12RB1
19	2290499	C	T	LINGO3
19	2396611	C	T	TMPRSS9
19	2405456	C	T	TMPRSS9
19	2408530	G	A	TMPRSS9
19	2416796	A	G	TMPRSS9
19	2425184	A	G	TMPRSS9
19	2425196	G	A	TMPRSS9
19	30021023	G	C	VSTM2B
19	30021131	G	A	VSTM2B
19	33355055	A	G	SLC7A9
19	33696350	C	T	LRP3
19	35524607	G	A	SCN1B
19	35718891	C	T	FAM187B
19	35718938	C	T	FAM187B
19	35719020	C	T	FAM187B
19	35719076	C	T	FAM187B
19	35719106	A	G	FAM187B
19	35719438	G	A	FAM187B
19	35790645	C	A	MAG
19	35850022	A	G	FFAR3
19	35850264	G	A	FFAR3
19	35863028	T	C	GPR42
19	36231288	C	T	IGFLR1
19	36330422	C	G	NPHS1
19	36336437	T	C	NPHS1
19	36339247	C	T	NPHS1
19	36349752	G	A	KIRREL2
19	36350474	G	A	KIRREL2
19	38851193	T	G	CATSPERG

Chr	Position	ref	alt	Gene
19	38851455	A	C	CATSPERG
19	39993470	T	A	DLL3
19	39993560	T	G	DLL3
19	39994711	T	C	DLL3
19	41884327	T	C	TMEM91
19	42083739	A	AG AC	CEACAM21
19	42083849	C	A	CEACAM21
19	42085873	A	G	CEACAM21
19	42132193	T	C	CEACAM4
19	42301685	A	C	CEACAM3
19	42301689	T	A	CEACAM3
19	42301763	A	C	CEACAM3
19	42301854	A	G	CEACAM3
19	42863035	A	G	MEGF8
19	42874901	G	A	MEGF8
19	43031248	T	G	CEACAM1
19	43031267	G	A	CEACAM1
19	43031369	G	A	CEACAM1
19	43031434	T	A	CEACAM1
19	43031514	G	T	CEACAM1
19	45150614	G	A	PVR
19	45161070	G	A	PVR
19	45179567	C	T	CEACAM19
19	45321841	G	A	BCAM
19	45322744	A	G	BCAM
19	47127439	A	G	PTGIR
19	47823038	G	A	C5AR1
19	47823871	G	T	C5AR1
19	49693558	A	C	TRPM4
19	49699758	TG CT GC GG GG GC C	T	TRPM4
19	49930180	T	C	GFY
19	49930295	C	G	GFY
19	49930352	C	A	GFY
19	49930721	T	C	GFY
19	49930786	C	A	GFY
19	501719	T	C	MADCAM1
19	501767	C	T	MADCAM1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
19	501801	A	C	MADCAM1
19	501802	G	C	MADCAM1
19	501900	C	A	MADCAM1
19	50462298	G	C	SIGLEC11
19	50462860	C	T	SIGLEC11
19	50463030	C	A	SIGLEC11
19	50463035	G	T	SIGLEC11
19	50463044	T	C	SIGLEC11
19	50463982	T	G	SIGLEC11
19	50464171	C	G	SIGLEC11
19	50464184	T	C	SIGLEC11
19	51294098	C	T	ACPT
19	51628529	A	G	SIGLEC9
19	51628622	A	C	SIGLEC9
19	51630482	C	A	SIGLEC9
19	51630485	C	A	SIGLEC9
19	51728641	A	G	CD33
19	51728815	T	C	CD33
19	51729594	T	C	CD33
19	51841312	C	T	VSIG10L
19	51841417	C	T	VSIG10L
19	51918360	A	G	SIGLEC10
19	51919263	G	T	SIGLEC10
19	51919302	T	C	SIGLEC10
19	51919949	G	A	SIGLEC10
19	51920103	G	C	SIGLEC10
19	51920112	C	T	SIGLEC10
19	51920115	C	T	SIGLEC10
19	51920196	G	T	SIGLEC10
19	51960940	A	G	SIGLEC8
19	52000672	G	A	SIGLEC12
19	52001485	G	A	SIGLEC12
19	52004743	G	A	SIGLEC12
19	52004747	G	T	SIGLEC12
19	52004903	G	A	SIGLEC12
19	52033038	T	G	SIGLEC6
19	52033158	C	T	SIGLEC6
19	52033206	G	A	SIGLEC6
19	52130925	G	A	SIGLEC5
19	52131119	A	G	SIGLEC5
19	52133292	A	G	SIGLEC5
19	5223018	G	A	PTPRS
19	52249680	T	A	FPR1
19	52250216	A	G	FPR1

Chr	Position	ref	alt	Gene
19	53518178	C	G	ERVV-1
19	53554075	A	G	ERVV-2
19	53761740	T	C	VN1R2
19	54578105	C	T	TARM1
19	54675643	G	A	TMC4
19	54724061	A	G	LILRB3
19	54724407	C	T	LILRB3
19	54724443	G	A	LILRB3
19	54724458	A	G	LILRB3
19	54724635	C	T	LILRB3
19	54724979	C	T	LILRB3
19	54725798	G	T	LILRB3
19	54725864	C	T	LILRB3
19	54725907	G	C	LILRB3
19	54725913	C	T	LILRB3
19	54725930	C	T	LILRB3
19	54726162	T	G	LILRB3
19	54726241	C	T	LILRB3
19	54726299	A	C	LILRB3
19	54726324	G	C	LILRB3
19	54726347	T	A	LILRB3
19	54743824	T	G	LILRA6
19	54744159	T	C	LILRA6
19	54744195	G	A	LILRA6
19	54744210	A	G	LILRA6
19	54744387	C	T	LILRA6
19	54744794	G	A	LILRA6
19	54744799	G	C	LILRA6
19	54744919	T	A	LILRA6
19	54745497	G	A	LILRA6
19	54745550	G	T	LILRA6
19	54745576	C	G	LILRA6
19	54745907	A	G	LILRA6
19	54745993	C	T	LILRA6
19	54746051	A	C	LILRA6
19	54759361	T	C	LILRB5
19	54782670	T	C	LILRB2
19	54782724	G	A	LILRB2
19	54784122	T	C	LILRB2
19	54848121	C	T	LILRA4
19	54848157	A	G	LILRA4
19	54849399	T	C	LILRA4
19	54849942	G	A	LILRA4
19	54871664	G	C	LAIR1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
19	54872594	G	A	LAIR1
19	54872611	A	T	LAIR1
19	54872698	C	G	LAIR1
19	55085770	C	A	LILRA2
19	55086296	G	A	LILRA2
19	55086890	C	T	LILRA2
19	55087313	T	G	LILRA2
19	55087403	G	C	LILRA2
19	55087462	C	T	LILRA2
19	55087490	T	C	LILRA2
19	55098677	G	C	LILRA2
19	55098680	A	C	LILRA2
19	55098686	C	A	LILRA2
19	55098695	A	G	LILRA2
19	55106663	A	G	LILRA1
19	55106778	G	A	LILRA1
19	55106865	T	C	LILRA1
19	55107223	G	T	LILRA1
19	55107227	A	G	LILRA1
19	55107343	T	C	LILRA1
19	55107363	G	T	LILRA1
19	55143083	T	C	LILRB1
19	55143157	G	A	LILRB1
19	55143199	C	T	LILRB1
19	55143208	A	C	LILRB1
19	55143209	G	C	LILRB1
19	55143452	T	C	LILRB1
19	55143491	G	T	LILRB1
19	55144100	A	G	LILRB1
19	55144207	C	G	LILRB1
19	55144208	G	A	LILRB1
19	55144556	G	A	LILRB1
19	55144623	C	T	LILRB1
19	55144710	A	T	LILRB1
19	55144711	G	C	LILRB1
19	55145454	G	A	LILRB1
19	55146106	C	G	LILRB1
19	55176262	A	G	LILRB4
19	55237603	G	A	KIR3DL3
19	55237677	C	T	KIR3DL3
19	55239168	G	T	KIR3DL3
19	55239176	G	A	KIR3DL3
19	55253450	T	G	KIR2DL3
19	55253567	A	G	KIR2DL3

Chr	Position	ref	alt	Gene
19	55258808	C	T	KIR2DL3
19	55285072	A	G	KIR2DL1
19	55286650	T	C	KIR2DL1
19	55317456	G	A	KIR2DL4
19	55317612	G	A	KIR2DL4
19	55317633	G	A	KIR2DL4
19	55317669	C	G	KIR2DL4
19	55329021	A	G	KIR3DL1
19	55329854	G	A	KIR3DL1
19	55330019	C	T	KIR3DL1
19	55333193	C	A	KIR3DL1
19	55333256	C	T	KIR3DL1
19	55333275	G	T	KIR3DL1
19	55363482	C	T	KIR3DL2
19	55363504	G	A	KIR3DL2
19	55365240	C	A	KIR3DL2
19	55365320	G	T	KIR3DL2
19	55396913	G	A	FCAR
19	55418054	A	C	NCR1
19	55527081	C	T	GP6
19	55536595	G	A	GP6
19	55710074	G	A	PTPRH
19	55713535	G	A	PTPRH
19	55715309	C	T	PTPRH
19	55715359	A	G	PTPRH
19	55716742	C	T	PTPRH
19	55953906	T	G	SHISA7
19	5733874	T	C	CATSPERD
19	5744499	T	C	CATSPERD
19	5749171	A	G	CATSPERD
19	5772897	C	T	CATSPERD
19	5772950	G	A	CATSPERD
19	6531285	G	T	TNFSF9
19	6534841	G	A	TNFSF9
19	6665020	T	C	TNFSF14
19	6901891	G	A	EMR1
19	6903920	A	G	EMR1
19	6904137	C	T	EMR1
19	6913707	C	T	EMR1
19	6913811	A	G	EMR1
19	6919624	A	C	EMR1
19	6919753	A	G	EMR1
19	6921868	G	A	EMR1
19	6926378	T	C	EMR1

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
19	7743821	A	G	C19orf59
19	7743845	T	G	C19orf59
19	7831061	A	G	CLEC4M
19	7831628	G	A	CLEC4M
19	7981568	C	T	TGFBR3L
19	7982478	C	G	TGFBR3L
19	8367709	C	T	CD320
19	8841461	G	A	OR2Z1
19	8962389	G	A	MUC16
19	8979223	G	C	MUC16
19	8982271	G	A	MUC16
19	8987218	G	T	MUC16
19	8993018	G	A	MUC16
19	8996460	A	C	MUC16
19	8999445	C	T	MUC16
19	8999449	G	T	MUC16
19	9001833	A	G	MUC16
19	9002504	T	C	MUC16
19	9002519	G	C	MUC16
19	9002576	T	C	MUC16
19	9002597	C	T	MUC16
19	9002612	T	G	MUC16
19	9002636	C	T	MUC16
19	9002659	C	T	MUC16
19	9002660	C	T	MUC16
19	9003615	C	T	MUC16
19	9003618	C	T	MUC16
19	9003640	G	T	MUC16
19	9003645	G	A	MUC16
19	9005616	G	T	MUC16
19	9006749	C	T	MUC16
19	9015670	C	T	MUC16
19	9016693	A	AG GT	MUC16
19	9017468	C	T	MUC16
19	9018441	G	C	MUC16
19	9018508	T	G	MUC16
19	9018516	G	T	MUC16
19	9018540	A	G	MUC16
19	9020153	C	T	MUC16
19	9024871	C	T	MUC16
19	9028373	G	A	MUC16
19	9045821	G	A	MUC16
19	9045897	G	T	MUC16

Chr	Position	ref	alt	Gene
19	9046876	G	T	MUC16
19	9047267	C	T	MUC16
19	9048035	G	C	MUC16
19	9048170	G	A	MUC16
19	9048320	C	T	MUC16
19	9048335	G	T	MUC16
19	9048342	C	T	MUC16
19	9048895	A	C	MUC16
19	9049149	G	A	MUC16
19	9049322	G	A	MUC16
19	9049910	C	T	MUC16
19	9056941	A	G	MUC16
19	9056982	G	A	MUC16
19	9056989	C	G	MUC16
19	9057408	A	G	MUC16
19	9057687	A	G	MUC16
19	9057721	C	T	MUC16
19	9057750	T	A	MUC16
19	9058624	G	A	MUC16
19	9058907	G	C	MUC16
19	9058942	C	T	MUC16
19	9059159	T	G	MUC16
19	9059181	G	A	MUC16
19	9059232	T	C	MUC16
19	9059307	C	T	MUC16
19	9060059	A	C	MUC16
19	9060085	C	T	MUC16
19	9060541	C	T	MUC16
19	9060774	A	G	MUC16
19	9060915	C	T	MUC16
19	9061560	G	A	MUC16
19	9062544	T	G	MUC16
19	9062622	G	A	MUC16
19	9062847	A	G	MUC16
19	9065203	T	C	MUC16
19	9066132	G	A	MUC16
19	9066140	T	G	MUC16
19	9066259	T	C	MUC16
19	9066874	G	T	MUC16
19	9068374	A	G	MUC16
19	9069792	G	A	MUC16
19	9069892	A	C	MUC16
19	9070186	C	T	MUC16
19	9070194	A	G	MUC16

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
19	9070225	G	C	MUC16
19	9070402	G	T	MUC16
19	9070837	G	A	MUC16
19	9071763	T	A	MUC16
19	9072313	A	G	MUC16
19	9072742	T	C	MUC16
19	9072975	G	A	MUC16
19	9074265	G	A	MUC16
19	9074950	G	T	MUC16
19	9075021	G	A	MUC16
19	9075217	T	A	MUC16
19	9075346	T	A	MUC16
19	9075565	C	T	MUC16
19	9075969	C	T	MUC16
19	9076083	G	A	MUC16
19	9076293	T	C	MUC16
19	9076728	C	T	MUC16
19	9076858	C	T	MUC16
19	9076929	T	G	MUC16
19	9076950	C	A	MUC16
19	9077196	C	T	MUC16
19	9077436	G	A	MUC16
19	9077581	G	T	MUC16
19	9077803	T	C	MUC16
19	9080462	C	G	MUC16
19	9080543	G	A	MUC16
19	9082465	GG TG TG AA GG TT AA CG TC T	G	MUC16
19	9083143	G	A	MUC16
19	9083174	G	A	MUC16
19	9083314	G	A	MUC16
19	9083457	C	T	MUC16
19	9083576	C	T	MUC16
19	9083791	G	A	MUC16
19	9084197	T	G	MUC16
19	9084233	C	T	MUC16

Chr	Position	ref	alt	Gene
19	9084299	T	C	MUC16
19	9084481	C	T	MUC16
19	9084953	C	A	MUC16
19	9085004	T	C	MUC16
19	9085958	A	G	MUC16
19	9086060	C	T	MUC16
19	9086123	A	C	MUC16
19	9086145	G	C	MUC16
19	9086269	T	C	MUC16
19	9086318	G	A	MUC16
19	9086533	T	C	MUC16
19	9087758	G	A	MUC16
19	9088330	G	A	MUC16
19	9088694	G	A	MUC16
19	9088721	A	T	MUC16
19	9088772	T	C	MUC16
19	9090182	T	C	MUC16
19	9090784	C	T	MUC16
19	9091772	G	A	MUC16
19	919933	G	A	KISS1R
19	9213396	G	A	OR7G2
19	9225940	G	A	OR7G1
19	9225973	G	A	OR7G1
19	9226159	TA A	T	OR7G1
19	9226192	A	G	OR7G1
19	9237622	T	C	OR7G3
19	9325252	G	A	OR7D4
19	9325278	G	A	OR7D4
19	9362297	C	T	OR7E24
20	10622501	G	C	JAG1
20	10626016	T	G	JAG1
20	13830137	A	C	SEL1L2
20	13912309	G	A	SEL1L2
20	14306773	T	G	FLRT3
20	1546911	C	G	SIRPB1
20	1552430	T	C	SIRPB1
20	1559259	C	T	SIRPB1
20	1616137	G	A	SIRPG
20	1616206	A	G	SIRPG
20	1895889	G	C	SIRPA
20	1895949	A	AG T	SIRPA
20	1895963	A	G	SIRPA

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
20	1895965	C	A	SIRPA
20	1895990	G	A	SIRPA
20	1896051	CC GA	C	SIRPA
20	1896059	G	A	SIRPA
20	1896060	T	C	SIRPA
20	19261606	T	C	SLC24A3
20	19261623	G	A	SLC24A3
20	23016692	G	C	SSTR4
20	23016970	T	G	SSTR4
20	23028686	C	A	THBD
20	23028724	G	A	THBD
20	23029695	G	C	THBD
20	23065209	G	A	CD93
20	24964558	T	C	APMAP
20	25282967	C	T	ABHD12
20	2945759	C	T	PTPRA
20	33447314	C	T	GGT7
20	3452041	C	T	ATRN
20	3528101	A	C	ATRN
20	3541382	T	C	ATRN
20	3565356	T	G	ATRN
20	3577062	G	A	ATRN
20	3654128	G	A	ADAM33
20	3655219	T	C	ADAM33
20	3675333	G	A	SIGLEC1
20	3675498	T	G	SIGLEC1
20	3682126	C	T	SIGLEC1
20	3684729	T	C	SIGLEC1
20	3686436	C	T	SIGLEC1
20	3686676	C	G	SIGLEC1
20	41818289	C	G	PTPRT
20	4229375	G	T	ADRA1D
20	4229505	CG CC CG CG CT	C	ADRA1D
20	4229514	T	C	ADRA1D
20	44751363	C	T	CD40
20	44869878	C	T	CDH22
20	45174465	C	T	OCSTAMP
20	45354829	C	G	SLC2A10
20	55066633	T	G	GCNT7

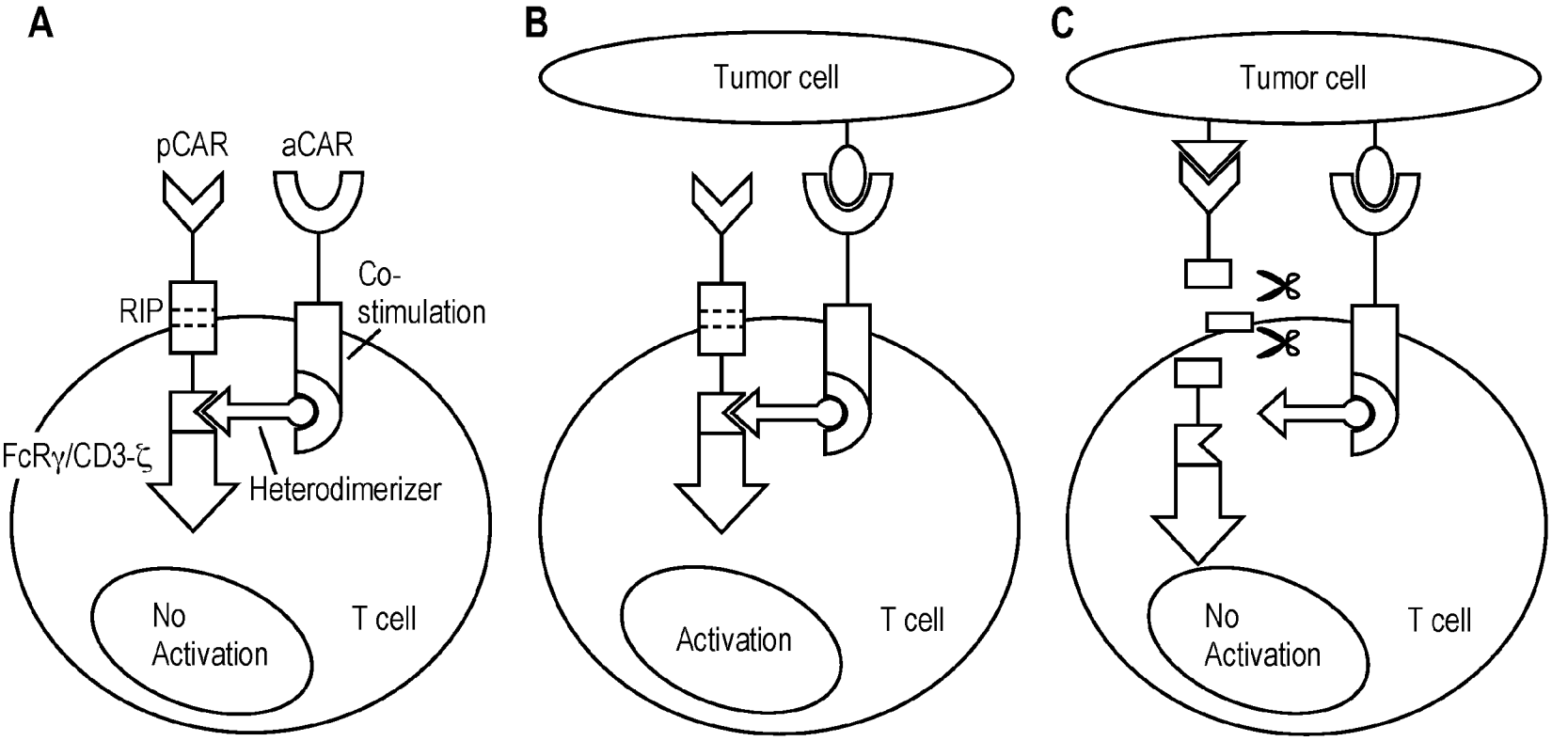
Chr	Position	ref	alt	Gene
20	55070268	A	T	GCNT7
20	58558015	T	C	CDH26
20	6031477	C	T	LRRN4
20	6033004	G	A	LRRN4
20	6033033	G	A	LRRN4
20	60503350	A	G	CDH4
20	62737568	T	C	NPBWR2
21	19651307	G	A	TMPRSS15
21	19670118	G	A	TMPRSS15
21	19687517	T	G	TMPRSS15
21	19704422	T	A	TMPRSS15
21	19756040	C	G	TMPRSS15
21	19770562	T	C	TMPRSS15
21	22710849	G	A	NCAM2
21	22746187	T	C	NCAM2
21	31587793	A	G	CLDN8
21	34715699	G	C	IFNAR1
21	34721782	C	T	IFNAR1
21	34787312	A	G	IFNGR2
21	39671476	G	A	KCNJ15
21	41137507	G	C	IGSF5
21	41142892	T	A	IGSF5
21	41142932	C	T	IGSF5
21	41143018	T	A	IGSF5
21	41725625	C	T	DSCAM
21	41725630	G	C	DSCAM
21	42852497	C	T	TMPRSS2
21	43505436	G	A	UMODL1
21	43529749	A	C	UMODL1
21	43531403	C	G	UMODL1
21	43531553	A	C	UMODL1
21	43531632	T	C	UMODL1
21	43531731	C	T	UMODL1
21	43539293	G	A	UMODL1
21	43546509	G	A	UMODL1
21	43803167	T	C	TMPRSS3
21	43808627	C	T	TMPRSS3
21	43809092	C	T	TMPRSS3
21	45656774	C	T	ICOSLG
21	45656920	C	T	ICOSLG
21	45821582	T	G	TRPM2
21	46309312	G	A	ITGB2
22	16449784	C	A	OR11H1
22	19951271	G	A	COMT

Figure 23 (Continued)

Chr	Position	ref	alt	Gene
22	20784050	T	A	SCARF2
22	20785639	G	A	SCARF2
22	21377650	G	A	P2RX6
22	24582041	A	G	SUSD2
22	24622648	T	C	GGT5
22	24628908	G	A	GGT5
22	25019155	T	C	GGT1
22	26688831	G	T	SEZ6L
22	26695077	G	T	SEZ6L
22	26709766	T	G	SEZ6L
22	29469192	G	A	KREMEN1
22	29517463	G	T	KREMEN1
22	29533572	C	G	KREMEN1
22	37319327	A	C	CSF2RB
22	37326443	G	C	CSF2RB
22	37466992	G	A	TMPRSS6
22	37471208	G	A	TMPRSS6
22	37485724	T	C	TMPRSS6
22	37538508	G	A	IL2RB
22	37603745	G	A	SSTR3
22	40066958	C	T	CACNA1I
22	41075543	A	G	MCHR1
22	41076970	G	A	MCHR1
22	42322716	G	C	TNFRSF13C
22	46655948	T	C	PKDREJ
22	46656242	A	G	PKDREJ
22	46656479	A	G	PKDREJ
22	46658438	A	C	PKDREJ
22	46780521	T	C	CELSR1
22	46782382	C	T	CELSR1
22	46786315	T	C	CELSR1
22	46787694	A	G	CELSR1
22	46787697	A	G	CELSR1
22	46793592	A	G	CELSR1
22	46860063	C	T	CELSR1
22	46929692	A	G	CELSR1
22	46930013	C	A	CELSR1

Chr	Position	ref	alt	Gene
22	46931077	G	C	CELSR1
22	46931309	T	C	CELSR1
22	46931402	G	C	CELSR1
22	46932788	C	T	CELSR1
22	46932854	G	A	CELSR1
22	46932946	G	A	CELSR1
22	50721492	C	T	PLXNB2
22	50721497	T	C	PLXNB2
22	50722134	T	C	PLXNB2
22	50722167	T	C	PLXNB2
22	50722408	T	C	PLXNB2
22	50728062	T	C	PLXNB2
X	102979486	T	C	GLRA4
X	108708516	C	T	GUCY2F
X	108708552	A	G	GUCY2F
X	135426693	A	G	GPR112
X	135426968	C	A	GPR112
X	135429503	C	A	GPR112
X	135430483	T	C	GPR112
X	135431236	T	C	GPR112
X	135474444	AG AT	A	GPR112
X	147088249	C	T	FMR1NB
X	153032658	G	A	PLXNB3
X	153035798	G	A	PLXNB3
X	153039502	G	C	PLXNB3
X	153689893	G	A	PLXNA3
X	153694334	C	G	PLXNA3
X	2724760	T	C	XG
X	40450585	C	G	ATP6AP2
X	65244971	C	T	VSIG4
X	65382685	T	C	HEPH
X	65822607	T	C	EDA2R
X	65824986	C	T	EDA2R
X	69478942	T	G	P2RY4
X	77268502	G	C	ATP7A
X	78216024	A	C	P2RY10

Figure 2


A
pCAR
aCAR
RIP
Co-
stimulation
FcRγ/CD3-ζ
Heterodimerizer
No
Activation
T cell
B
Tumor cell
Activation
T cell
C
Tumor cell
No
Activation
T cell