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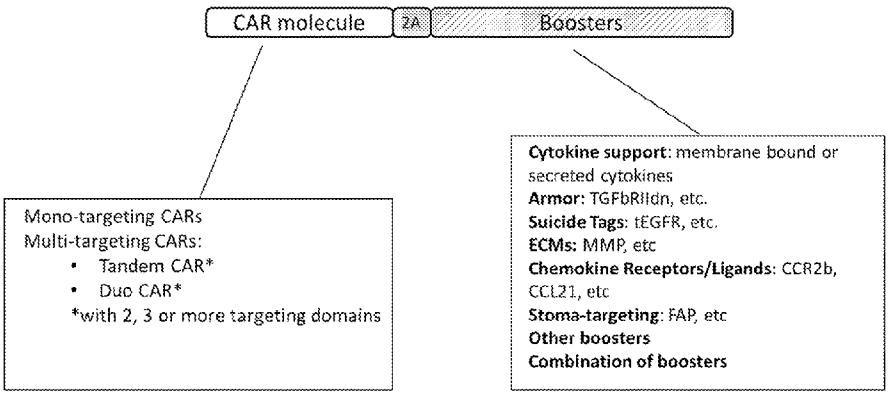

FIGURE 1

(57) Abstract: Novel anti-effector moiety antibodies or antigen binding domains thereof and CARs that contain such effector moiety antigen binding domains, either with or without one or more booster elements, and host cells expressing the receptors, and nucleic acid molecules encoding the receptors are provided herein, as well as methods of use of same in a patient-specific immunotherapy that can be used to treat solid tumor cancers and other diseases and conditions.

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CHIMERIC ANTIGEN RECEPTOR THERAPIES FOR TREATING SOLID TUMORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Number 63/393,088, filed on July 28, 2022, and to U.S. Provisional Patent Application Number 63/433,632, filed on December 19, 2022, the entire contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE DISCLOSURE

This application relates to the field of cancer, particularly to a composition encoding functional chimeric antigen receptors and methods of use of same in patient-specific immunotherapy to treat solid tumors.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on [[XXX]], 2022, is named Sequence Listing.txt and is [[XXX]] kilobytes in size.

BACKGROUND OF THE INVENTION

Cancer is one of the deadliest threats to human health. In the U.S. alone, cancer affects nearly 1.3 million new patients each year, and is the second leading cause of death after cardiovascular disease, accounting for approximately 1 in 4 deaths. Solid tumors are responsible for most of those deaths. Although there have been significant advances in the medical treatment of certain cancers, the overall 5-year survival rate for all cancers has improved only by about 10% in the past 20 years. Cancers, or malignant tumors, metastasize and grow rapidly in an uncontrolled manner, making treatment extremely difficult.

Chimeric Antigen Receptors are hybrid molecules comprising three essential units: (1) an extracellular antigen-binding motif, (2) linking/transmembrane motifs, and (3) intracellular T-cell signaling motifs (Long AH, Haso WM, Orentas RJ. Lessons learned from a highly-active CD22-specific chimeric antigen receptor. Oncoimmunology. 2013; 2 (4): e23621). The antigen-binding motif of a CAR is commonly fashioned after a single chain Fragment variable (scFv), the minimal binding domain of an

immunoglobulin (Ig) molecule. Alternate antigen-binding motifs, such as receptor ligands (*i.e.*, IL-13 has been engineered to bind tumor expressed IL-13 receptor), intact immune receptors, library-derived peptides, and innate immune system effector molecules (such as NKG2D) also have been engineered. Tandem, or even triple- or quadruple targeting domains may be constructed by linking multiple antigen-binding motifs sequentially, and attaching them to CAR hinge, transmembrane domain and intracellular sequences. Alternate cell types for CAR expression (such as NK, NKT, iNKT, or gamma-delta T cells) are also under development (Brown CE *et al.* Clin Cancer Res. 2012;18(8):2199–209; Lehner M *et al.* PLoS One. 2012; 7 (2): e31210). There remains significant work with regard to defining the most active T-cell population to transduce with CAR vectors, determining the optimal culture and expansion techniques, and defining the molecular details of the CAR protein structure itself.

The linking motifs of a CAR can be a relatively stable structural domain, such as the constant domain of IgG, or designed to be an extended flexible linker. Structural motifs, such as those derived from IgG constant domains, can be used to extend the scFv binding domain away from the T-cell plasma membrane surface. This may be important for some tumor targets where the binding domain is particularly close to the tumor cell surface membrane (such as for the disialoganglioside GD2; Orentas *et al.*, unpublished observations). To date, the signaling motifs used in CARs always include the CD3-ζ chain because this core motif is the key signal for T cell activation. The first reported second-generation CARs featured CD28 signaling domains and the CD28 transmembrane sequence. This motif was used in third-generation CARs containing CD137 (4-1BB) signaling motifs as well (Zhao Y *et al.* J Immunol. 2009; 183 (9): 5563–74). With the advent of new technology, the activation of T cells with beads linked to anti-CD3 and anti-CD28 antibody, the presence of the canonical "signal 2" from CD28 was no longer required to be encoded by the CAR itself. Using bead activation, third-generation vectors were found to be not superior to second-generation vectors in *in vitro* assays, and they provided no clear benefit over second-generation vectors in mouse models of leukemia (Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, Dimitrov DS, Morgan RA, FitzGerald DJ, Barrett DM, Wayne AS, Mackall CL, Orentas RJ. Anti-CD22-chimeric antigen receptors targeting B cell precursor acute lymphoblastic leukemia. Blood. 2013; 121 (7):1165–74; Kochenderfer JN *et al.* Blood. 2012; 119 (12):2709–20). This is borne out by the clinical success of CD19-specific CARs that are in a second generation CD28/CD3-ζ (Lee DW *et al.* American Society of Hematology Annual Meeting. New Orleans, LA; December 7-10, 2013) and a CD137/CD3-ζ signaling format (Porter DL *et al.* N Engl J Med. 2011; 365 (8): 725–33). In addition to CD137, other tumor necrosis factor receptor superfamily members such as OX40 also are able to provide important persistence signals in CAR-transduced T cells (Yvon E *et al.* Clin Cancer Res. 2009;15(18):5852–60). Equally important are the culture conditions under which the CAR T-cell populations were cultured.

Chimeric antigen receptor (CAR) T-cell therapy is a promising approach in treating both hematological and solid tumors, however the desired treatment benefits in solid tumors have not been achieved yet, whereas treatment of hematologic malignancies has proven highly effective, yielding several US Food and Drug Administration (FDA)- approvals for CAR T products for B cell malignancies and multiple myeloma (Gill S, et al., Blood Rev. 2016;30(3):157–1671; Victor E. et al., J Immunol April 1, 2021, 206 (7) 1561-1568; Wagner J, et al., Mol Ther. 2020 Nov 4;28(11):2320-2339; He C, et al., Cancers. 2020; 12(7):196). CAR cells are recombinant receptors for antigens, which redirect the specificity and function of T lymphocytes and other immune cells toward intended tumor targets (Sadelain M, et al., Cancer Discov. 2013;3:388–98). Engineered CAR T molecules redirect the immune activity towards desired antigens and depending on the quantity and quality of this interaction can have a lasting desired effect against tumor cells. Solid tumors present a challenge to current CAR T targeting approaches. Challenges to this therapeutic modality include tumor antigen escape, insufficient persistence of the engineered CAR molecules, and reduced effectiveness within the solid tumor environment. Additionally, CAR T cell – mediated toxicity resulting in cytokine release syndrome (CRS) and Immune Effector Cell Associated Neurotoxicity (ICANS), as well as off-target, and on-target off-tumor CAR reactivity, hamper further advancement of the CAR therapies in solid tumors. Optimization of CAR design remains largely empiric, and small modifications to the modular design can have a significant impact on a particular therapy (Guedan S, et al., Mol Ther Methods Clin Dev. 2018 Dec 31;12:145-156). Co-expression of multiple CAR molecules in the same effector cell, and optimization of CAR architecture and co-stimulatory domains is feasible, and may improve CAR T effector function an persistence (Schneider D, et al., Sci Transl Med. 2021 Mar 24;13(586)). In addition, third generation CAR T cells combining the signaling potential of two costimulatory domains may lead to improved CAR survival, expansion and effectiveness (Subklewe M, et al., Transfus Med Hemother. 2019;46(1):15-24; Maria-Luisa Schubert, MD et al., Blood. 2019. 134 (Supplement_1)).

Tumor antigen escape and tumor target heterogeneity are common causes of CAR therapy failure, and recent studies suggest this may be an especially important factor in the treatment of solid tumors (Majzner RG, and Mackall CL., Cancer Discov. 2018 Oct;8(10):1219-1226). Single targeting CAR therapies have shown effective in treating various cancers including B-ALL and multiple myeloma, but rates of relapse in some instances are as high as 60% (Walsh Z, et al., Curr Hematol Malig Rep 14, 451–459 (2019). By targeting multiple antigens simultaneously, risk of relapse and resistance are diminished (Schneider D, et al., Sci Transl Med. 2021 Mar 24;13(586)). Several options to avoid this outcome have been tested clinically, including sequential mono CAR treatment or co-infusions with CD19 and CD22 CARs, but proved ultimately ineffective (Shalabi H, et al., Haematologica Italy. 2018;103:e215–8). Ongoing or completed studies with multi-targeting CARs focused on various combinations of CD19,

CD20, CD22, HER2, TSLPR, IL-13Rα2 and more (Walsh, Z. et al., Curr Hematol Malig Rep 14, 451–459 (2019); Shalabi H, et al., Haematologica Italy. 2018;103:e215–8; Bielamowicz K, et al.,Neuro-Oncology. 2018;20(4): 506–18; Han X, et al., J Hematol Oncol 12, 128 (2019). Further clinical evidence is needed to determine which of the multi-targeting CAR therapies will prove most effective.

As noted, the efficacy of CAR therapies may be curtailed by short CAR T cell persistence and eventually being overwhelmed by the rebounding tumor burden, and this is especially true of solid tumors. The use of Interleukin (IL)-7 and CCR2b in in-vivo experiments has proved efficacious, showing both improved persistence and improved anti- tumor activity in neuroblastoma and melanoma models (Guangchao Li, et al., Frontiers in Oncology. 2021; 11: 2021). In a study with hematologic cancer, multiplex targeting and co-stimulation through the combination of a CAR and a chimeric costimulatory receptors (CCRs) has shown to be an effective method of by enhancing cytotoxic efficacy and persistence, thus preventing relapses of tumor clones and ultimately to improving clinical outcomes of CAR T cell treatment (Katsarou A, et al., Sci Transl Med. 2021 Dec 8;13(623); Pietrobon, V., et al., Int. J. Mol. Sci. 2021, 22, 10828).

Thus, while it may be believed that CARs can trigger T-cell activation in a manner similar to an endogenous T-cell receptor, a major impediment to the clinical application of CAR-based technology to date has been limited by *in vivo* expansion of CAR+ T cells, rapid disappearance of the cells after infusion, disappointing clinical activity, relapse of the underlying medical disease or condition. Many of these issues arise due to tumor target heterogeneity and tumor-mediated resistance to therapy, including the impact of tumor microenvironment and tumor stromal factors, and may be addressed by CAR T cell engineering.

Solid tumors present a challenging environment for CARs including an immunosuppressive environment characterized by physical, functional, and dynamic barriers hindering T-cell function. The tumor micro environment (TME) can prove difficult for successful CAR function and targeting. Tumors can employ strategies to resist the targeted effects of the CARs by increasing the production of inhibitory cytokines (Lindo L, et al., Front Immunol. 2021 Feb 10;11:618387). To counteract this increasingly hostile environment, research of "armored" CARs has been developing. Alabanza et al. (Front Immunol. 2022 Feb 9;13:832645) used this approach by co-expressing a TGFβ Receptor II dominant-negative form ("armor") on BCMA – targeting CAR T cells, in order to resist the suppressive effects of TGFβ in the multiple myeloma bone marrow niche. This resulted in functional persistence despite sustained exposure to TGFβ in animal models of Multiple Myeloma. The TME comprises a varied cell population that proves difficult to target. Yeku et al. (2017) study of ovarian cancer showed previous CAR T cell therapy for ovarian cancer directed against the folate receptor were largely unsuccessful in clinical trials due in part to action of immunosuppressive cytokines such as IL-4, IL-6, LIF, IL-10, TGFβ, myeloid derived suppressor cells, tumor associated macrophages (TAMs) and regulatory T cells which suppress the effects

of the targeting CARs. By creating an IL-12 armored CAR T cell they showed treatment could overcome the inhibitory microenvironment, alter the ascitic cytokine and TAM microenvironment, and overcome PD-L1-mediated inhibition (Yeku OO, et al., Sci Rep 7, 10541 (2017).

In addition to a challenging TME, the tumor stroma barrier presents challenges for effective CAR penetration. Solid tumors have a dense extracellular matrix (ECM) formed by cancer-associated fibroblasts (CAFs) which inhibits T cells from infiltrating the deep area of the tumor, thus negating continuous contact between tumor cells and CAR-T cells (Zhang, B. L et al., Sci. China Life Sci. 2016, 59 (4), 340−348). One approach is to facilitate the degradation of the ECM, thus allowing for effective CAR-T cell infiltration into the solid tumors' matrix. Engineering hyaluronidase (HAase) and the checkpoint blocking antibody α-PDL1 on the CAR-T cell surface has shown enhanced tumor infiltration and antitumor efficacy in solid tumors. (Yangyang Zhao, et al., ACS Central Science 2022 8 (5), 603-614). Similarly, an approach to engineer CARs to express the enzyme heparanase (HPSE), showed improve capacity to breakdown the ECM (Caruana I, et al., Nat Med. 2015 May; 21(5): 524–529).

Along with the various strategies discussed earlier that aim to improve the persistence and effectiveness of the CAR therapy, measures continue to be developed to improve the safety profile of CAR T therapies. Widespread adoption and application of CAR-T therapies has been limited because of the many challenges including tumor lysis syndrome, neurotoxicity syndrome, and cytokine release syndrome. Cytokine release syndrome (CRS) is a systemic inflammatory response triggered by T-cell activation. CRS is mainly caused by the activated CAR-T cell resulting in a notable increase in the secretion of proinflammatory factors (e.g., IL-6, IFN-γ, and TNF-α) by immune cells that disrupt the balance between proinflammatory and anti-inflammatory responses (Hay KA, et al., Blood. 2017;130: 2295–306). The use of suicide genes to prevent undue off target activity and improving the safety of CAR T cells is becoming increasingly important. Suicide genes, as a controlling gene, which are co-expressed with the CAR construct and are able to induce cell death when activated by an additional agent such as a drug or antibody. By design, the best possible agent for suicide gene activation will be biologically inert, have sufficient bio-availability and bio-distribution profiles, and be characterized by negligible or absent toxicity. (Jones BS, et al., Front Pharmacol. 2014; 5:254). Proof of concept of this was shown in a study by Kao et al., (2019) using truncated epidermal growth factor receptor (EGFRt) as a suicide gene system co-delivered with anti-CD19 CAR. Both in-vitro and in-vivo analysis showed positive results (Kao Roy L, et al. Human Gene Therapy. Apr 2019;413-428). Clinical evaluation of these strategies is ongoing but holds promise to unlock a wide array of safer CAR T therapeutic strategies.

Accordingly, there is an urgent and long felt need in the art for discovering compositions and methods for treatment of cancer using a CAR-based therapy that can exhibit cancer-specific intended therapeutic attributes without the aforementioned short comings.

The present invention addresses these ongoing unmet needs by providing boosted CAR compositions that exhibit one or more of the following characteristics: i) a high surface expression on transduced T cells, ii) a high degree of cytolysis and transduced T cell in vivo expansion and persistence, iii) multi-targeting to overcome antigen escape, iv) armor so as to overcome immunosuppression in TME, v) cytokine stimulated element to promote autonomous T cell stimulation with cytokines, resulting in heightened anti-tumor cytotoxicity, expansion, memory formation, cytokine secretion, persistence, vi) digestive enzymes to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration, and vii) an on-switch or off-switch, to control the expression of the CAR, or the co-expressed functional "booster" element(s), as well as therapeutic methods of using such boosted CARs that can be used to treat solid tumors, including tumors expressing a targetable antigen, such as CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B 1, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv

fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof, or other antigens, or any combination thereof, as well as other diseases and/or conditions expressing CAR-relevant targets.

SUMMARY OF THE INVENTION

Novel anti-effector moiety antibodies or antigen binding domains thereof and chimeric antigen receptors (CARs) that contain such effector moiety antigen binding domains are provided herein, as well as host cells (*e.g.*, T cells) expressing the receptors, and nucleic acid molecules encoding the receptors. CAR may consist either of a single molecule expressed on the effector cell surface, or a CAR comprised of an effector cell-expressed signaling module and a soluble targeting module, such as when the soluble targeting module binds to the cell-expressed signaling module, a complete functional CAR is formed. The CARs exhibit a high surface expression on transduced T cells, with a high degree of cytolysis and transduced T cell expansion and persistence *in vivo*. Methods of using the disclosed CARs, host cells, and nucleic acid molecules are also provided, for example, to treat a cancer in a subject.

In its broadest aspect, novel chimeric antigen receptors (CARs) are provided herein comprising a boosted CAR comprising a CAR construct with a main effector moiety molecule followed by one or more 2A sequences, in frame to one or more additional "booster" elements for improved function, including enhanced tumor penetration, to improve the therapeutic effect of CAR-T cells in solid tumors, hematologic tumors, autoimmune disease, hereditary disease, or other relevant indications.

In yet another broad aspect, novel chimeric antigen receptors (CARs) are provided herein comprising a boosted CAR wherein the functional co-expressed boosted CAR elements are expressed from a single multi-cistronic vector at high transduction efficiency, thereby simplifying the CAR manufacturing and release and reducing cost for market implementation. In one aspect, the boosted CAR compositions comprise one or more of the following characteristics: i) a high surface expression on transduced T cells, ii) multi-targeting to overcome antigen escape, iii) one or more armor elements so as to overcome immunosuppression in TME, iv) one or more cytokine stimulated elements (including, for example, and not by way of limitation, chemo attractive-receptors and/or secretion of chemotactic molecules) to promote autonomous T cell stimulation with cytokines, resulting in heightened anti-tumor cytotoxicity, expansion, memory formation, cytokine secretion, persistence, v) one or more digestive enzymes to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration, vi) one or more pro-inflammatory immune activators, and vii) one or more on-switches or off-switches, to control the expression of the CAR, wherein the boosted CARs achieve a high degree

of cytolysis and transduced T cell in vivo expansion and persistence to promote in vivo expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer or autoimmune, alloimmune, or autoaggressive disease, or prevention or amelioration of relapse of cancer or autoimmune, alloimmune, or autoaggressive disease, or a combination thereof, in a patient-specific manner.

In yet another broad aspect, the novel chimeric antigen receptors (CARs) provided herein may comprise single, tandem, or multi-targeting CAR constructs (including those in a DuoCAR format), or any combination thereof.

In certain aspects, the novel boosted CARs are under the control of one or more constitutive promoters, tissue specific promoters, or inducible promoters, or any combination thereof.

In certain aspects, the one or more switches comprising a tag, a kill switch, an on switch, an off switch, and/or an adapter switch, or any combination thereof.

In certain embodiments, the single, tandem, multi-targeting CARs, and DuoCARs (either with or without one or more booster elements) novel chimeric antigen receptors (CARs) are provided are used to transduce effector cells for the treatment of solid and hematologic tumors and other diseases through targeted antigens (for example, and not by way of limitation, CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B l, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6,

TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof.

In certain aspects, the effector cells comprise T cells, natural killer (NK) cells, natural killer T (NKT) cells, invariant natural killer T (iNKT) cells, dendritic cells (DCs), gamma delta T cells, monocytes, macrophages, stem cells, and induced pluripotent stem (iPS) cells.

In yet another broad aspect, one or more of the above-identified novel boosted chimeric antigen receptors (CARs) provided *supra* with respect to SEQ ID NOs: 151 to 256 may comprise either a single, tandem, or multi-targeting CAR construct (including those in a DuoCAR *format*), or any combination thereof.

For each of the various aspects and embodiments of the single, tandem, multi-targeting CARs, and DuoCARs, (either with or without one or more booster elements) CAR constructs specifically contemplated herein, the nucleotide sequences encoding the functional CAR (either with or without one or more booster elements) comprise the nucleotide sequence of SEQ ID NO: 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 245, 247, 249, 251, 253, or 255, or any combination thereof.

For each of the various aspects and embodiments of the single, tandem, multi-targeting CARs, and DuoCARs, (either with or without one or more booster elements) CAR constructs specifically contemplated herein, each vector encodes a functional CAR (either with or without one or more booster elements) comprising the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof.

In yet another broad aspect, one or more of the above-identified novel boosted chimeric antigen receptors (CARs) provided *supra* with respect to SEQ ID NOs: 127 to 149 may comprise either a single, tandem, or multi-targeting CAR construct (including those in a DuoCAR *format*), or any combination thereof.

For each of the various aspects and embodiments, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN antibody or a fragment thereof is provided comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149.

For each of the various aspects and embodiments, an isolated polynucleotide encoding a fully human anti-HER2, FOLR1, MUC16, CD276, EGFR, GD2, NKGD2 antibody or a fragment thereof is provided comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 127, 129, 131, 133, 135, 137, 139, and 141.

For each of the various aspects and embodiments, novel single, tandem, DuoCARs, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-ROR1 and/or anti-MSLN antigen binding domain comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain.

For each of the various aspects and embodiments, novel single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-HER2, FOLR1, MUC16, CD276, EGFR, GD2, and/or NKGD2 antigen binding domain comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 127, 129, 131, 133, 135, 137, 139, and 141; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain. For each of the various aspects and embodiments, novel single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-ROR1 and/or anti-MSLN antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain.

For each of the various aspects and embodiments, novel single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-HER2, FOLR1, MUC16, CD276, EGFR, GD2, and/or NKGD2 antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 128, 130, 132, 134, 136, 138, 140, and 142; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain.

In one embodiment, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti GD2 antibody or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, and 149.

In one embodiment, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti GD2 antibody or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, and 150.

In one aspect, an isolated nucleic acid molecule encoding a single, tandem, DuoCAR, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more boosting elements) is provided comprising, from N-terminus to C-terminus, at least one anti-ROR1 and/or anti-MSLN antigen binding domain encoded by a nucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, at least one transmembrane domain, and at least one intracellular signaling domain.

In one aspect, an isolated nucleic acid molecule encoding a single, tandem, DuoCAR, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more boosting elements) is provided comprising, from N-terminus to C-terminus, at least one anti-ROR1 and/or anti-MSLN antigen binding domain encoded by a nucleotide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment, the targeting domain of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is expressed separately in the form of monoclonal antibody, ScFv Fab, Fab'2 and is containing an antigen-targeting domain comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, coupled to an additional binding tag or epitope, whereas the effector-cell expressed component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) contains a binding domain specifically directed to bind the tag or epitope expressed on the soluble single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) module, such as specific binding on the soluble component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) to the cell bound component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) forms the full

functional single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) structure.

In yet another embodiment, an isolated nucleic acid molecule encoding the single, tandem, Duo, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) extracellular anti-ROR1 and/or anti-MSLN antigen binding domain further comprises at least one lipocalin-based antigen binding antigen (anticalins) that binds to ROR1 and/or MSLN.

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular anti-ROR1 and/or anti-MSLN antigen binding domain is connected to the transmembrane domain by a linker domain.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded anti-ROR1 and/or anti-MSLN extracellular antigen binding domain is preceded by a sequence encoding a leader or signal peptide.

In one aspect, the single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more boosting elements) provided herein further comprise a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular anti-ROR1 and/or anti-MSLN antigen binding domain, the intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded linker domain is derived from the extracellular domain of IgG1, IgG2, IgG3 or IgG4, CD8, TNFRSF19, or CD28, and is linked to a transmembrane domain.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) further comprises a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or a combination thereof.

In yet another aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multi-targeting, chimeric antigen receptor (CAR) construct, wherein the CAR

comprises at least one extracellular antigen binding domain comprising an anti-MSLN and/or anti-ROR1 antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain, wherein the T cells are T cells of a human having a cancer or an autoimmune, alloimmune, or autoaggressive disease. The cancer includes, inter alia, a hematological cancer such as leukemia *(e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma) or multiple myeloma, or a combination thereof.

In another aspect, methods of making single, tandem, DuoCAR, or multiple-targeting CAR construct-containing T cells (hereinafter "CAR-T cells") (either with or without one or more booster elements) are provided. The methods include transducing a T cell with a vector or nucleic acid molecule encoding a disclosed CAR that specifically binds MSLN and/or ROR1, thereby making the CAR-T cell.

In yet another aspect, a method of generating a population of RNA-engineered cells is provided that comprises introducing an *in vitro* transcribed RNA or synthetic RNA of a nucleic acid molecule encoding a disclosed single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more booster elements) into a cell of a subject, thereby generating a single, tandem, DuoCAR, or multiple-targeting CAR cell (either with or without one or more booster elements).

In yet another aspect, a method for diagnosing a disease, disorder or condition associated with the expression of MLSN and/or ROR1 on a cell, is provided comprising a) contacting the cell with a human anti-MSLN and/or anti-ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; and b) detecting the presence of MSLN and/or ROR1 wherein the presence of MSLN and/or ROR1 diagnoses for the disease, disorder or condition associated with the expression of MSLN and/or ROR1.

In another embodiment, a method of inhibiting MSLN and/or ROR1-dependent T cell inhibition, is provided comprising contacting a cell with a human anti-MSLN and/or anti-ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150. In one embodiment, the cell is selected from the group consisting of a MSLN and/or ROR1-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another aspect, a method is provided for inducing an anti-tumor immunity in a mammal comprising administering to the mammal a therapeutically effective amount of a T cell transduced with vector or nucleic acid molecule encoding a disclosed single, tandem, or multiple-targeting CAR (either with or without one or more booster elements).

In another embodiment, a method of treating or preventing cancer in a mammal is provided comprising administering to the mammal one or more of the disclosed single, tandem, or multiple-targeting CARs (either with or without one or more booster elements), in an amount effective to treat or prevent cancer in the mammal. The method includes administering to the subject a therapeutically effective amount of host cells expressing a disclosed single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) that specifically binds MSLN and/or ROR1 and/or one or more of the aforementioned antigens, under conditions sufficient to form an immune complex of the antigen binding domain on the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) and the extracellular domain of MSLN and/or ROR1 and/or one or more of the aforementioned antigens in the subject.

In yet another embodiment, a method is provided for generating a persisting population of genetically engineered T cells in a human diagnosed with cancer. In one embodiment, the method comprises administering to a human a T cell genetically engineered to express a single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) comprises at least one MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof; at least one transmembrane domain; and at least one intracellular signaling domain wherein the persisting population of genetically engineered T cells, or the population of progeny of the T cells, persists in the human for at least one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

In yet another aspect, a kit is provided for making a chimeric antigen receptor T-cell as described *supra* or for preventing, treating, or ameliorating any of the cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen in a subject as described *supra*, comprising a container comprising any one of the nucleic acid molecules, vectors, host cells, or compositions disclosed *supra* or any combination thereof, and instructions for using the kit.

In one aspect of the present invention, an immunotherapy composition is provided comprising a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more booster elements) which immunotherapy composition may be used to transduce autologous lymphocytes to generate active patient-specific anti-tumor lymphocyte cell populations that can be infused directly back into the patient to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In one embodiment, a pharmaceutical composition is provided wherein the at least one transmembrane domain of the single, tandem, DuoCAR, or multi-targeting CAR (either with or without one or more boosting elements) contains a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

It will be understood that the single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more booster elements), host cells, nucleic acids, and methods are useful beyond the specific aspects and embodiments that are described in detail herein. The foregoing features and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIGURE 1 depicts the structure of boosted CAR. Boosted CAR comprised of a CAR molecule followed by a 2A sequence, in frame to a boosting element. CAR molecule represented mono CARs and multi-targeting tandem or Dual CARs. Boosting elements various from cytokines (membrane bound IL7), armors (TGFβRIIdn), suicide tag (tEGFR), extracellular matrix enzymes (ECMs), chemokine receptors (CXCL8, CCL2), stroma targeting molecules (FAP), *et al.*

FIGURES 2A-2E depict the mIL7 armed ROR1 and/or MSLN CAR structure and surface expression on transduced primary T cell.(Figure 2A) Mono CAR armed with membrane bound IL7 (mIL7) comprised a ROR1 or MSLN scFv binding domain, IgG4 or CD8 hinge domain, CD8 transmembrane domain, 41BB or CD28 co-stimulatory domain, a CD3ζ activation domain, followed by a 2A peptide, and in frame to membrane bound IL7. Tandem boosted CAR constructs comprised of a MSLN-ROR1 tandem scFv targeting domain, IgG4 short hinge, CD8 or CD28 transmembrane domain, a single 4-1BB or tandem CD28_4-1BB co-stimulatory domain, a CD3ζ activation domain, and a 2A sequence connected mIL7. DuoCAR constructs contained a mono ROR1 CAR, followed by 2A sequence, a mono MSLN CAR with different co-stimulatory domain or transmembrane domain, in frame to 2A peptide connected m IL7. Mono ROR or MSLN CARs and tandem CARs were included as control constructs. Primary T cells from healthy

donor were activated with TransAct in the presence of IL-2, and transduced with lentiviral vectors encoding ROR1 and/or MSLN CAR constructs. Transduced T cells were assayed for CAR surface expression with ROR1 Fc and/or MSLN His staining followed by anti-Fc-AF647 or anti-His APC with flow cytometry. (Figure 2B) Percentage of ROR1 CAR expression in T cells transduced with CAR constructed encoding ROR1 binders was plotted. (Figure 2C) Percentage of MSLN CAR expression in T cells transduced with MSLN binder containing CAR was quantified. Mean fluoresce intensity of ROR1 binder expression (Figure 2D) and MSLN binder expression (Figure 2E) were presented as bar figures. Data represented one independent experiment from two different donors.

FIGURES 3A-3C depict the cytotoxicity of ROR1 and/or MSLN CAR constructs *in vitro*. Luciferase-based cytotoxicity assays were performed using $ROR1^+$ $MSLN^+$ tumor line OVCAR3 with (Figure 3A) CARs containing ROR1 scFv, (Figure 3B) CARs containing MSLN scFv, and $ROR1^-MSLN^-$ tumor line (Figure 3C) HL-60. All target lines were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at the 10 series effector to target (E:T) ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Non-linear EC50 shift, x is log concentration was used for curve fit. Data represented one independent experiment from two different donors.

FIGURES 4A-4C depict the relative potency of ROR1 and/or MSLN CAR constructs *in vitro*. Luciferase-based cytotoxicity assays were performed using $ROR1^+$ $MSLN^+$ tumor lines. CAR T cells and tumor cells were co-cultured overnight at the 10 different effector to target (E:T) ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Relative potency comparing to ROR1 CAR LTG2529 was calculated using non-linear EC50 shift, x is log concentration function in GraphPad Prism. Relative potency of each constructs targeting $ROR1^+$ $MSLN^+$ tumor lines : (Figure 4A) OVCAR-3, (Figure 4B) NCI-H226, (Figure 4C) CAPAN-1 was plotted as bar figures. Data represented one independent experiment from one to two different donors.

FIGURES 5A-5C depict CAR T cytokine release in response to NCI-H226 lung carcinoma cell lines. Culture supernatants of CAR T cells was evaluated after overnight incubation alone or with $ROR1^+MSLN^+$ NCI-H226 target cells at 10 different E:T ratios. Cytokine production of (Figure 5A) IFNγ, and (Figure 5B) TNFα, (Figure 5C) IL-2, were analyzed by ELISA. Mean± SEM of two technical replicates. Data show one experiment performed with technical triplicates from one donor, representing results from three independent experiments in separate donors.

FIGURES 6A-6C depict the membrane bound IL7 expression and its functionality of sustaining CAR T-cell growth after IL-2 withdrawal. (Figure 6A) Expression of membrane bound IL7 was determined by western using IL7 antibody followed with goat anti mouse HPR conjugated secondary antibody. GAPDH measured by anti-GAPDH and goat anti-Rabbit secondary antibody was included as loading

control. CAR T cells were transduced with lentivirus encoding ROR1 and/or MSLN CAR constructs with or without mIL7 at MOI 20. Transduced CAR T cells were washed and cultivated at 1e6/ml using TexMACS medium without IL-2 supplement. long term target cell stimulation. Cell expansion (Figure 6B) and T cell size (Figure 6C) were monitored weekly until no cell expansion observed for continuously 2-3 weeks. Data represented one independent experiment from two separate donors.

FIGURES 7A and 7B depict the time to 50% target cell killing (KT50) (Figure 7A) and the relative potency of MLSN CAR T cells before and after IL-2 withdrawal (Figure 7B). MSLN CAR with mIL7 D0245 and ROR2/MSLN DuoCAR with mIL7 D0282 were cultivated with TexMACS medium without IL-2 supplement for 69 days. Cytotoxicity of CAR D0245 and D0282 were measured by xCELLigence RTCA instrument using $ROR1^{+}MSLN^{+}$ pancreatic cancer cell line AsPC-1. MSLN CAR D0181, CAR D0245 and D0282 without IL-2 withdraw were included as controls. CAR T cells and target cells were cocultured at ET ratio 2:1. Percentage specific target lysis was assessed by impeded electron flow. KT50 represents the coincubation time necessary to achieve 50 % of the target cells cytolysis. Relative potency calculated based on KT50 of MSLN CAR D0181 without IL-2 withdrawal. Data represented one independent experiment from two separate donors.

FIGURES 8A-8E depict *in vitro* characterization of TGFβRIIdn boosted MSLN CARs. (Figure 8A) MSLN CAR D0181 comprised of MSLN scFv binding domain, CD8 hinge domain and transmembrane domain, 41BB co-stimulatory domain and a CD3ζ activation domain. Boosted CAR D0211 comprised of a mono MSLN CAR, a 2A peptide linker and in frame to a dominant negative TGFβ receptor II (TGFβRIIdn). Primary T cells from healthy donors were activated with TransAct in the presence of IL-2, and transduced with lentiviral vectors encoding MSLN CAR D0181 and boosted MSLN CAR D0211 constructs. (Figure 8B) CAR surface expression was assessed by flow cytometry using MSLN-His followed by anti-His-APC staining. The TGFβRIIdn expression of was determined by biotinylated TGFβR and streptavidin PE staining. Histogram overlay of UTD, CAR D0181 and D0211 was shown in right. (Figure 8C) Luciferase-based cytotoxicity assays were performed using MSLN+ tumor line NCI-H226, A431-MSLN and a MSLN- A431. All target lines were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at the 10 series effector to target (E:T) ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Non-linear EC50 shift, x is log concentration was used for curve fit. (Figure 8D) Culture supernatants of CAR T cells was evaluated after overnight incubation with MSLN+ NCI-H226 target cells at 10 different E:T ratios. Cytokine production of IFNγ, and TNFα, were analyzed by ELISA. Mean ± SEM of two technical replicates. Data represented one independent experiment from four separate donors. (Figure 8E) Kinetic killing assay testing the functionality of MSLN and ROR1 CAR T cells boosted with TGFβRIIdn -armor against AsPc-1 tumor cell line, in the presence or absence of TGFβ.

FIGURES 9A-9D depicts in expression and cytotoxicity of ROR1 CARs with TGFβRIIdn on an overnight endpoint killing assay at a range of effector to target cell ratios. Primary T cells from a healthy donor were activated with TransAct in the presence of IL-2, and transduced with lentiviral vectors encoding ROR1 CAR LTG2529 and boosted, TGFβRIIdn-armored ROR1CAR D0228 constructs. CAR surface expression was assessed by flow cytometry using ROR1 Fc followed by anti-Fc-AF647 staining. Percentage of CAR expression was plotted in panel (Figure 9A). ROR1+ target lines, OVAR3 (Figure 9B) , CAPAN-2(Figure 9C) and NCI-H226 (Figure 9D) were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at the various effector to target (E:T) ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Non-linear EC50 shift, x is log concentration function in Prism was used for curve fit. Data represented one independent experiment from 1 different donor.

FIGURES 10A and 10B depict the structure of MSLN and ROR1 CAR with ECM booster and surface expression in human primary T cells. (Figure 10A) MSLN targeting CAR comprised of a fully human MSLN scFv targeting domain, a CD8 hinge and transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ activation domain. ROR1 targeting CAR comprised of a fully human ROR1 scFv9 targeting domain, a IgG4 short hinge, CD8 transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ activation domain. Booster CARs contained mono targeting CARs, followed by 2A peptide, in frame to an ECM molecule. Heparanase (HPSE), Metalloproteinase (MMP2), Hyaluronidase PH-20 were selected as booster molecules . (Figure 10B) Primary T cells from healthy donor were activated with TransAct in the presence of IL-2, and transduced with lentiviral vectors encoding CAR constructs. Transduced T cells were assayed for CAR surface expression with ROR1 Fc or MSLN-His staining followed by anti-Fc-AF647 or anti-His APC respectively with flow cytometry. CD4 staining was included to identify CD4+ and CD8+ population. Percentage of CAR positivity was listed above the plot. UTD – untransduced control.

FIGURES 11A-11D depicts the cytotoxicity of MSLN and ROR1 CAR constructs *in vitro*. Luciferase-based cytotoxicity assays were performed using $ROR1^{+}$ $MSLN^{+}$ tumor lines: (Figure 11A) MEC-1 $ROR1^{Hi}$ $MSLN^{Hi,}$, (Figure 11C) NCI H226 and $ROR1^{-}MSLN^{-}$ tumor line, (Figure 11B) MEC-1, and (Figure 11D) HL-60. All target lines were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at the indicated effector to target (E:T) ratios: 1.25:1, 5:1, or 10:1. Percentage specific target lysis was assessed by luminometry. Data represented one independent experiment from two different donors. Mean ± SEM of three technical replicates. Representative experiment from one donor was shown in the panel.

FIGURES 12A and 12B depict the expression of HPSE in booster CARs and its capacity to facilitate CAR T cell migration *in vitro*. (Figure 12A) Secreted HPSE by CAR D0344 and CAR D0347 was measured by ELISA, mono CAR D0181, CAR D0290 and un-transduced T cell (UTD) from same donor were

included as control. Culture supernatants of CAR T cells was evaluated after overnight incubation. (Figure 12B) HPSE functionality was evaluated by migration assay using 0, 2.5 or 5 mg/ml Cultrex coated transwell. One million thawed CAR T cells were seed into precoated transwell. After 24hr, the total CAR T cells migrated into bottom chamber was quantify using Absolute counting beads by flow cytometer.

FIGURES 13A and 13B depict the *in vivo* activity of CAR T constructs in JeKo-1 xenograft model. NSG mice were implanted with $5x10^5$ JeKo-1 cells stably transduced with luciferase, via tail vein on Day 0. Tumor burden was determined using bioluminescent imaging. Mice with comparable mean tumor burden were randomly distributed into each group and injected with $5x10^6$/mouse CAR+ T cells or UTD on day 7 . Tumor kinetics were measured at day 13, 20, 27, 34, 41, and 48. (Figure 13A) representative mouse bioluminescent images were shown at indicated time points. (Figure 13B) Time course of tumor growth based on mouse whole body bioluminescence (radiance) were quantified as photons per second per cm^2 per steradian. TA-tumor alone, UTD – non-transduced T cell control. N=6, mean ± SEM.

FIGURE 14 depicts the body weight changes of mice during JeKo-1 xenograft study. NSG mice bearing JeKo-1 mantle cell lymphoma were treated with $5x10^6$ CART+ cells per mouse and mouse weights were recorded three times/week. Body weight change was calculated as the percentage of change from study initiation. Mean ± SEM. TA-tumor alone, UTD – non-transduced T cell control. N=6 mice/group.

FIGURES 15A and 15B depict the *in vivo* activity of CAR T constructs in OVCAR-3 xenograft model. NSG mice were injected intraperitoneally with $1x10^7$ OVCAR-3-luciferase cells on Day 0. Tumor burden was measured using bioluminescent imaging by IVIS-S5 instrument. Mice with comparable tumor burden were randomly distributed into each group, and treated with $5x10^6$/mouse CAR+ T cells or UTD on day 7. Kinetics of tumor development were measured at day10, 17, 24, 31, 38, 45, and 52. (Figure 15A) Mouse bioluminescent images were shown at indicated time points. (Figure 15B) Time course of tumor growth based on mouse whole body bioluminescence (radiance) were quantified as photons per second per cm^2 per steradian and plotted. TA-tumor alone, UTD – non-transduced T cell control. N=4~5, mean ± SEM.

FIGURE 16 depicts the body weight changes of mice during OVCAR-3 study. NSG mice bearing disseminated OVCAR-3 tumors were treated with $5x10^6$ CAR T-positive (CAR T+) cells per mouse and mouse weights were recorded three times/week. Body weight change was calculated as the percentage of change from study initiation. Mean ± SEM. TA-tumor alone, UTD – non-transduced T cell control. N=4~5 mice/group.

FIGURE 17 depicts the structure of ROR1 and FolR1 CAR with ECM booster and surface expression in human primary T cells. A) ROR1 targeting CAR comprised of a fully human ROR1 scFv9 targeting domain, a IgG4 short hinge, CD8 transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ activation domain. FolR1 targeting CAR comprised of a fully human Farle scFv targeting domain, a CD8 hinge and transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ activation domain

under the PGK or EF1α promoter. Booster CARs contained mono targeting CARs, followed by 2A peptide, in frame to an ECM molecule. Matrix Metalloproteinase-2 (MMP-2), Matrix Metalloproteinase-9 (MMP-9), Hyaluronidase (PH-20), and Heparanase (HPSE), were selected as booster molecules. For the ROR1 CAR set expressing hyaluronidase, PH-20 is expressed under the native or tPA signaling peptide in the presence, absence or retains 7 amino acids of the GPI anchor. B) Primary T cells from a healthy donor were activated with TransAct in the presence of IL-2, and transduced with lentiviral vectors encoding CAR constructs. Transduced T cells were assayed for CAR surface expression with ROR1-Fc or FolR1-Fc staining followed by anti-Fc-AF647 with flow cytometry. CD4 staining was included to identify CD4+ and CD8+ population. Percentage of CAR positivity was listed above the plot. UTD – un-transduced control.

FIGURES 18A-18E depict the cytotoxicity and cytokine release of ROR1 and FolR1 CARs constructs *in vitro*. A, B) Luciferase-based cytotoxicity assays were performed using antigen-specific tumor lines. ROR1 CARs were tested against ROR1+ lines NCI-H226 and MEC-1 ROR1Hi, MEC-1 was used as a negative control line which expresses basal levels of ROR1. CAR-T cells and tumor cells were co-cultured overnight at the indicated effector to target (E:T) ratios: 10:1, 5:1, or 1.25:1. Percentage specific target lysis was assessed by luminometry. (Figure 18A) Data represented one independent experiment from 3 different donors. Mean ± SD of three technical replicates. Representative experiment from one donor was shown in the panel. (Figure 18B) Data represented one independent experiment from 1 donor. Mean ± SD of three technical replicates. (Figure 18C) FolR1 CARs were tested against FolR1+ line OVCAR3 and HL-60 was used as a negative control for nonspecific killing. CAR-T cells and tumor cells were co-cultured overnight at the indicated effector to target (E:T) ratios: 10:1, 2.5:1, or 1.25:1. Percentage specific target lysis was assessed by luminometry. Data represented one independent experiment from 3 different donors. Mean ± SD of three technical replicates. Representative experiment from one donor was shown in the panel. All target lines were stably transduced with firefly luciferase. (Figure 18D, Figure 18E) Cytokine production of IFNγ, and TNFα, were analyzed by ELISA. (Figure18D) Culture supernatants of CAR-T cells was evaluated after overnight incubation with ROR1+ NCI-H226 target cells at E:T ratios 10:1, 5:1, 1.25:1. Mean ± SD of three technical replicates. Data represents 3 independent experiments from 3 separate donors. (Figure 18E) Culture supernatants of CAR-T cells was evaluated after overnight incubation with FolR1+ OVCAR3 target cells at E:T ratios 10:1, 2.5:1, 1.25:1. Mean ± SD of three technical replicates. Data represents 3 independent experiments from 3 separate donors.

FIGURES 19A-19D depict the expression of enzymes in booster CARs and its capacity to facilitate CAR-T cell migration *in vitro*. (Figure 19A) Left: Concentration of secreted MMP-9 by ROR1 co-expressing MMP-9 (D0373). Un-transduced and CAR D0290 were also measured by MMP-9 ELISA. Data represents one independent experiment out of 2 different donors tested. Right: HPSE by CAR D0368 and D0369 was measured by ELISA, CAR D0351 and un-transduced T cell (UTD) from same donor were

included as controls. Culture supernatants of CAR-T cells was evaluated from final day of CAR-T production. (Figure 19B) MMP-2, MMP-9 functionality was evaluated by migration assay using 0 or 5 mg/ml Cultrex™ coated transwell. Half a million thawed CAR-T cells were seeded into precoated transwells. After 24hr, the total CAR-T cells migrated into bottom chamber was quantify using Absolute counting beads by flow cytometer. (Figure 19C) HPSE and PH-20 functionality was evaluated by migration assay using 0 or 5 mg/ml Cultrex™ coated (Figure 19C) or hyaluronan coated (Figure 19D) transwell, respectively. Half a million thawed CAR-T cells were seeded into precoated transwells. After 24hr, the total CAR-T cells migrated into bottom chamber was quantify using Absolute counting beads for Cultrex™ coated by flow cytometer.

FIGURES 20A-20C depict the in vivo activity of FolR1 CAR-T co-expressing HPSE or PH-20 in an OVCAR3 xenograft model. NSG mice were implanted with $1x10^7$ OVCAR3 cells stably transduced with luciferase, via intraperitoneal injection. Tumor burden was determined using bioluminescent imaging by IVIS-S5 instrument. Mice with comparable mean tumor burden were randomly distributed into each group and injected with $5x10^6$/mouse CAR+ T cells or UTD on day 8. Tumor kinetics were measured at day 11, 18, 25, 32, and 39. (Figure 20A) Representative mouse bioluminescent images were shown at indicated time points. (Figure 20B) Time course of tumor growth based on mouse whole body bioluminescence (radiance, photons/sec/cm2/sr) were quantified and plotted as shown. TA-tumor alone, UTD – un-transduced T cell control. N= 4, mean ± SD. (Figure 20C) Body weight changes of mice during OVCAR3 xenograft study. OVCAR3 bearing mice were treated with CAR-T cells weights were recorded three times/week. Body weight change was calculated as the percentage of change from study initiation. Mean ± SEM. TA-tumor alone, UTD – non-transduced T cell control. N=4 mice/group.

FIGURES 21A and 2B depict characterization of boosted Farle CAR-T with ECM enzymes HPSE or PH-20 *in vivo*. (Figure 21A) CAR-T infiltration, CAR expression (percent and gMFI), and CD4:CD8 ratios were measured in the bone marrow (top) and spleen (middle) at study end of life. All samples were normalized by volume and Absolute counting beads. Spleen weights were measured to have no significant difference between treatment groups. (Figure 21B) Memory phenotype of CAR-T cells in the bone marrow (top) and spleen (bottom). Naïve, central memory, effector memory and effector cells were measured for CAR-T+ cells (left), CAR+ CD4+ (middle) and CAR+CD8+ (right). Mean ± SD. Statistical difference was calculated using One-Way ANOVA in Prism software. For B, the statistical difference of the effector population was measured between different treatment groups. TA-tumor alone, UTD – non-transduced T cell control. N=4 mice/group.

FIGURES 22A-22C depict ROR1 and CD276 CAR structure and surface expression on transduced primary T cells. (Figure 22A) ROR1 or CD276 CAR comprised a ROR1 or CD276 scFv binding domain, IgG4 or CD8 hinge domain, CD8 transmembrane domain, 41BB co-stimulatory domain, a CD3ζ activation

domain. (Figure 22B) Representative flow plots of CAR expression on transduced T cells. CAR and CAR/CCR T cells were stained with ROR1-Fc followed by anti Fc AF647 for ROR1 CAR detection, and with CD276-His for CD276 CCR detection. (Figure 22C) Average CAR expression in T cells from three healthy donors. Error bars represented mean ± SEM.

FIGURES 23A-23C depict the cytotoxicity of ROR1 or CD276 CAR constructs in vitro. Luciferase-based cytotoxicity assays were performed using ROR1+ CD276+ tumor line (Figure 23A) OVCAR3; (Figure 23B) AsPC-1 ; (Figure 23C) NCI-H226. All target lines were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at the 10 series effector to target (E:T) ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Nonlinear EC50 shift, where x is log concentration was used for curve fit. Data represent one independent experiment out of three experiments in T cells from different donors. Error bars represent mean ± SEM.

FIGURES 24A-24C depict the structure and primary human T cell surface expression of ROR1 CAR boosted with CD276 CCR. (Figure 24A) CD276 CCR boosted ROR1 CAR comprises a ROR1 CAR in frame to a CD276 CCR, linked by P2A ribosomal skip element. (Figure 24B) Transduced primary T cells were gated based on forward and side scatter, doublet exclusion, and viability dye negativity. Surface CAR expression of the ROR1-targeting or the CD276-targeting domains of each binder was detected by co-staining ROR1-Fc and CD276-His, followed by anti-Fc and anti-His FL conjugate. Representative flow plots of ROR1 CAR and CD276 CCR co-expression are shown. (Figure 24C) CAR and CCR co-expression on T cell surface was quantified. Mean of results from transduction of T cells from three healthy donors are shown, error bars indicate ± SEM.

FIGURES 25A-25D depict the cytotoxicity of ROR1 CAR alone, without the CD276 CCR constructs in vitro. Luciferase-based cytotoxicity assays were performed using (Figure 25A) ROR1+ CD276+ tumor line OVCAR3; (Figure 25B) ROR1- CD276- tumor line RS4;11; and single target positive cell line (Figure 25C) ROR1+ CD276- RS4;11-ROR1; (Figure 25D) ROR1- CD276+ RS4;11-CD276. All target lines were stably transduced with firefly luciferase. CAR T cells and tumor cells were co-cultured overnight at a series of 10 effector to target ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of CAR expression. Nonlinear EC50 shift, where x is log of concentration, was used for curve fit. Data represent one independent experiment from three experiments performed on T cells from 3 different donors. Error bar = Mean ± SEM

FIGURES 26A and 26B depict the relative potency of ROR1 CAR/CD276 CCR constructs in vitro. CAR T cells and ROR1+ tumor cells were co-cultured overnight at 10 different effector to target ratios. Percentage specific target lysis was assessed by luminometry and normalized to percentage of ROR1 CAR expression. Relative potency comparing to ROR1 CAR LTG2529 was calculated using nonlinear EC50

shift, function in GraphPad Prism, where x is log concentration. Relative potency of each constructs targeting ROR1+ in tumor lines : (Figure 26A) OVCAR-3, (Figure 26B) RS4;11-ROR1 was plotted as bar figures. Data represent Mean ± SEM of independent experiments using T cells from 3 different donors.

FIGURES 27A-27K depict that the novel anti-ROR1 LTG2529 (with scFV9 binder) demonstrated higher expression & cytokine secretion vs LTG2527 (with the control R12 binder) whereas exhibiting comparable cytotoxic potency in vitro and efficacy in vivo against hematologic tumors. (Figure 27A) Schematic diagram of CAR constructs. (Figure 27B) Left: flow plot examples of percentage of CAR^+T-cells; Center: percentage of CAR^+T-cells (n = 3 donors); right: CAR density (n = 3 donors); all were at day 8 of transduction. (Figure 27C) Quantification of ROR1 molecules per cell in different hematologic cell lines, the experiment was performed in duplicates employing anti-ROR1 Ab from BD Biociences; a separate experiment was also performed in duplicates using anti-ROR-1 Abs from Miltenyi Biotec and R&D systems with similar results. (Figure 27D) In vitro cytotoxic activity of CAR-Ts when co-cultured for 18 hrs with MCL cell line Jeko-1; left: a representative Killing curve; right: relative potency of LTG2529 vs LTG2527 (n=3 donors).(Figure 27E) Quantification of cytokines secreted in 18-hr co-culture of CAR Ts with Jeko-1 cell line by ELISA, a representative data from 3 donors was shown. (Figures 27F-27K): NSG mice were implanted with Jeko-1 cells (i.v., 0.5e6 cells/mouse; 6 mice/group) at day # -6, followed by staging at day #-1, CAR T cells were administered (i.v., 3e6 CAR^+T cells/mouse) at day # 0 (Figure 27F); tumor progression was quantified by Bioluminescence Imaging (Figure 27G, Figure 27H), body weight was monitored (Figure 27I), blood was sampled at the indicated time points and the tumor cells (Figure 27J) or T-cells (Figure 27K) were quantified by Flow Cytometry. Notes: *: $p<0.05$; **: $p<0.01$; n/s: not significant.

FIGURES 28A-28I depict that LTG2529, not LTG2527, was effective in suppressing solid tumor progression in *in vivo* ovarian cancer OVCAR-3 xenograft model despite exhibiting comparable *in vitro* cytotoxic activity (with higher cytokine production). (A) Quantification of ROR1 expression on surface of various solid tumor cancer cell lines; the experiment was performed in duplicates employing anti-ROR1 Ab from BD Biociences; a separate experiment was also performed in duplicates using anti-ROR-1 Abs from Miltenyi Biotec and R&D systems with similar results. (B) A representative Killing curve of CAR-Ts against various solid cancer cell lines in an 18-hr co-culture with relative cytotoxic potency of LTG2529 vs LTG2527 (n=3 donors) on the right. (C) Quantification of cytokines secreted in 18-hr co-culture of CAR Ts with OVCAR-3 cell line by ELISA, a representative data was shown, 3 independent experiments were performed employing 3 donors with similar results. (D-I): Efficacy of CAR-Ts in *in vivo* ovarian cancer OVCAR-3 xenograft model: NSG mice (5 mice/group) were implanted (i.p.) with OVCAR-3 cell line (10e6 cells/mouse) at day -7, followed by staging at day -1; CAR-Ts (5e6 CAR^+T-cells/mouse) were administered (i.v.) at day 0 (D); tumor progression was quantified by Bioluminescence imaging (E, F); body weight was monitored (G); blood was sampled at the indicated time points to quantify CAR+T cells in both CD8 and CD4 subpopulations (H) as well as memory T cells (I).

FIGURES 29A-29K depict that Dominant negative TGFbRII (DN) obstructed TGFb1 signaling in T cells transduced with LTG2529 and reduced the inhibitory effect of TGFb1 on CAR-Ts' cytotoxic activity against pancreatic cancer cell line AsPC-1 *in vitro*. (Figure 29A) schematic diagram of constructs of LTG2529 alone and

LTG2529 armored with DN (namely D0228). (Figure 29B) At day 8 of transduction, CAR expression (left: flow plots, center: graph from the flow plots) and memory phenotype (right) of both $CD8^+$ and $CD4^+$T-cells transduced with LTG2529 or D0228 were analyzed by Flow cytometry; 3 independent experiments were performed, employing 3 donors, with similar results. (Figure 29C) Expression of TGFbRII in T-cells transduced with LTG2529 or D0228 was assessed by Flow cytometry; 3 independent experiments were performed, employing 3 donors, with similar results. (Figure 29D) CAR-Ts were IL-2 starved for 22 hrs to synchronize the cells followed by treatment with TGFb1 (10 ng/mL) for 0.5 or 2 hrs; cells were then stained with pSmad2/3 and subject to Flow analysis; upper panel: flow plots: lower panel: graph from the plots in the upper panel; the data is the representative of 3 independent experiments performed on 3 donors. (Figure 29E) Expression of ROR1 on AsPC-1 cell line was assessed by flow cytometry. (Figure 29F) AsPC-1 was co-cultured with CAR-Ts without or with TGFb1 (1 or 10 ng/mL); tumor cell lysis was measured by xCELLigence; left: % cytolysis; center: Time at which 50% tumor cells were killed (KT_{50}); right: cytotoxic relative potency of CAR-Ts treated with TGFb1 vs non-treatment; 2 independent experiments employing 2 donors were performed in triplicates with similar results. (Figure 29G) Cytokine production from the experiments in (Figure 29E) was quantified by ELISA; 2 independent experiments employing 2 donors were performed in triplicates with similar results. (Figure 29H, Figure 29I): Production of TGFb1 either in active or latent form by various solid tumor cell lines (Figure 29H) or by AsPC-1 ectopically overexpressing TGF1 (Figure 29I) was assessed by ELISA; data are representative of 2 independent experiments with similar results. (Figure 29J) AsPC-1 overexpressing TGFb1 (AsPC-1/TGFb) or AspC-1 ctrl was co-cultured with CAR-Ts, % cytolysis of tumor cells was shown. (Figure 29K) Cytokine production from the experiments in (Figure 29E) was quantified by ELISA; 2 independent experiments employing 2 donors were performed in triplicates with similar results. Notes: *: $p<0.05$; **: $P<0.01$; ***: $P<0.001$.

FIGURES 30A-30J depict that TGFbRIIDN showed higher frequency of CAR+T cells in Pancreatic cancer xenograft model employing AsPC-1 which produced low level of TGFb1. (Figures 30A-30D): Efficacy of CAR-Ts in *in vivo* pancreatic cancer AsPC-1 xenograft model: NSG mice (5 mice/group) were implanted subcutaneously with AsPC-1 cells (1e6 cells/mouse) at day -17, followed by staging and CAR-T infusion (i.v., 5e6 CAR^+T-cells/mouse) at day 0 (Figure 30A); tumor volume was measured (Figure 30B)(left: tumor volume from mice across all groups; right: tumor volume from mice treated with armored and non-armored CARs started at day 10 post T cell dosing); body weight was monitored (Figure 30C); blood from mice were sampled and quantified for CD8 subpopulation of CAR+T cells (Figure 30D). (Figures 30E-J): At day 73 post T cell dosing, mice (4 from the non-armored CAR-treated group, and 3 from the armored CAR-treated group; notes: 1 mouse from the armored group were euthanized due to excessive weight loss at day 60 post T cell infusion) were re-challenged with AsPC-1 cells (1e6 cells/mouse, on the left flank; as the first challenge was on the right flank)(Figure 30E); tumor volume on both flank (Figure 30F), and survival rate (Figure 30G) were monitored; blood from mice were sampled at the indicated time points to quantify T cell memory phenotype (Figure 30H), percentage of CAR+cells (Figure 30I); T-cells isolated from spleen and bone marrow at the terminated time point were also analyzed for CAR+ T-cell components by flow cytometry (Figure 30J).

FIGURES 31A-31G depict the attenuation of the inhibitory effect of TGFb by TGFbRIIDN-armored ROR1 CAR T cells in Pancreatic cancer xenograft model employing AsPC-1 overexpressing TGFb. NSG mice (5

mice/group) were implanted subcutaneously with AsPC-1/TGFb cells (1e6 cells/mouse) at day -15, followed by staging and CAR-T infusion (i.v., 5e6 CAR^+T-cells/mouse) at day 0 (Figure 31A); tumor volume was monitored (Figure 31B); blood from mice was sampled at day 5 and day 15 post T cell infusion and was quantified for cytokines (Figure 31C), including TGFb1 (left), IFNg (center), and GM-CSF (right); T-cells isolated from blood at the indicated time points were quantified for total cell number (Figure 31D), CAR+components in both CD8 and CD4 subpopulations (Figure 31E). At the terminated time point (day 49 post T cell infusion), T cells from blood, spleen, and bone marrow were harvested and quantified for CAR+components (Figure 31F) and memory phenotype (Figure 31G) in both CD4 and CD8 subpopulations.

DETAILED DESCRIPTION

Definitions

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an antigen" includes single or plural antigens and can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." Thus, "comprising an antigen" means "including an antigen" without excluding other elements. The phrase "and/or" means "and" or "or." It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of +/- 20%, +/- 10%, or more preferably +/- 5%, or +/- 1%, or still more preferably +/- 0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

Unless otherwise noted, the technical terms herein are used according to conventional usage. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 1999; Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994; and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995; and other similar references.

Novel anti-effector moiety antibodies or antigen binding domains thereof and chimeric antigen receptors (CARs) that contain such effector moiety antigen binding domains are provided herein, as well as host cells (*e.g.*, T cells) expressing the receptors, and nucleic acid molecules encoding the receptors.

CAR may consist either of a single molecule expressed on the effector cell surface, or a CAR comprised of an effector cell-expressed signaling module and a soluble targeting module, such as when the soluble targeting module binds to the cell-expressed signaling module, a complete functional CAR is formed. The CARs exhibit a high surface expression on transduced T cells, with a high degree of cytolysis and transduced T cell expansion and persistence *in vivo*. Methods of using the disclosed CARs, host cells, and nucleic acid molecules are also provided, for example, to treat a cancer in a subject.

In its broadest aspect, novel chimeric antigen receptors (CARs) are provided herein comprising a boosted CAR comprising a CAR construct with a main effector moiety molecule followed by one or more 2A sequences, in frame to one or more additional "booster" elements for improved function, including enhanced tumor penetration, to improve the therapeutic effect of CAR-T cells in solid tumors, hematologic tumors, autoimmune disease, hereditary disease, or other relevant indications.

In yet another broad aspect, novel chimeric antigen receptors (CARs) are provided herein comprising a boosted CAR wherein the functional co-expressed boosted CAR elements are expressed from a single multi-cistronic vector at high transduction efficiency, thereby simplifying the CAR manufacturing and release and reducing cost for market implementation. In one aspect, the boosted CAR compositions comprise one or more of the following characteristics: i) a high surface expression on transduced T cells; ii) multi-targeting to overcome antigen escape; iii) one or more armor elements so as to overcome immunosuppression in TME; iv) one or more cytokine stimulated elements to promote autonomous T cell stimulation with cytokines, resulting in heightened anti-tumor cytotoxicity, expansion, memory formation, cytokine secretion, persistence; v) one or more digestive enzymes to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration; vi) one or more pro-inflammatory immune activators; and vii) one or more on-switches or off-switches, to control the expression of the CAR; or any combination thereof, wherein the boosted CARs achieve a high degree of cytolysis and transduced T cell in vivo expansion and persistence to promote in vivo expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In yet another broad aspect, the novel chimeric antigen receptors (CARs) provided herein may comprise single, tandem, or multi-targeting CAR constructs (including those in a DuoCAR format), or any combination thereof.

In certain aspects, the novel boosted CARs are under the control of one or more constitutive promoters, tissue specific promoters, or inducible promoters, or any combination thereof.

In certain aspects, the novel boosted CARs may comprise one or more pro-inflammatory immune activators.

In certain aspects, the one or more pro-inflammatory immune activators may comprise boosters that turn "cold' immune environment to "hot", such as neutrophil-activating protein (NAP) from bacteria such as *Helicobacter pylori*, bacterial lipopolysaccharide (LPS) components, or Polyinosine-polycytidylic acid (poly(I:C), or soluble inflammatory factors such as FLT3 Ligand, or oncolytic viruses, or TNF family cytokines, including CD40 ligand (CD40L), tumor necrosis factor (TNF) and receptor activator of nuclear factor-κB (RANKL)/TRANCE which can trigger or enhance exogenous bystander responses against solid cancers.

In one aspect, such elements when used as a booster to CAR T cell therapy may reduce or ablate tumor growth, and/or increase survival rates, regardless of target antigen, tumor type and host haplotype. Such boosters may act by supporting dendritic cell maturation and bystander responses, leading to epitope spreading and infiltration of CD8+ cells targeting tumor associated antigens other than CAR T-targeted antigen.

In certain aspects, the one or more switches comprises a tag, a kill switch, an on switch, an off switch, and/or an adapter switch, or any combination thereof.

In certain aspects, the novel boosted CARs switch may be a tag (CD19, CD34, CD22, EGFR), or a kill switch (iCAS9), or an [ON] switch, or an [OFF] switch, or adapter switch, or any combination thereof.

In certain embodiments, the single, tandem, multi-targeting, DuoCARs (either with or without one or more booster elements) novel chimeric antigen receptors (CARs) are provided are used to transduce effector cells for the treatment of solid and hematologic tumors and other diseases through targeted antigens (for example, and not by way of limitation, CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B l, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17,

LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof.

In one embodiment, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti-GD2, and/or anti CD276, and/or anti GPC2, and/or anti FGFR2, and/or anti PSMA, and/or anti MUC1, and/or anti MUC16, and/or anti IL13R alpha antibody, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an $F(ab')_2$ fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv).

In one embodiment, an isolated polynucleotide encoding an anti-GD2, anti-GD3, anti-GM2, anti-Ley, anti-polysialic acid, anti-fucosyl GMl, anti-GM3, anti-Tn, anti-STn, anti-sLe(animal), anti-GloboH, anti-CD5, anti-CD7, anti-CD19, anti-CD20, anti-CD22, anti-CD25, anti-CD37, anti-CD30, anti-CD33, anti-CD38, anti-CD123, anti-CD45, anti-CAMPATH-1, anti-BCMA, anti-CS-1, anti-PD-L1, anti-CD276/B7-H3, anti-B7-H4, anti-B7-DC, anti-HLA-DR carcinoembryonic antigen (CEA), anti-TAG-72, anti-EpCAM, anti-folate-binding protein, anti-folate receptor alpha (FOLR1), anti-folate receptor beta (FOLR2), anti-A33, anti-G250, anti-prostate-specific membrane antigen (PSMA), anti-ferritin, anti-CA-125, anti-CA19-9, anti-CD44v6, anti-epidermal growth factor, anti-pl85, anti-IL-2 receptor, anti-interleukin 1 receptor accessory protein (IL1RAP), anti-EGFRvIII (de2-7), anti-fibroblast activation protein, anti-tenascin, anti-a metalloproteinase, anti-endosialin, anti-vascular endothelial growth factor, anti-$\alpha v\beta 3$, anti-WT1, anti-LMP2, anti-HPV E6, anti-HPV E7, anti-Her-2/neu, anti-p53 nonmutant, anti-NY-ESO-1, anti-MelanA/MART 1, anti-Ras mutant, anti-gp100, anti-FGFR1, anti-FGFR2, anti-FGFR3, anti-FGFR4, anti-GPC1, anti-GPC2, anti-GPC3, anti-p53 mutant, anti-PR1, anti-bcr-abl, anti-tyrosinase, anti-survivin, anti-PSA, anti-hTERT, anti-Sarcoma translocation breakpoint fusion protein, anti-EphA2, anti-PAP, anti-ML-IAP, anti-AFP, anti-ERG, anti-NA17, anti-PAX3, anti-ALK, anti-androgen receptor,

anti-cyclin B 1, anti-MYCN, anti-RhoC, anti-TRP-2, anti-mesothelin, anti-PSCA, anti-MAGE Al, anti-MAGE A3, anti-CYP1B 1, anti-PLAV1, anti-BORIS, anti-ETV6-AML, anti-NY-BR-1, anti-RGS5, anti-SART3, anti-Carbonic anhydrase IX, anti-PAX5, anti-OY-TES 1, anti-Sperm protein 17, anti-LCK, anti-HMWMAA, anti-AKAP-4, anti-SSX2, anti-XAGE 1, anti-B7H3, anti-Legumain, anti-Tie 3, anti-PAGE4, anti-VEGFR2, anti-MAD-CT-1, anti-PDGFR-B, anti-MAD-CT-2, anti-TRAIL 1, anti-MUC1, anti-MUC16/CA125, anti-MAGE A4, anti-MAGE C2, anti-GAGE, anti-EGFR, anti-EGFR1, anti-EGFR2/Her2, anti-CMET, anti-HER3, anti-CA6, anti-NAPI2B, anti-TROP2, anti-TEM1, anti-TEM7, anti-TEM8, anti-FAP, anti-LAP, anti-CLDN6, anti-CLDN8, anti-CLDN16, anti-CLDN18.2, anti-RON, anti-LY6E, anti-DLL3, anti-PTK7, anti-UPK1B, anti-STRA6, anti-TMPRSS3, anti-TMRRSS4, anti-TMEM238, anti-Clorfl86, anti-LIV1, anti-ROR1, anti-ROR2, anti-Fos-related antigen 1, anti-VEGFR1, anti-endoglin, anti-CD90, anti-CD326, anti-CD70, anti-SSEA4, anti-CD318, anti-CLA, anti-TSPAN8, anti-GPRC5D, anti-EpCAM, anti-Thy1, anti-IL13Ra2, anti-BDCA1, anti-BDCA2, anti-BDCA3, anti-GD2, anti-PSMA, anti-FAP, anti-CLL1, anti-SLAMF7/CS1, anti-CD147, anti-DPPA5, anti-GRP78, anti-CD66c, VISTA, LRRC5, LRRC15 antibody, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an $F(ab')_2$ fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded extracellular ROR1 and/or MSLN antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to ROR1 and/or MSLN.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded extracellular ROR1 and/or MSLN antigen binding domain comprises at least one heavy chain variable region of an antibody that binds to ROR1 and/or MSLN.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded extracellular ROR1 and/or MSLN antigen binding domain comprises an ScFv.

In yet another broad aspect, one or more of the above-identified novel boosted chimeric antigen receptors (CARs) provided *supra* with respect to SEQ ID NOs: 151 to 256 may comprise either a single, tandem, or multi-targeting CAR construct (including those in a DuoCAR *format*), or any combination thereof.

For each of the various aspects and embodiments of the single, tandem, multi-targeting, DuoCARs, (either with or without one or more booster elements) CAR constructs specifically contemplated herein, the nucleotide sequences encoding the functional CAR (either with or without one or more booster elements) comprise the nucleotide sequence of SEQ ID NO: 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 245, 247, 249, 251, 253, or 255, or any combination thereof.

For each of the various aspects and embodiments of the single, tandem, multi-targeting, DuoCARs, (either with or without one or more booster elements) CAR constructs specifically contemplated herein, each vector encodes a functional CAR (either with or without one or more booster elements) comprising the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof.

For each of the various aspects and embodiments, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti GD2 antibody or a fragment thereof is provided comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, 149.

For each of the various aspects and embodiments, novel single, tandem, DuoCARs, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-ROR1 and/or anti-MSLN antigen binding domain comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain.

For each of the various aspects and embodiments, novel single, tandem, DuoCARs, or multiple-targeting CARs (either with or without one or more booster elements) are provided herein comprising a single, tandem, DuoCAR, or multiple-targeting CAR molecule (either with or without one or more booster elements) comprising at least one extracellular antigen binding domain comprising an anti-ROR1 and/or anti-MSLN antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain.

In one embodiment, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti GD2 antibody or a fragment thereof is provided, wherein the antibody or

a fragment thereof comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149.

In one embodiment, an isolated polynucleotide encoding a fully human anti-ROR1 and/or anti-MSLN anti-ROR1 and/or anti-MSLN and/or anti FolR1, and/or anti HER2/ERBB2, and/or anti GPC3, and/or anti-FGFR4, and /or anti GD2 antibody or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150.

In one aspect, an isolated nucleic acid molecule encoding a single, tandem, DuoCAR, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more boosting elements) is provided comprising, from N-terminus to C-terminus, at least one ROR1 and/or MSLN antigen binding domain encoded by a nucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, at least one transmembrane domain, and at least one intracellular signaling domain.

In one aspect, an isolated nucleic acid molecule encoding a single, tandem, DuoCAR, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more boosting elements) is provided comprising, from N-terminus to C-terminus, at least one ROR1 and/or MSLN antigen binding domain encoded by a nucleotide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment, the targeting domain of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is expressed separately in the form of monoclonal antibody, ScFv Fab, Fab'2 and is containing an antigen-targeting domain comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, coupled to an additional binding tag or epitope, whereas the effector-cell expressed component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) contains a binding domain specifically directed to bind the tag or epitope expressed on the soluble single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) module, such as specific binding on the soluble component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) to the cell bound component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) forms the full functional single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) structure.

In another embodiment, the targeting domain of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is expressed separately in the form of a

monoclonal antibody, ScFv Fab, Fab'2 and contains an antigen-targeting domain comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, and an additional ScFv, whereas the effector-cell expressed component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) contains a tag or epitope specifically reactive with the additional ScFv expressed on the soluble single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) module, such as specific binding on the soluble component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) to the cell bound component of the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) forms the full functional single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) structure.

In yet another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) extracellular ROR1 and/or MSLN antigen binding domain further comprises at least one lipocalin-based antigen binding antigen (anticalins) that binds to ROR1 and/or MSLN.

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular ROR1 and/or MSLN antigen binding domain is connected to the transmembrane domain by a linker domain.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded ROR1 and/or MSLN extracellular antigen binding domain is preceded by a sequence encoding a leader or signal peptide.

In yet another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided comprising at least one ROR1 and/or MSLN antigen binding domain encoded by a nucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 143, 145, 147, and 149, and wherein the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) additionally encodes an extracellular antigen binding domain targets an antigen that includes, but is not limited to, CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta

(FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B 1, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof.

In certain embodiments, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the additionally encoded extracellular antigen binding domain comprises an anti-CD19 ScFv antigen binding domain, an anti-CD20 ScFv antigen binding domain, an anti-CD22 ScFv antigen binding domain, anti-BCMA ScFv antigen binding domain, anti-CD5 ScFv antigen binding domain, an anti-CD33 ScFv antigen binding domain, an anti-CD38 ScFv antigen binding domain, an anti-CD123 (IL3RA) ScFv antigen binding domain, an anti-CD138 ScFv antigen binding domain, an anti-GPC2 ScFv antigen binding domain, an anti-GPC3 ScFv antigen binding domain, an anti-FGFR4 ScFv antigen binding domain, an anti-c-Met ScFv antigen binding domain, an anti-PSMA ScFv antigen binding domain, an anti-glycolipid F77 ScFv antigen binding domain, an anti-EGFRvIII ScFv antigen binding domain, an anti-GD-2 ScFv antigen binding domain, an anti-NY-ESO-1 TCR ScFv antigen binding domain, an anti-MAGE A3 TCR ScFv antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one aspect, the single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more boosting elements) provided herein further comprise a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular ROR1 and/or MSLN antigen binding domain, the intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded linker domain is derived from the extracellular domain of IgG1, IgG2, IgG3 or IgG4, CD8, TNFRSF19, or CD28, and is linked to a transmembrane domain.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) further comprises a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19, Fc epsilon R, or a combination thereof.

In yet another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded intracellular signaling domain further comprises a CD3 zeta intracellular domain.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded intracellular signaling domain is arranged on a C-terminal side relative to the CD3 zeta intracellular domain.

In another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, or a combination thereof.

In another embodiment, an immunotherapy composition is provided wherein the at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), PD-1, GITR, CTLA-4, or any combination thereof.

In one embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided that further

contains a leader sequence or signal peptide wherein the leader or signal peptide nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 13, SEQ ID NO: 39, SEQ ID NO: 41, or SEQ ID NO: 43.

In yet another embodiment, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the encoded leader sequence comprises the amino acid sequence of SEQ ID NO: 14, SEQ ID NO: 40, SEQ ID NO: 42, or SEQ ID NO: 44.

In one aspect, a single, tandem, DuoCAR, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more boosting elements) is provided herein comprising, from N-terminus to C-terminus, at least one ROR1 and/or MSLN antigen binding domain, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular ROR1 and/or MSLN antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to the antigen, or at least one heavy chain variable region of an antibody that binds to the antigen, or a combination thereof.

In another embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the at least one transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In some embodiments, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) additionally encodes an extracellular antigen binding domain comprising anti-CD19, anti-CD20, anti-CD22, anti-CD33, anti-CD38, anti-CD123 (IL3RA), anti-CD138, anti-GPC2, anti-GPC3, anti-FGFR4, anti-c-Met, anti-PSMA, anti-Glycolipid F77, anti-EGFRvIII, anti-GD-2, anti-NY-ESO-1 TCR, anti-MAGE A3 TCR, anti-GD2, anti-GD3, anti-GM2, anti-Ley, anti-polysialic acid, anti-fucosyl GMl, anti-GM3, anti-Tn, anti-STn, anti-sLe(animal), anti-GloboH, anti-CD5, anti-CD7, anti-CD19, anti-CD20, anti-CD22, anti-CD25, anti-CD37, anti-CD30, anti-CD33, anti-CD38, anti-CD123, anti-CD45, anti-CAMPATH-1, anti-BCMA, anti-CS-1, anti-PD-L1, anti-CD276/B7-H3, anti-B7-H4, anti-B7-DC, anti-HLA-DR carcinoembryonic antigen (CEA), anti-TAG-72, anti-EpCAM, anti-folate-binding protein, anti-folate receptor alpha (FOLR1), anti-folate receptor beta (FOLR2), anti-A33, anti-G250, anti-prostate-specific membrane antigen (PSMA), anti-ferritin, anti-CA-125, anti-CA19-9, anti-CD44v6, anti-epidermal growth factor, anti-pl85, anti-IL-2 receptor, anti-interleukin 1 receptor accessory protein (IL1RAP), anti-EGFRvIII (de2-7), anti-fibroblast activation

protein, anti-tenascin, anti-a metalloproteinase, anti-endosialin, anti-vascular endothelial growth factor, anti-αvβ3, anti-WT1, anti-LMP2, anti-HPV E6, anti-HPV E7, anti-Her-2/neu, anti-p53 nonmutant, anti-NY-ESO-1, anti-MelanA/MART 1 , anti-Ras mutant, anti-gp100, anti-GPRC5D, anti-FGFR1, anti-FGFR2, anti-FGFR3, anti-FGFR4, anti-GPC1, anti-GPC2, anti-GPC3, anti-p53 mutant, anti-PR1, anti-bcr-abl, anti-tyrosinase, anti-survivin, anti-PSA, anti-hTERT, anti-a Sarcoma translocation breakpoint fusion protein, anti-EphA2, anti-PAP, anti-ML-IAP, anti-AFP, anti-ERG, anti-NA17, anti-PAX3, anti-ALK, anti-androgen receptor, anti-cyclin B 1, anti-MYCN, anti-RhoC, anti-TRP-2, anti-mesothelin, anti-PSCA, anti-MAGE Al, anti-MAGE A3, anti-CYP1B 1, anti-PLAV1, anti-BORIS, anti-ETV6-AML, anti-NY-BR-1, anti-RGS5, anti-SART3, anti-Carbonic anhydrase IX, anti-PAX5, anti-OY-TES 1, anti-Sperm protein 17, anti-LCK, anti-HMWMAA, anti-AKAP-4, anti-SSX2, anti-XAGE 1, anti-B7H3, anti-Legumain, anti-Tie 3, anti-PAGE4, anti-VEGFR2, anti-MAD-CT-1, anti-PDGFR-B, anti-MAD-CT-2, anti-TRAIL 1, anti-MUC1, anti-MUC16/CA125, anti-MAGE A4, anti-MAGE anti-C2, anti-GAGE, anti-EGFR, anti-EGFR1, anti-EGFR2/Her2, anti-CMET, anti-HER3, anti-CA6, anti-NAPI2B, anti-TROP2, anti-TEM1, anti-TEM7, anti-TEM8, anti-FAP, anti-LAP, anti-CLDN6, anti-CLDN8, anti-CLDN16, anti-CLDN18.2, anti-RON, anti-LY6E, anti-DLL3, anti-PTK7, anti-UPK1B, anti-STRA6, anti-TMPRSS3, anti-TMRRSS4, anti-TMEM238, anti-Clorfl86, anti-LIV1, anti-ROR1, anti-ROR2, anti-Fos-related antigen 1, anti-VEGFR1, anti-endoglin, anti-CD90, anti-CD326, anti-CD70, anti-SSEA4, anti-CD318, anti-CLA, anti-TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, anti-CD147, anti-DPPA5, anti-GRP78, anti-CD66c, anti-VISTA, anti-LRRC5, anti-LRRC15 antibody, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an $F(ab')_2$ fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), , or a fragment of any of the preceding, or a molecule that is at least 80% 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain additionally comprises an anti-CD19 ScFv antigen binding domain, an anti-CD20 ScFv antigen binding domain, an anti-CD22 ScFv antigen binding domain, an anti-CD33 ScFv antigen binding domain, an anti-CD38 ScFv antigen binding domain, an anti-CD123 (IL3RA) ScFv antigen binding domain, an anti-CD138 ScFv antigen binding domain, an anti-GPC2 ScFv antigen binding domain, an anti-GPC3 ScFv antigen binding domain, an anti-FGFR4 ScFv antigen binding domain, an anti-c-Met ScFv antigen binding domain, an anti-PMSA ScFv antigen binding domain, an anti-glycolipid F77 ScFv antigen binding domain, an anti-EGFRvIII ScFv antigen binding domain, an anti-GD-2 ScFv antigen binding domain, an anti-NY-ESo-1

TCR ScFv antigen binding domain, an anti-MAGE A3 TCR ScFv antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain alternatively comprises an anti-CD19 ScFv antigen binding domain, an anti-CD20 ScFv antigen binding domain, an anti-CD22 ScFv antigen binding domain, an anti-CD33 ScFv antigen binding domain, an anti-CD38 ScFv antigen binding domain, an anti-CD123 (IL3RA) ScFv antigen binding domain, an anti-CD138 ScFv antigen binding domain, an anti-GPC2 ScFv antigen binding domain, an anti-GPC3 ScFv antigen binding domain, an anti-FGFR4 ScFv antigen binding domain, an anti-c-Met ScFv antigen binding domain, an anti-PMSA ScFv antigen binding domain, an anti-glycolipid F77 ScFv antigen binding domain, an anti-EGFRvIII ScFv antigen binding domain, an anti-GD-2 ScFv antigen binding domain, an anti-NY-ESo-1 TCR ScFv antigen binding domain, an anti-MAGE A3 TCR ScFv antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In another embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain additionally comprises an immunoglobulin variable heavy chain only (VH) anti-CD19 antigen binding domain, an anti-CD20 VH antigen binding domain, an anti-CD22 VH antigen binding domain, an anti-CD33 VH antigen binding domain, an anti-CD38 VH antigen binding domain, an anti-CD123 (IL3RA) VH antigen binding domain, an anti-CD138 VH antigen binding domain, an anti-GPC2 VH antigen binding domain, an anti-GPC3 VH antigen binding domain, an anti-FGFR4 VH antigen binding domain, an anti-c-Met VH antigen binding domain, an anti-PMSA VH antigen binding domain, an anti-glycolipid F77 VH antigen binding domain, an anti-EGFRvIII VH antigen binding domain, an anti-GD-2 VH antigen binding domain, an anti-NY-ESO-1 TCR VH antigen binding domain, an anti-MAGE A3 TCR VH antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In another embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain alternatively comprises an immunoglobulin variable heavy chain only (VH) anti-CD19 antigen binding domain, an anti-CD20 VH antigen binding domain, an anti-CD22 VH antigen binding domain, an anti-CD33 VH antigen binding domain, an anti-CD38 VH antigen binding domain, an anti-CD123 (IL3RA) VH antigen binding domain, an anti-CD138 VH antigen binding domain, an anti-GPC2 VH antigen binding domain, an anti-GPC3 VH antigen binding domain, an anti-FGFR4 VH antigen binding domain, an anti-c-Met VH antigen binding domain, an anti-PMSA VH antigen binding domain, an anti-glycolipid F77 VH antigen binding domain, an anti-EGFRvIII VH antigen binding domain, an anti-GD-2 VH antigen binding

domain, an anti-NY-ESO-1 TCR VH antigen binding domain, an anti-MAGE A3 TCR VH antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In another embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain additionally comprises a protein or a peptide (P) sequence capable of specifically binding target antigen, which may be derived from a natural or a synthetic sequence comprising anti-CD19 P antigen binding domain, an anti-CD20 P antigen binding domain, an anti-CD22 P antigen binding domain, an anti-CD33 P antigen binding domain, an anti-CD38 P antigen binding domain, an anti-CD123 (IL3RA) P antigen binding domain, an anti-CD138 P antigen binding domain, an anti-BCMA (CD269) P antigen binding domain, an anti-GPC2 P antigen binding domain, an anti-GPC3 P antigen binding domain, an anti-FGFR4 P antigen binding domain, an anti-c-Met P antigen binding domain, an anti-PMSA P antigen binding domain, an anti-glycolipid F77 P antigen binding domain, an anti-EGFRvIII P antigen binding domain, an anti-GD-2 P antigen binding domain, an anti-NY-ESO-1 TCR P antigen binding domain, an anti-MAGE A3 TCR P antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof. In another embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

In another embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain alternatively comprises a protein or a peptide (P) sequence capable of specifically binding target antigen, which may be derived from a natural or a synthetic sequence comprising anti-CD19 P antigen binding domain, an anti-CD20 P antigen binding domain, an anti-CD22 P antigen binding domain, an anti-CD33 P antigen binding domain, an anti-CD38 P antigen binding domain, an anti-CD123 (IL3RA) P antigen binding domain, an anti-CD138 P antigen binding domain, an anti-BCMA (CD269) P antigen binding domain, an anti-GPC2 P antigen binding domain, an anti-GPC3 P antigen binding domain, an anti-FGFR4 P antigen binding domain, an anti-c-Met P antigen binding domain, an anti-PMSA P antigen binding domain, an anti-glycolipid F77 P antigen binding domain, an anti-EGFRvIII P antigen binding domain, an anti-GD-2 P antigen binding domain, an anti-NY-ESO-1 TCR P antigen binding domain, an anti-MAGE A3 TCR P antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof. In another embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

In yet another embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more boosting elements) is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain comprising a functional signaling domain of a protein selected from the group consisting of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or a combination thereof.

In one embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 151. In one embodiment, the nucleic acid sequence encodes a boosted CAR comprising the amino acid sequence of SEQ ID NO: 152.

In another embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 153. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 154.

In another embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 155. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 156.

In another embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 157. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 158.

In another embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 159. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 160.

In another embodiment, the nucleic acid sequence encoding a boosted CAR comprises the nucleic acid sequence of SEQ ID NO: 161. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 162.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 163. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 164.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 165. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 166.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 167. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 168.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 179. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 180.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 181. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 182.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 183. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 184.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 185. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 186.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 187. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 188.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 189. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 190.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 191. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 192.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 193. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 194.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 195. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 196.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 197. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 198.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 226. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 225.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 228. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 227.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 230. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 229.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 232. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 231.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 234. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 233.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 236. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 235.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 238. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 237.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 240. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 239.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 242. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 241.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 244. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 243.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 245. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 246.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 247. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 248.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 249. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 250.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 251. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 252.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 253. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 254.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 255. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 256.

In one aspect, the single, tandem, DuoCARs, or multi-targeting CARs (either with or without one or more boosting elements) disclosed herein are modified to express or contain a detectable marker for use in diagnosis, monitoring, and/or predicting the treatment outcome such as progression free survival of cancer patients or for monitoring the progress of such treatment.

In one embodiment, the nucleic acid molecule encoding the disclosed single, tandem, DuoCARs, or multi-targeting CARs (either with or without one or more boosting elements) can be contained in a vector, such as a viral vector. The vector is a DNA vector, an RNA vector, a plasmid vector, a cosmid vector, a herpes virus vector, a measles virus vector, a lentivirus vector, adenoviral vector, or a retrovirus vector, or a combination thereof.

In certain embodiments, the vector further comprises a promoter wherein the promoter is an inducible promoter, a tissue specific promoter, a constitutive promoter, a suicide promoter or any combination thereof.

In yet another embodiment, the vector expressing the single, tandem, DuoCAR, or multi-targeting CAR (either with or without one or more boosting elements) can be further modified to include one or more operative elements to control the expression of single, tandem, DuoCAR, or multi-targeting CAR T cells (either with or without one or more boosting elements), or to eliminate single, tandem, DuoCAR, or multi-targeting CAR T cells (either with or without one or more boosting elements) cells by virtue of a suicide switch. The suicide switch can include, for example, an apoptosis inducing signaling cascade or a drug that induces cell death. In a preferred embodiment, the vector expressing the single, tandem, DuoCAR, or multi-targeting CAR (either with or without one or more boosting elements) can be further modified to express an enzyme such thymidine kinase (TK) or cytosine deaminase (CD).

In another aspect, host cells including the nucleic acid molecule encoding the single, tandem, DuoCAR, or multi-targeting CAR (either with or without one or more boosting elements) are also provided. In some embodiments, the host cell is a T cell, such as a primary T cell obtained from a subject. In one embodiment, the host cell is a CD8+ T cell.

In yet another aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multi-targeting, chimeric antigen receptor (CAR) construct, wherein the CAR comprises at least one extracellular antigen binding domain comprising a MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain, wherein the T cells are T cells of a human having a cancer. The cancer includes, inter alia, a hematological cancer such as leukemia *(e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma) or multiple myeloma, or a combination thereof.

In yet another aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multi-targeting, boosted chimeric antigen receptor (CAR) construct, wherein the boosted CAR comprises at least one extracellular antigen binding domain comprising a MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain followed by one or more 2A sequences, in frame to one or more armor molecules, one or more extracellular matrix enzymes, one or more chemokine receptors, one or more stroma-targeting molecules, one or more tumor microenvironment (TME)-digestive elements, one or more switch tag elements, one or more chemo attractive-receptors, one or more chemotactic molecule secretors, one or more switches, and/or one or more cytokines, or any combination thereof; and a pharmaceutically acceptable excipient, wherein the boosted CARs are used to genetically modify one or more human T cell lymphocyte populations, wherein the T cells are T cells of a human having a cancer. The cancer includes, inter alia, a hematological cancer such as leukemia *(e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma) or multiple myeloma, or a combination thereof.

In one embodiment, a pharmaceutical composition is provided wherein the at least one transmembrane domain of the CAR (either with or without one or more booster elements) contains a

transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In another embodiment, a pharmaceutical composition is provided wherein the human cancer includes an adult carcinoma comprising oral and pharynx cancer (tongue, mouth, pharynx, head and neck), digestive system cancers (esophagus, stomach, small intestine, colon, rectum, anus, liver, intrahepatic bile duct, gallbladder, pancreas), respiratory system cancers (larynx, lung and bronchus), bones and joint cancers, soft tissue cancers, skin cancers (melanoma, basal and squamous cell carcinoma), pediatric tumors (neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma), tumors of the central nervous system (brain, astrocytoma, glioblastoma, glioma), and cancers of the breast, the genital system (uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, penis, endometrium), the urinary system (urinary bladder, kidney and renal pelvis, ureter), the eye and orbit, the endocrine system (thyroid), and the brain and other nervous system, or any combination thereof.

In yet another embodiment, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells of a human having a cancer wherein the cancer is a refractory cancer non-responsive to one or more chemotherapeutic agents. The cancer includes hematopoietic cancer, myelodysplastic syndrome pancreatic cancer, head and neck cancer, cutaneous tumors, minimal residual disease (MRD) in multiple myeloma (MM), smoldering multiple myeloma (SMM), monoclonal gammopathy of undetermined significance (MGUS), adult and pediatric hematologic malignancies, including acute lymphoblastic leukemia (ALL), CLL (Chronic lymphocytic leukemia), non-Hodgkin's lymphoma (NHL), including follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Hodgkin's lymphoma (HL). chronic myelogenous leukemia (CML), lung cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, melanoma or other hematological cancer and solid tumors, or any combination thereof.

In another aspect, methods of making single, tandem, DuoCAR, or multiple-targeting CAR construct-containing T cells (hereinafter "CAR-T cells") (either with or without one or more booster elements) are provided. The methods include transducing a T cell with a vector or nucleic acid molecule encoding a disclosed CAR that specifically binds MSLN and/or ROR1, thereby making the CAR-T cell.

In yet another aspect, a method of generating a population of RNA-engineered cells is provided that comprises introducing an *in vitro* transcribed RNA or synthetic RNA of a nucleic acid molecule encoding a disclosed single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more booster elements) into a cell of a subject, thereby generating a single, tandem, DuoCAR, or multiple-targeting CAR cell (either with or without one or more booster elements).

In yet another aspect, a method for diagnosing a disease, disorder or condition associated with the expression of MLSN and/or ROR1 on a cell, is provided comprising a) contacting the cell with a human anti- MLSN and/or ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; and b) detecting the presence of MSLN and/or ROR1 wherein the presence of MSLN and/or ROR1 diagnoses for the disease, disorder or condition associated with the expression of MSLN and/or ROR1.

In one embodiment, the disease, disorder or condition associated with the expression of MSLN and/or ROR1 is cancer including hematopoietic cancer, myelodysplastic syndrome pancreatic cancer, head and neck cancer, cutaneous tumors, minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adult B cell malignancies including, CLL (chronic lymphocytic leukemia), CML (chronic myelogenous leukemia), non-Hodgkin's lymphoma (NHL), pediatric B cell malignancies (including B lineage ALL (acute lymphocytic leukemia)), multiple myeloma lung cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, melanoma or other hematological cancer and solid tumors, or any combination thereof.

In another embodiment, a method of diagnosing, prognosing, or determining risk of a MSLN and/or ROR1-related disease in a mammal, is provided comprising detecting the expression of MSLN and/or ROR1 in a sample derived from the mammal comprising: a) contacting the sample with a human anti-MSLN and/or anti-ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; and b) detecting the presence of MSLN and/or ROR1 wherein the presence of MSLN and/or ROR1 diagnoses for a MSLN and/or ROR1-related disease in the mammal.

In another embodiment, a method of inhibiting MSLN and/or ROR1-dependent T cell inhibition, is provided comprising contacting a cell with a human anti-MSLN and/or ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150. In one embodiment, the cell is selected from the group consisting of a MSLN and/or ROR1-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another embodiment, a method of blocking T-cell inhibition mediated by a MSLN and/or ROR1-expressing cell and altering the tumor microenvironment to inhibit tumor growth in a mammal, is provided comprising administering to the mammal an effective amount of a composition comprising an isolated anti-MSLN and/or anti-ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150. In one embodiment, the cell is selected from the group consisting of a MSLN and/or ROR1-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another embodiment, a method of inhibiting, suppressing or preventing immunosuppression of an anti-tumor or anti-cancer immune response in a mammal, is provided comprising administering to the mammal an effective amount of a composition comprising an isolated anti-MSLN and/or anti-ROR1 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150. In one embodiment, the antibody or fragment thereof inhibits the interaction between a first cell with a T cell, wherein the first cell is selected from the group consisting of a MSLN and/or ROR1-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another aspect, a method is provided for inducing an anti-tumor immunity in a mammal comprising administering to the mammal a therapeutically effective amount of a T cell transduced with vector or nucleic acid molecule encoding a disclosed single, tandem, or multiple-targeting CAR (either with or without one or more booster elements).

In another embodiment, a method of treating or preventing cancer in a mammal is provided comprising administering to the mammal one or more of the disclosed single, tandem, or multiple-targeting CARs (either with or without one or more booster elements), in an amount effective to treat or prevent cancer in the mammal. The method includes administering to the subject a therapeutically effective amount of host cells expressing a disclosed single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) that specifically binds MSLN and/or ROR1 and/or one or more of the aforementioned antigens, under conditions sufficient to form an immune complex of the antigen binding domain on the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) and the extracellular domain of MSLN and/or ROR1 and/or one or more of the aforementioned antigens in the subject.

In yet another embodiment, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) includes at least one extracellular MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of the subject having cancer.

In yet another embodiment, a method is provided for treating cancer in a subject in need thereof comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective

amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) comprises at least one MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having cancer. In some embodiments of the aforementioned methods, the at least one transmembrane domain comprises a transmembrane the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In yet another embodiment, a method is provided for treating a mammal having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) includes at least one extracellular MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of the subject having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition.

In yet another embodiment, a method is provided for treating autoimmune, alloimmune, or autoaggressive diseases in a subject in need thereof comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) comprises at least one MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition. In some embodiments of the aforementioned methods, the at least one transmembrane domain comprises a transmembrane the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5,

CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In yet another embodiment, a method is provided for treating a mammal having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) includes at least one extracellular MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of the subject having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition.

In yet another embodiment, a method is provided for treating autoimmune, alloimmune, or autoaggressive diseases in a subject in need thereof comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a single, tandem, or multiple-targeting chimeric antigen receptor (CAR) (either with or without one or more booster elements), wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) comprises at least one MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having an autoimmune, alloimmune, or autoaggressive disease, disorder or condition. In some embodiments of the aforementioned methods, the at least one transmembrane domain comprises a transmembrane the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

For each of the various aspects and embodiments of the methods for treating autoimmune, alloimmune, or autoaggressive diseases in a subject in need thereof, the single, tandem, multi-targeting, DuoCAR, (either with or without one or more booster elements) CAR constructs specifically contemplated *supra* and/or *infra*, the nucleotide sequences encoding any of the aforementioned functional CARs (either

with or without one or more booster elements) referenced *supra* and/or *infra*, may be used to treat an autoimmune, alloimmune, or autoaggressive disease, disorder or condition.

For each of the various aspects and embodiments of the methods for treating autoimmune, alloimmune, or autoaggressive diseases in a subject in need thereof, the single, tandem, multi-targeting, DuoCAR, (either with or without one or more booster elements) CAR constructs specifically contemplated *supra* and/or *infra*, the amino acid sequences encoding any of the aforementioned functional CARs (either with or without one or more booster elements) referenced *supra* and/or *infra*, may be used to treat an autoimmune, alloimmune, or autoaggressive disease, disorder or condition.

For the various aspects and embodiments of the methods for treating autoimmune, alloimmune, or autoaggressive diseases described herein, exemplary non-limiting examples of autoimmune diseases include chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, Goodpasture's, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purpura, neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cyroglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, Am. J. Transplant., 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy. [0034] Antigen binding domains that are specific for a ligand on B cells, plasma cells or plasmablasts are useful in the methods of treating autoimmune diseases, alloimmune diseases, or autoaggressive diseases as described herein. For example, a CAR construct can contain an antigen binding domain that is specific for, without limitation, CD19, CD20, CD22, CD138, BCMA, CD319, CD10, CD24, CD27, CD38, or CD45R. In addition, a CAR construct can contain an antigen binding domain that is specific for, without limitation, an autoimmune specific antigen. Autoimmune specific antigens include, for example, the antigen that results in systemic lupus erythematosus (SLE), Graves' disease, celiac disease, diabetes mellitus type 1, rheumatoid arthritis (RA), sarcoidosis, Sjogren's syndrome, polymyositis (PM), and dermatomyositis (DM), mucocutaneous pemphigus vulgaris, myasthenia gravis. See, for example, Ellebrecht et al., 2016, Science, 353:179-84.

In yet another embodiment, a method is provided for generating a persisting population of genetically engineered T cells in a human diagnosed with cancer. In one embodiment, the method comprises

administering to a human a T cell genetically engineered to express a single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) wherein the single, tandem, or multiple-targeting CAR (either with or without one or more booster elements) comprises at least one MSLN and/or ROR1 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 144, 146, 148, and 150, or any combination thereof; at least one transmembrane domain; and at least one intracellular signaling domain wherein the persisting population of genetically engineered T cells, or the population of progeny of the T cells, persists in the human for at least one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

In one embodiment, the progeny T cells in the human comprise a memory T cell. In another embodiment, the T cell is an autologous T cell.

In all of the aspects and embodiments of methods described herein, any of the aforementioned cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen that may be treated or prevented or ameliorated using one or more of the single, tandem, or multiple-targeting CARs (either with or without one or more booster elements) disclosed herein,

In yet another aspect, a kit is provided for making a chimeric antigen receptor T-cell as described *supra* or for preventing, treating, or ameliorating any of the cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen in a subject as described *supra*, comprising a container comprising any one of the nucleic acid molecules, vectors, host cells, or compositions disclosed *supra* or any combination thereof, and instructions for using the kit.

In one aspect of the present invention, an immunotherapy composition is provided comprising a single, tandem, DuoCAR, or multiple-targeting CAR (either with or without one or more booster elements) which immunotherapy composition may be used to transduce autologous lymphocytes to generate active patient-specific anti-tumor lymphocyte cell populations that can be infused directly back into the patient to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In yet another aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one extracellular antigen binding domain comprising an anti-ROR1 and/or anti-MSLN antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 144, 146, 148, and 150; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain; and at least one boosting element comprising one or more armor molecules (TGFβRIIdn, truncated PD-1 (decoy), PD-

1 dominant-negative (PD-1dn), synthetic PD-1 activating receptor, truncated CTLA-4, truncated Tim-3, truncated TIGIT, TIGIT neutralizing antibody, TIGIT intrabody, TIGIT shRNA), one or more extracellular matrix enzymes (ECMs), one or more chemokine receptors (CXCL8, CCL2) one or more stroma-targeting molecules (FAP, LRRC15, CD276/B7-H3, TEM7, TEM8, TEM1), one or more TME-digestive element (heparanase (HPSE), MMP (MMP-1, MMP-2, MMP-9, MMP-12, MMP-13) and hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, PH-20, and hyaluronoglucosaminidase pseudogene 1 (HYALP1), tissue inhibitors of metalloproteinases (TIMPs) (TIMP-1, TIMP-2, TIMP-3, TIMP-4), hyaluronidase), one or more switches (tag, kill switch, on switch, off switch, adapter switch, truncated EGF receptor, truncated CD19, truncated CD20, CD20 mimotope, truncated CD34, truncated LNGF receptor), chimeric costimulatory receptor (CCR), and/or one or more cytokines (membrane-bound or soluble IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21, TNFα, IFNγ (each of the aforementioned may be with or without FC (antibody fragment crystallizable) element)), or a combination of membrane bound receptor and tethered cytokine ligand (mbIL15, mbIL7, mbIL-21), innate system-inducting ligands (TLR ligands, LPS, bacterial products), or any combination thereof, wherein the T cells are T cells of a human having a cancer. The cancer includes, inter alia, a hematological cancer such as leukemia *(e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma) or multiple myeloma, or a combination thereof.

In one embodiment, a pharmaceutical composition is provided wherein the at least one transmembrane domain of the single, tandem, DuoCAR, or multi-targeting CAR (either with or without one or more boosting elements) contains a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

It will be understood that the single, tandem, DuoCAR, or multiple-targeting CARs (either with or without one or more booster elements), host cells, nucleic acids, and methods are useful beyond the specific aspects and embodiments that are described in detail herein. The foregoing features and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

In one aspect of the above-identified invention, the DuoCARs (either with or without one or more boosters) disclosed herein comprise at least two vectors, each vector encoding a functional CAR (either with or without one or more boosters), whereby the combination of vectors results in the expression of two or more non-identical binding domains, herein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs, at least one

extracellular domain capable of binding to an antigen, at least one transmembrane domain, and at least one intracellular domain.

In certain aspects of the boosted CARs of the present invention, an immunotherapy composition is provided comprising one or more isolated nucleic acid molecules encoding at least two vectors, each vector encoding a functional DuoCAR (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs, which immunotherapy composition may be used to transduce autologous lymphocytes to generate active patient-specific anti-tumor lymphocyte cell populations that can be infused directly back into the patient to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner. Novel adoptive immunotherapy compositions comprising such two or more vector-transduced lymphocytes are provided herein as well as are methods of use of same in a patient-specific combination immunotherapy that can be used to treat cancers and other diseases and conditions.

Thus, in one aspect, lentiviral vectors expressing Duo chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) are provided herein, as well as nucleic acid molecules encoding the lentiviral vectors expressing DuoCARs (either with or without one or more booster elements). Methods of using the disclosed lentiviral vectors expressing DuoCARs (either with or without one or more booster elements), host cells, and nucleic acid molecules are also provided, for example, to treat a cancer in a subject.

In one aspect, an immunotherapy composition is provided comprising one or more isolated nucleic acid molecules encoding at least two vectors (DuoCARs) (either with or without one or more booster elements), each vector encoding a functional CAR (either with or without one or more booster elements), wherein at least one binding domain(s) in one of the vectors are non-identical, and whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs.

In one embodiment, an immunotherapy composition is provided comprising one or more isolated nucleic acid molecules encoding at least three vectors (TrioCARs) (either with or without one or more booster elements), each vector encoding a functional CAR (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs.

In one embodiment, an immunotherapy composition is provided comprising one or more isolated nucleic acid molecules encoding at least four vectors (QuatroCARs) (either with or without one or more booster elements), each vector encoding a functional CAR (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs.

In yet another embodiment, an immunotherapy composition is provided comprising one or more isolated nucleic acid molecules encoding at least two, three, four, five, six, seven, eight, nine, or ten vectors (*e.g.*, an "nCAR") (either with or without one or more booster elements), each vector encoding a functional CAR (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs, wherein each unique member of the nCAR set when assembled into a CAR product constitutes a unique CAR composition referred to herein as "nCAR" (either with or without one or more booster elements) (*e.g.*, DuoCAR, TrioCAR, QuatroCAR, PentaCAR, HexaCAR, HeptaCAR, OctaCAR, NonaCAR, and DecaCAR, etc.).

In another aspect, the DuoCARs (either with or without one or more boosters) are used to enhance the immune response to tumor mediated by the therapeutic T cell population. The immune response is enhanced in multiple ways.

First, DuoCARs enable multi-targeting of tumor cells, reducing the risk of tumor antigen escape and enabling efficient elimination of antigen-heterogeneous tumors. This feature is especially important in targeting solid tumors, which often display antigen heterogeneity and antigen loss. Table 1, *infra*, exemplifies CARs with dual targeting capacity of solid tumor antigens mesothelin and ROR1.

In addition, the DuoCAR format, allows for introduction of multiple co-stimulatory domains in CAR architecture, so that stronger overall stimulation can be provided for CAR T cell effector functions, differentiation and memory formation, and persistence. For example, same CAR T cell can benefit form CD28-stimulation required for potent CAR T cell activation, expansion and cytokine production, and 4-1BB stimulation to extend CAR T cell survival and persistence in the patient. Each DuoCAR chain may be a 2nd or a 3rd generation DuoCAR, and may incorporate one or two co-stimulatory domains. By providing a third T cell activating sequence on a separate vector CAR construct (either with or without one or more boosters), the inventors are able to regain the advantage of expressing two or more targeting domains, improved co-stimulation, and a booster payload, without incurring the disadvantage of the decreased expression of the CAR at the T cell surface at the CAR% level.

In a second aspect, the DuoCARs (either with or without one or more boosters) of the present invention may target cell-types other than the tumor that mediate immunosuppressive effects. For example, if immunosuppressive cells expressing one of the targeted antigens are present in the tumor lesion and also inhibit an anti-tumor immunity, as by the production of IL-10 or other mediators, the second benefit to the use of the DuoCAR-expressing (either with or without one or more boosters) tumor-specific T cell population is that the immunosuppressive cell population is also removed.

For example, if immunosuppressive B cells are present within a solid tumor lesion, these could be eliminated by the use of a B cell-specific DuoCAR (such as CD19-specific DuoCARs, either with or without one or more boosters). If immunosuppressive fibroblast-like cells are present, these could be removed by stromal-specific DuoCARs (either with or without one or more boosters) (for example by targeting fibroblast activating protein-alpha (FAP)). If malformed vasculature is responsible for the lack of an efficacious immune response a DuoCAR specific for these types of vascular or lymph vessel specific targets (such as anti-VEGFR) may also improve therapeutic outcome.

In a third aspect, the DuoCARs (either with or without one or more boosters) of the present invention target an immunosuppressive population that is distal to the tumor, *i.e.* present in another compartment in the body. For example, using a DuoCAR (either with or without one or more boosters) to target myeloid derived suppressor cells (MDSCs), that may be present either in the tumor lesion itself or in the regional lymph nodes or bone marrow. It is well established that tumor-draining lymph nodes can either be loci of immune activation or immune suppression. This depends upon the overall inflammatory tone of the lymph node as well as distal dendritic cell differentiation prior to migration to the lymph node. If a tumor-draining lymph node is populated with myeloid-derived suppressor cells (MDSC) or miss-differentiated antigen presenting cells such as dendritic cells, a DuoCAR (either with or without one or more boosters) that targets these cell types, although distal to the tumor itself, may also improve therapeutic outcome. Beyond the cancer-specific DuoCAR (either with or without one or more boosters) immunotherapeutic applications, a second application of DuoCARs (either with or without one or more boosters) would be the prevention or treatment of autoimmune, alloimmune, autoaggressive and/or inflammatory diseases. The difference from oncologic-based applications is that T-regulatory cells (Treg), or induced T-regulatory cells (iTreg), or other cells cultured in conditions that promote Th-2-like immune responses, would be the cellular substrate. For oncologic application Th-1 like cells are the cellular substrate. In therapeutic applications as diverse as graft-versus-host disease (GvHD) following hematopoietic stem cell transplantation (HSCT), allergic airway, gut, or other mucosal inflammation, or skin allergies, the presence of CAR-modified lymphocytes that produce immune-inhibitory cytokines, such as transforming growth factor-beta (TFG-beta), would serve to exert a broad tolerogenic signal that ameliorates the autoimmune-, alloimmune-, autoaggressive- or inflammation-driven disease. This approach

includes neurological inflammatory conditions of the periphery or central nervous system (CNS) such as Alzheimer's disease, multiple sclerosis, traumatic brain injury, Parkinson's disease, and CTE (chronic traumatic encephalopathy due to repeated concussions or micro-concussions), or connective tissue diseases such as Rheumatoid arthritis, Scleroderma, Granulomatosis with polyangiitis, Churg-Strauss syndrome, Lupus, Microscopic polyangiitis, Polymyositis/dermatomyositis, Marfan syndrome, or Epidermolysis bullosa acquisita. This approach also includes progressive scarring diseases such as COPD (chronic obstructive pulmonary disease) or fibrotic diseases of the lung, heart, kidney, or liver. For example, systemic scleroderma is a progressive, rare disease that causes fibrosis not only in the skin but also in tissues throughout the body, including the heart, lungs and kidneys.

In the treatment of inflammatory diseases, lymphocytes specific for tissue antigens, distress markers on the surface of inflamed cells, or misfolded proteins (such as tau protein or beta-amyloid) would be created by generating DuoCAR (either with or without one or more boosters) expression vectors that are specific for these targets. Single antibody-based therapy for Alzheimer's is already in clinical development (*i.e.*, Solanezumab by Eli Lilly and Company and Aducanumab by Biogen, Inc.). In Alzheimer's disease, antibody to monomeric or aggregated beta-amyloid could be used in a CAR (either with or without one or more boosters) format in lieu of binders to cell surface proteins. Binders to tau protein or tau-peptides bound by MHC molecules could also be used as binding motifs for CARs (either with or without one or more boosters). Receptors that mediate the homing of lymphocytes to specific peripheral tissues can also be included in a CAR (either with or without one or more boosters) format, in order to render regional specificity to the CAR-expressing (either with or without one or more boosters) Treg population. Adhesion receptor domains known to drive lymphocyte infiltration into specific tissues and cytokine sequences or cytokine or chemokine receptors or binders could be used as part of the CAR (either with or without one or more boosters) domain. Adhesion molecules such as CD44 and integrin alpha-4 are known to target lymphocytes to the CNS, thus including domains from adhesion molecules know to mediate CNS migratory behavior of lymphocyte populations could also be used to target CAR-expressing (either with or without one or more boosters) lymphocytes to regions of disease. The same would hold true for the gut (*i.e.* binders to MAdCAm-1, expression of a CCR9, or anti-CCL25, etc.), lung (*i.e.* P-selectin or mesothelin), skin (*i.e.* binders to E-selectin), or other mucosal surfaces.

To use this approach, a patient with an inflammatory condition or whose disease could be treated by mitigation of inflammatory pathology, such as Alzheimer's disease, would be admitted to the clinic and peripheral blood harvested. Treg cells could be selected directly by immunomagnetic beads (Regulatory T cell isolation kit, Miltenyi Biotec), or induced by culture in the appropriate cytokine milieu. These Treg or iTreg would then be transduced with a DuoCAR (either with or without one or more boosters) vector and if required expanded *in vitro* (Treg expansion kit, Miltenyi Biotec). The DuoCAR (either with or without

one or more boosters) binding domains would be derived from antibodies or receptors that mediate tissue specific homing and disease-associated binders, such as anti-beta amyloid. The engineered immune effector cells thus generated would be targeted to the appropriate site, and produce cytokines consistent with their Th2 or Treg differentiation pattern. It is also known that CAR-T cells can be engineered to secrete specific genetic payloads upon activation of the CAR receptor (either with or without one or more boosters). In addition to the DuoCAR (either with or without one or more boosters) payload expressed from the vector, additional therapeutic proteins or peptides could be expressed or secreted by the engineered T cell populations such as: i) one or more A-beta DPs (amyloid beta degrading proteases), ii) one or more matrix proteases (such as MMP-9 and MMP9), iii) one or more peptides or soluble antibody-like binders that interfere with plaque formation, iv) one or more cytokines (such as TGF-beta, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21), v) one or more armor elements so as to overcome immunosuppression in TME, vi) one or more digestive enzymes to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration, vii) one or more pro-inflammatory immune activators, and viii) one or more on-switches or off-switches, or any combination thereof, to control the expression of the CAR, wherein the boosted CARs achieve a high surface expression on transduced T cells, a multi-targeting activity to overcome antigen escape, a high degree of cytolysis and transduced T cell *in vivo* expansion and persistence to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer or autoimmune, alloimmune, or autoaggressive disease, or prevention or amelioration of relapse of cancer or autoimmune, alloimmune, or autoaggressive disease, or a combination thereof, in a patient-specific manner. In reference to the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs, the functional boosting element portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs.

MiRNAs could also be expressed within cells to modulate T cell function. Examples of miRNAs are miR-92a, miR-21, miR-155, miR-146a, miR-3162, miR-1202, miR-1246 and miR-4281, miR-142, miR-17-92. Also shRNAs to miRNAs could be developed. Examples are shRNAs targeted to miR-28, miR-150 and miR-107, which normally bind to PD1 and increase its expression.

Beyond oncology-based and inflammatory and autoimmune, alloimmune, or autoaggressive disease-based applications, a third application of the DuoCAR (either with or without one or more boosters) technology is the generation of therapeutic lymphocyte populations specific for viral, bacterial, or fungal antigens. Thus, as for oncology applications described for B cell malignancies, the targeting of infectious disease would allow the DuoCAR (either with or without one or more boosters) products to mediate immunoprotective or immunotherapeutic activity against the infective agents or the diseased tissues where

they reside based upon recognition of microbial antigens. Unlike T cell receptor (TCR)-based approaches, where the T cell receptor itself mediates the recognition of pathogen encoded peptides, the DuoCAR (either with or without one or more boosters) approach would utilize binding proteins expressed in a CAR (either with or without one or more boosters) vector format that would give antibody-like recognition (that is, not requiring antigen processing) to the transduced T cell population. The activation of the therapeutic T cell population would result in an immune activating locus able to eliminate the infected cells, and if the microbial antigen is not cell associated, to release soluble mediators like interferon-gamma that would enable an effective immune response to be mounted against the infectious agent.

For example, HIV is known to be highly variable, and yet specific clades or families can be categorized and antibody to clade-specific viral envelope protein (env, gp120) created. Using the DuoCAR (either with or without one or more boosters) approach, three or more clade-specific antibody-like binders are included in the CAR (either with or without one or more boosters) constructs resulting in broad anti-HIV immune activity. In addition to viral proteins, bacterial protein can be targeted. A current medical challenge is the treatment of antibiotic resistant bacterial strains that often arise in healthcare settings. These include VRE (vancomycin resistant enterococci), MRSA (methicillin-resistant staphylococcus aureus), KPC (Klebsiella pneumoniae carbapenemase producing gram-negative bacteria, also CRKP), and others. Klebsiella cell surface antigens include the O antigen (9 variants) and the K antigen (appx. 80 variants). The O antigen spectrum could readily be covered with a small DuoCAR (either with or without one or more boosters) library, as could a number of the K antigens. For use, CAR constructs (either with or without one or more boosters) would be created that feature antibodies that bind to different K or O serotypes, and these CAR vectors (either with or without one or more boosters) used to transduce a Th1-like effector cell population, isolated and activated as for oncology applications. In fungal diseases, the work of L. Cooper *et al.* (Kumasesan, P.R., 2014, PNAS USA, 111:10660) demonstrated that a fungal binding protein normally expressed on human cells, dectin-1, can be reconfigured as a CAR (either with or without one or more boosters), and used to control fungal growth *in vitro*. The human disease aspergillosis occurs in severely immunosuppressed individuals and is caused by the fungus A. fumigatus. Multiple groups have produced monoclonal antibodies specific for the antigenic components of the aspergillus cell surface, thus opening the door to adoptive immunotherapy with DuoCARs (either with or without one or more boosters) that target three or more aspergillus antigens on the fungal surface. Thus, in all of these infectious disease applications, the ability to create immunoglobulin-like binders to microbial antigens allows a plurality of antigens to be targeted by CAR-expressing (either with or without one or more boosters) effector lymphocyte populations.

What follows is a detailed description of the DuoCARs (either with or without one or more boosters) that may be used in the patient-specific autologous anti-tumor lymphocyte cell population(s) disclosed

herein, including a description of their extracellular domain, the transmembrane domain and the intracellular domain, along with additional description of the DuoCARs (either with or without one or more boosters), antibodies and antigen binding fragments thereof, conjugates, nucleotides, expression, vectors, and host cells, methods of treatment, compositions, and kits employing the disclosed DuoCARs (either with or without one or more boosters). While the compositions and methods of the present invention have been illustrated with reference to the generation and utilization of DuoCARs (either with or without one or more boosters), it is contemplated herein that the compositions and methods are specifically intended to include the generation and utilization of TrioCARs (either with or without one or more boosters) and QuatroCARs (either with or without one or more boosters).

In one embodiment, an immunotherapy composition is provided comprising: (a) at least two vectors, each comprising nucleic acid sequences that are functional in cells; (b) wherein each vector encodes a functional CAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements) comprises of at least one binding domain, a single transmembrane domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domains in one of the vectors are non-identical; and (e) wherein the at least one binding domain, a single transmembrane domain, at least one linker domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of vectors are used to genetically modify one or more lymphocyte populations.

In another embodiment, an immunotherapy composition is provided comprising: (a) at least two vectors, each comprising nucleic acid sequences that are functional in cells; (b) wherein each vector encodes a functional CAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements)comprises at least one binding domain, a single transmembrane domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domain(s) in each vector are non-identical; (e) wherein the at least one signaling motif combinations are non-identical between each of the vectors; and (f) wherein the at least one binding domain, a single transmembrane domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of two or more vectors are used to genetically modify one or more lymphocyte populations.

In another embodiment, an immunotherapy composition is provided wherein the linker or spacer domain of the CAR (either with or without one or more booster elements) is derived from the extracellular domain of IgG1, IgG2, IgG3 or IgG4, CD8, TNFRSF19, or CD28, and is linked to the transmembrane domain.

In another embodiment, an immunotherapy composition is provided wherein the CAR (either with or without one or more booster elements) further comprises a transmembrane domain that comprises a

transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19, Fc epsilon R, or any combination thereof.

In another embodiment, an immunotherapy composition is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, or any combination thereof.

In another embodiment, an immunotherapy composition is provided wherein the at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), PD-1, GITR, CTLA-4, or any combination thereof.

In another embodiment, an immunotherapy composition is provided wherein a single vector is used to encode all chimeric antigen receptors (*e.g.*, retroviral, adenoviral, SV40, herpes vector, POX vector, RNA, plasmid, cosmid, or any viral vector or non-viral vector), in combination with a CRISPR system for integration.

In another embodiment, an immunotherapy composition is provided wherein each vector is an RNA or DNA vector, alone or in combination with a transfection reagent or a method to deliver the RNA or DNA into the cell, a non-limiting example being electroporation.

In another embodiment, an immunotherapy composition is provided wherein at least one vector expresses a nucleic acid molecule that modulates the expression of a nucleic acid in the cell.

In another embodiment, an immunotherapy composition is provided wherein the nucleic acid molecule inhibits or deletes the expression of an endogenous gene.

In certain embodiments, an immunotherapy composition is provided wherein the active patient-specific autologous anti-tumor lymphocyte cell population is generated within one day, two days, three days, four days, five days, seven days, ten days, twelve days, fourteen days, twenty-one days, or one month of lymphocyte harvest or tumor biopsy and wherein the active patient-specific autologous anti-tumor lymphocyte cell population that can be infused back into a patient suffering from cancer and is capable of promoting *in vivo* expansion, persistence of patient-specific anti-tumor lymphocyte cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In one aspect, isolated nucleic acid molecules encoding the aforementioned chimeric antigen receptors (including the DuoCARs recited, *supra*) are provided herein.

In one aspect, the CARs (either with or without one or more booster elements) used in the patient-specific autologous lymphocyte population(s) of the immunotherapy composition of the present invention, the CARs (either with or without one or more booster elements) are modified to express or contain a

detectable marker for use in diagnosis, monitoring, and/or predicting the treatment outcome such as progression free survival of cancer patients or for monitoring the progress of such treatment. In one embodiment of the CARs (either with or without one or more booster elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), the nucleic acid molecules encoding the disclosed CARs (either with or without one or more booster elements) can be contained in a vector, such as a viral or non-viral vector. The vector is a DNA vector, an RNA vector, a plasmid vector, a cosmid vector, a herpes virus vector, a measles virus vector, a lentiviral vector, adenoviral vector, or a retrovirus vector, or a combination thereof.

In certain embodiments of the CARs (either with or without one or more booster elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), the two or more lentiviral vectors are pseudotyped with different viral glycoproteins (GPs) including for example, and not by way of limitation, amphotropic murine leukemia virus [MLV-A], a baboon endogenous virus (BaEV), GP164, gibbon ape leukemia virus [GALV], RD114, feline endogenous virus retroviral-derived GPs, and vesicular stomatitis virus [VSV], measles virus, fowl plague virus [FPV], Ebola virus [EboV], lymphocytic choriomeningitis virus [LCMV]) non retroviral-derived GPs, as well as chimeric variants thereof including, for example, and not by way of limitation, chimeric GPs encoding the extracellular and transmembrane domains of GALV or RD114 GPs fused to the cytoplasmic tail (designated TR) of MLV-A GP.

In certain embodiments of the CARs (either with or without one or more booster elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), the vector further comprises a promoter wherein the promoter is an inducible promoter, a tissue specific promoter, a constitutive promoter, a suicide promoter or any combination thereof.

In yet another embodiment of the CARs (either with or without one or more booster elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), the vector expressing the CAR (either with or without one or more booster elements) can be further modified to include one or more operative elements to control the expression of CAR T cells, or to eliminate CAR-T cells by virtue of a suicide switch. The suicide switch can include, for example, an apoptosis inducing signaling cascade or a drug that induces cell death. In a preferred embodiment, the vector expressing the CAR (either with or without one or more booster elements) can be further modified to express an enzyme such thymidine kinase (TK) or cytosine deaminase (CD).

In another aspect of the CARs (either with or without one or more booster elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), host cells including the nucleic acid molecule(s) encoding the CARs (either with or without one or more booster elements) are also provided. In some embodiments, the host cell is a T cell, such as a primary T cell obtained from a subject. In one embodiment, the host cell is a CD8+ T cell. In one embodiment the host cell is a CD4+ T cell. In one

embodiment the host cells are selected CD4+ and CD8+ lymphocytes purified directly from a patient product without regard to proportionality. In another embodiment the number of CD4+ and CD8+ T cells in the product are specific. In another embodiment specific subsets of T cells are utilized as identified by phenotypic markers including T naïve cells (Tn), T effector memory cells (Tem), T central memory cells (Tcm), T regulatory cells (Treg), induced T regulatory cells (iTreg), T suppressor cells (Ts), T stem cell memory cells (Tscm), Natural Killer (NK) cells, invariant Natural Killer T (iNKT) cells, and lymphokine activated killer (LAK) cells.

In one embodiment, as used herein, invariant Natural Killer T cells are a small population of αβ T lymphocytes highly conserved from mice to humans. iNKT cells have been suggested to play important roles in regulating many diseases, including cancer, infections, allergies, and autoimmunity. When stimulated, iNKT cells rapidly release a large amount of effector cytokines like IFN-γ and IL-4, both as a cell population and at the single-cell level. These cytokines then activate various immune effector cells, such as natural killer (NK) cells and dendritic cells (DCs) of the innate immune system, as well as CD4 helper and CD8 cytotoxic conventional αβ T cells of the adaptive immune system via activated DCs. Because of their unique activation mechanism, iNKT cells can attack multiple diseases independent of antigen- and MHC-restrictions, making them attractive universal therapeutic agents. Notably, because of the capacity of effector NK cells and conventional αβ T cells to specifically recognize diseased tissue cells, iNKT cell-induced immune reactions result in limited off-target side effects.

In one aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells comprising novel single, tandem, or multi-targeting CAR constructs, or any combination thereof, comprising a CAR molecule followed by one or more 2A sequences, in frame to one or more armor molecules, one or more extracellular matrix enzymes, one or more chemokine receptors, one or more stroma-targeting molecules, one or more tumor microenvironment (TME)-digestive elements, one or more switch tag elements, one or more chemo attractive-receptors, one or more chemotactic molecule secretors, one or more switches, and/or one or more cytokines, or any combination thereof; and a pharmaceutically acceptable excipient, wherein the boosted CARs are used to genetically modify one or more human T cell lymphocyte populations.

In yet another embodiment, a pharmaceutical composition is provided comprising an anti-tumor effective amount of an immunotherapy composition comprising a population of patient-specific autologous anti-tumor lymphocyte cell population(s) of a human having a cancer, wherein the cells of the population include cells comprising nucleic acid molecules encoding at least two vectors, each vector encoding a functional CAR (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector

encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs.

In yet another embodiment, a pharmaceutical composition is provided comprising an anti-tumor effective amount of an immunotherapy composition comprising a population of patient-specific autologous anti-tumor lymphocyte cell population(s) of a human having a cancer, wherein the cells of the population include cells comprising (a) nucleic acid molecules encoding two or more vectors; (b) wherein each vector encodes a functional CAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements) comprises of at least one binding domain, at least one transmembrane domain, at least one linker domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domains in one of the vectors are non-identical; and (e) wherein the at least one binding domain, a single transmembrane domain, at least one linker domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of vectors are used to genetically modify one or more lymphocyte populations.

In yet another embodiment, a pharmaceutical composition is provided comprising an anti-tumor effective amount of an immunotherapy composition comprising a population of patient-specific autologous anti-tumor lymphocyte cell population(s) of a human having a cancer, wherein the cells of the population include cells comprising (a) nucleic acid molecules encoding two or more vectors; (b) wherein each vector encodes a functional CAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements) comprises at least one binding domain, at least one transmembrane domain, at least one linker domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domain(s) in each vector are non-identical; (e) wherein the at least one signaling motif combinations are non-identical between each of the vectors; and (f) wherein the at least one binding domain, a single transmembrane domain, at least one linker domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of two or more vectors are used to genetically modify one or more lymphocyte populations.

In one embodiment, the cancer is a refractory cancer non-responsive to one or more chemotherapeutic agents. The cancer includes hematopoietic cancer, myelodysplastic syndrome, pancreatic cancer, head and neck cancer, cutaneous tumors, minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), lung cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, melanoma or other hematological cancer and solid tumors, or any combination thereof. In another embodiment, the cancer includes a hematological cancer such as leukemia (*e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma) or multiple myeloma, or any combination thereof.

In yet another embodiment, the cancer includes an adult carcinoma comprising coral and pharynx cancer (tongue, mouth, pharynx, head and neck), digestive system cancers (esophagus, stomach, small intestine, colon, rectum, anus, liver, intrahepatic bile duct, gallbladder, pancreas), respiratory system cancers (larynx, lung and bronchus), bones and joint cancers, soft tissue cancers, skin cancers (melanoma, basal and squamous cell carcinoma), pediatric tumors (neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma), tumors of the central nervous system (brain, astrocytoma, glioblastoma, glioma), and cancers of the breast, the genital system (uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, penis, endometrium), the urinary system (urinary bladder, kidney and renal pelvis, ureter), the eye and orbit, the endocrine system (thyroid), and the brain and other nervous system, or any combination thereof.

In another aspect, a pharmaceutical composition is provided comprising an autologous lymphocyte cell population transduced with two or more lentiviral vectors encoding single or multiple chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements), thereby generating a patient-specific autologous anti-tumor lymphocyte cell population capable of promoting *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In another aspect, a pharmaceutical composition is provided comprising an autologous T cell population transduced with one or more lentiviral vectors encoding single or multiple chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) to generate an patient-specific autologous anti-tumor lymphocyte cell population capable of promoting *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In another aspect, methods of making active patient-specific autologous anti-tumor Duo (either with or without one or more booster elements) CAR-containing lymphocyte cells are provided. The methods include transducing a lymphocyte cell with two or more vectors or nucleic acid molecule encoding two or more chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) that specifically bind an antigen, thereby making active patient-specific autologous anti-tumor DuoCAR-containing lymphocyte cells.

In yet another aspect, a method of generating a population of RNA-engineered lymphocyte cells is provided that comprises introducing an *in vitro* transcribed RNA or synthetic RNA of a nucleic acid molecule encoding a two or more chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) into a cell population of a subject, thereby generating an patient-specific autologous anti-tumor lymphocyte cell population capable of promoting *in vivo* expansion, persistence of

patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In another aspect, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of an autologous lymphocyte cell population transduced with one or more lentiviral vectors encoding single or multiple chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) thereby generating an patient-specific autologous anti-tumor lymphocyte cell population capable of promoting *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer, or prevention or amelioration of relapse of cancer, or a combination thereof, in a patient-specific manner.

In another aspect, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of an autologous lymphocyte cell population transduced with two or more lentiviral vectors encoding single or multiple chimeric antigen receptors (DuoCARs) (either with or without one or more booster elements) to generate an patient-specific autologous anti-tumor lymphocyte cell population which can be infused directly back into the patient to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, or remission of cancer, or prevention or amelioration of relapse of cancer, or any combination thereof, in a patient-specific manner.

In one embodiment, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising at least two vectors, each vector encoding a functional CAR (DuoCARs) (either with or without one or more booster elements), whereby the combination of vectors results in the expression of two or more non-identical binding domains, wherein each vector encoded binding domain(s) are covalently linked to a transmembrane domain and one or more non-identical intracellular signaling motifs, and a pharmaceutically acceptable excipient, wherein the combination of vectors are used to genetically modify one or more lymphocyte populations.

In another embodiment, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising (a) nucleic acid molecules encoding two or more vectors; (b) wherein each vector encodes a functional DuoCAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements) comprises

of at least one binding domain, at least one transmembrane domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domains in one of the vectors are non-identical; and (e) wherein the at least one binding domain, a single transmembrane domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of vectors are used to genetically modify one or more lymphocyte populations.

In yet another embodiment, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising (a) nucleic acid molecules encoding two or more vectors; (b) wherein each vector encodes a functional DuoCAR (either with or without one or more booster elements); (c) wherein each CAR (either with or without one or more booster elements) comprises at least one binding domain, at least one transmembrane domain, and at least one intracellular signaling motif; (d) wherein the at least one binding domain(s) in each vector are non-identical; (e) wherein the at least one signaling motif combinations are non-identical between each of the vectors; and (f) wherein the at least one binding domain, a single transmembrane domain, and at least one intracellular signaling motif are covalently linked in each said vector, wherein the combination of two or more vectors are used to genetically modify one or more lymphocyte populations.

In certain embodiments, the genetically modified lymphocytes are autologous T cell lymphocytes, and wherein the autologous or allogeneic T cell lymphocytes are infused directly back into the patient so as to prevent or ameliorate relapse of malignant disease.

In certain other embodiments, the genetically modified lymphocytes are autologous T cell lymphocytes, and wherein the autologous lymphocytes are infused directly back into the patient to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cell lymphocytes resulting in tumor stabilization, reduction, elimination, or remission of cancer, or prevention or amelioration of relapse of cancer, or any combination thereof, in a patient-specific manner.

In yet another embodiment, the T cell has been preselected by virtue of expressing specific activation or memory-associated surface markers.

In yet another embodiment, the T cell is derived from a hematopoietic stem cell donor, and wherein the procedure is carried out in the context of hematopoietic stem cell transplantation.

In certain embodiments, a method is provided wherein the lymphocyte cell has been preselected by virtue of expressing specific activation or memory-associated surface markers.

In certain embodiments, a method is provided herein wherein the lymphocyte cell is a T cell and is derived from a hematopoietic stem cell donor, and wherein the procedure is carried out in the context of hematopoietic stem cell transplantation.

In yet another aspect, a method is provided for generating a persisting population of genetically engineered patient-specific autologous anti-tumor lymphocyte cell population(s) in a human diagnosed with cancer. In one embodiment, the method comprises administering to a human patient in need thereof one or more patient-specific autologous anti-tumor lymphocyte cell population(s) described herein, wherein the persisting population of patient-specific autologous anti-tumor lymphocyte cell population(s), or the population of progeny of the lymphocyte cells, persists in the human for at least one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

In one embodiment, the progeny lymphocyte cells in the human comprise a memory T cell. In another embodiment, the T cell is an autologous T cell.

In all of the aspects and embodiments of methods described herein, any of the aforementioned cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen that may be treated or prevented or ameliorated using a patient-specific autologous anti-tumor lymphocyte cell population(s) comprising one or more of the DuoCAR (either with or without one or more booster elements) immunotherapeutic compositions as disclosed herein.

In yet another aspect, a kit is provided for making a DuoCAR immunotherapeutic composition (either with or without one or more booster elements) comprising a patient-specific autologous anti-tumor lymphocyte cell population(s) as described *supra* or for preventing, treating, or ameliorating any of the cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen in a subject as described *supra*, comprising a container comprising any one of the nucleic acid molecules, vectors, host cells, or compositions disclosed *supra* or any combination thereof, and instructions for using the kit.

While the compositions and methods of the present invention have been illustrated with reference to the generation and utilization of DuoCARs (either with or without one or more booster elements), it is contemplated herein that the compositions and methods are specifically intended to include the generation and utilization of TrioCARs and QuatroCARs (either with or without one or more booster elements).

In yet another aspect, an immunotherapy composition comprising one or more isolated nucleic acids encoding at least one vector, wherein said vector contains a nucleic acid sequence that results in at least one messenger RNA (*i.e.*, a multi-cistronic nucleic acid or a nucleic acid resulting in more than one transcript) encoding a DuoCAR (either with or without one or more booster elements), resulting in the ability to bind two or more non-identical antigen targets, thereby generating multiple antigen specificities residing in a single cell expressing said vector.

In yet another aspect, an immunotherapy composition comprising one or more isolated nucleic acids encoding at least two vectors, as described *supra*, wherein each vector further encodes a functional tag or

anti-tag binding moiety (AT-CAR) (either with or without one or more booster elements) that reconstitutes a functional chimeric antigen receptor upon co-incubation or co-administration of a soluble binder (such as a tagged scFv, or a scFv linked to an anti-tag binder), whereby the combination of the two vectors results in the ability to bind two or more non-identical antigen binding domains, resulting in multiple antigen specificities residing in a cell expressing these two vectors.

In yet another aspect, an immunotherapy composition comprising one or more isolated nucleic acids encoding at least two vectors, as described *supra*, wherein each vector encoding a functional tag or anti-tag binding moiety (AT-CAR) (either with or without one or more booster elements) that reconstitutes a functional chimeric antigen receptor upon co-incubation or co-administration of a soluble binder (such as a tagged scFv, or a scFv linked to an anti-tag binder), wherein each vector expresses a unique tag (or anti-tag) that can bind soluble protein or protein modified structures resulting in multiple antigen specificities, or wherein each vector expresses a unique tag (or anti-tag) that binds only one of the soluble binding domains resulting in a specific linkage of the AT-CAR (either with or without one or more booster elements) encoded intracellular signaling motifs to the antigen-binding domains of the tagged (or anti-tagged) binder.

In a non-limiting embodiment for the manufacture of DuoCAR vectors (either with or without one or more booster elements), each of the compositions and methods disclosed in the embodiments and aspects referred to supra, the two vectors can be made separately and then added to the T cells sequentially or at the same time. In another non limiting embodiment, the plasmid DNA of the two or more vectors can be combined before or during transfection of production cells, or integrated in the production cells genome, to produce a mixture of viral vectors that contain the multiple DuoCAR (either with or without one or more booster elements) vector particles, subsequently used for the transduction and genetic modification of patient T Cells.

In each of the aforementioned aspects and embodiments described *supra*, for example, scFv binders have been created for mesothelin, as disclosed in Applicant's issued U.S. Patent No. 10,183,993, entitled Compositions and Methods for Treating Cancer with Anti-Mesothelin Immunotherapy, and assigned Lentigen Technology, Inc. matter number LEN_017, nucleotide sequence ScFv antigen SEQ ID NO: 149 and amino acid sequence SEQ ID NO: 150, respectively, that can be incorporated into functional CARs, nucleotide sequence SEQ ID NO: 39 and amino acid sequence SEQ ID NO: 40, respectively, and that can thereby be incorporated into a DuoCAR therapy.

In each of the aforementioned aspects and embodiments described *supra*, in addition to scFv sequences, single chain antigen binders (as opposed to scFv) can be incorporated into a single, tandem, DuoCAR, or multi-targeting CAR application. For example, the CD33-specific heavy chain only binder, as disclosed in Applicant's issued U.S. Patent No. 10,426,797,, entitled Compositions and Methods For

Treating Cancer With Anti-CD33 Immunotherapy, and assigned Lentigen Technology, Inc. matter number LEN_018, nucleotide sequence SEQ ID NO: 41 and amino acid sequence SEQ ID NO: 42, respectively, can be incorporated into a functional CAR, LTG1906, nucleotide sequence SEQ ID NO: 43 and amino acid sequence SEQ ID NO: 44, respectively, that targets CD33-expressing malignancies.

In each of the aforementioned aspects and embodiments described *supra*, one example of a single, tandem, DuoCAR, or multi-targeting CAR therapeutic application would be the treatment of leukemia that expresses the CD19, CD20, and TSLPR antigens. In this case, LTG1496 or LTG1497 (SEQ ID NOs: 35, 26, respectively) could be combined with a TSLPR-specific CAR (LTG1789), SEQ ID NO: 47 and amino acid sequence SEQ ID NO: 48, respectively, that had been created from TSLPR-specific scFV domains, nucleotide sequence SEQ ID NO: 45 and amino acid sequence SEQ ID NO: 46.

In each of the aforementioned aspects and embodiments described *supra*, another example of a single, tandem, DuoCAR, or multi-targeting CAR therapeutic application would be the treatment of cancer that expresses the CD38 antigen. For instance, the CD38-specific binders, as disclosed in Applicant's issued U.S. Patent No. 11,103,533; entitled Compositions and Methods For Treating Cancer With Anti-CD38 Immunotherapy; as filed on November 30, 2018; and assigned Lentigen Technology, Inc. matter number LEN_026; can be incorporated into one or more functional CARs that target CD38-expressing malignancies, as disclosed in Applicant's issued U.S. Patent No. 11,103,533, the entirety of which is incorporated by reference herein.

In each of the aforementioned aspects and embodiments described *supra*, another example of a single, tandem, DuoCAR, or multi-targeting CAR therapeutic application would be the treatment of cancer that expresses the CD123 antigen. For instance, the CD123-specific binders, as disclosed in Applicant's issued U.S. Patent No. 10,844,128; entitled Compositions and Methods For Treating Cancer With Anti-CD123 Immunotherapy; as filed on September 20, 2019; and assigned Lentigen Technology, Inc. matter number LEN_024; and claiming priority to Provisional Patent Application No. 62/734,106; as filed on September 20, 2018; can be incorporated into one or more functional CARs that target CD123-expressing malignancies, as disclosed in Applicant's issued U.S. Patent No. 10,844,128, the entirety of which is incorporated by reference herein.

In each of the aforementioned aspects and embodiments described *supra*, another example of a single, tandem, DuoCAR, or multi-targeting CAR therapeutic application would be the treatment of cancer that expresses the CD123 antigen. For instance, the CD123-specific binders, as disclosed in Applicant's U.S. co-pending Patent Application No. 17/685,132; entitled Compositions and Methods For Treating Cancer With Anti-CD123 Immunotherapy; as filed on March 2, 2022; and assigned Lentigen Technology, Inc. matter number MBG_99; can be incorporated into one or more functional CARs that target CD123-

expressing malignancies, as disclosed in Applicant's co-pending U.S. Patent Application No. 17/685,132, the entirety of which is incorporated by reference herein.

In each of the aforementioned aspects and embodiments described *supra*, another example of a single, tandem, DuoCAR, or multi-targeting CAR therapeutic application would be the treatment of cancer that expresses the BCMA antigen. For instance, the BCMA-specific binders, as disclosed in Applicant's issued U.S. Patent No. 11,052,112; entitled Fully Human BCMA CAR T Cells for the Treatment of Multiple Myeloma and Other BCMA-Positive Malignancies; as filed on May 30, 2019; and assigned Lentigen Technology, Inc. matter number MBG_13; can be incorporated into one or more functional CARs that target BCMA-expressing malignancies, as disclosed in Applicant's issued U.S. Patent No. 11,052,112, the entirety of which is incorporated by reference herein.

In each of the aforementioned aspects and embodiments described *supra*, examples of tandem-CARs (containing 2 scFv domains, as described in nucleotide sequence SEQ ID: 23 and amino acid sequence SEQ ID:24) on which this technology is based include the CD20_CD19 CAR LTG1497, nucleotide sequence SEQ ID NO: 25 and amino acid sequence SEQ ID NO: 26. In some cases reversing the order of the two binders may provide a better DuoCAR expression in target cells. Thus, LTG1497, where the CD19 scFv is more proximal, as shown in nucleotide sequence SEQ ID NO: 25 and amino acid sequence SEQ ID NO: 26; and LTG1496 where the CD19 scFV is more distal to the membrane, as shown in nucleotide sequence SEQ ID NO: 33 and amino acid sequence SEQ ID NO: 34, can both be used as one of the members of a DuoSet comprising a DuoCAR.

In each of the aforementioned aspects and embodiments described *supra*, one or more of the above-identified novel boosted chimeric antigen receptors (CARs) provided *supra* with respect to each of the aforementioned of applicant's co-pending patent applications or issued patents SEQ ID NOs: 23, 24, 25, 26, 33, 34, 35, 41, 42, 43, 44, 45, 46, 47, and 48 may comprise either a single, tandem, or multi-targeting CAR construct (including those in a DuoCAR *format*), or any combination thereof.

In each of the aforementioned aspects and embodiments described *supra*, Applicant's co-pending patent applications and/or issued patents demonstrate one or more additional characteristics of the DuoCAR constructs, including, for example, i) despite the reduction in MFI of the larger payload constructs, multi-targeting in the DuoCAR format was superior in tumor cell killing as compared to monoCAR targeting; ii) mesothelin boosted CARs with mbIL7 showed superior, antigen-dependent target cell killing as compared to the non-boosted mesothelin CARs; iii) in addition, the mIL7 boosted DuoCARs and tandem CARs demonstrated superior target killing as compared to the non-boosted CARs counterparts; iv) in addition mIL7-boosted DuoCARs and Tandem CARs demonstrated superior cytokine elaboration in response to tumor antigen, greater long-term persistence and expansion under cytokine-poor conditions, and better preservation of effector function; v) mesothelin CARs boosted with TGFBRIIdn armor demonstrated robust

transduction an expansion in culture, and robust killing of tumor lines expressing high, medium or low levels of mesothelin, despite the armor payload; and/or vi) mesothelin and ROR1 CARs with HPSE booster effectively digested the ECM in a transwell migration assay, and/or any combination thereof.

A. Chimeric Antigen Receptors (as present in single, tandem, DuoCARs, multiple-targeting CARs, either with or without one or more boosters)

A CAR is an artificially constructed hybrid protein or polypeptide containing the antigen binding domains of an antibody (*e.g.*, single chain variable fragment (scFv)) linked to T-cell signaling domains via a transmembrane domain. Characteristics of DuoCARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, and exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing DuoCARs the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, DuoCARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

As disclosed herein, the intracellular T cell signaling domains of the DuoCARs can include, for example, a T cell receptor signaling domain, a T cell costimulatory signaling domain, or both. The T cell receptor signaling domain refers to a portion of the CAR comprising the intracellular domain of a T cell receptor, such as, for example, and not by way of limitation, the intracellular portion of the CD3 zeta protein. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule, which is a cell surface molecule other than an antigen receptor or their ligands that are required for an efficient response of lymphocytes to antigen. In some instances, the activation domains can be attenuated by the mutation of specific sites of phosphorylation, *i.e.* the ITAM motifs in the CD3 zeta chain, thus carefully modulating the degree of signal transduction mediated by that domain.

1. Extracellular Domain

In one embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein, comprises a target-specific binding element otherwise referred to as an antigen binding domain or moiety. The choice of domain depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus,

examples of cell surface markers that may act as ligands for the antigen binding domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) include those associated with viral, bacterial and parasitic infections, autoimmune disease, alloimmune disease, autoaggressive disease and cancer cells.

In one embodiment, the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can be engineered to target a tumor antigen of interest by way of engineering a desired antigen binding domain that specifically binds to an antigen on a tumor cell. Tumor antigens are proteins that are produced by tumor cells that elicit an immune response, particularly T-cell mediated immune responses. The selection of the antigen binding domain will depend on the particular type of cancer to be treated. Tumor antigens are well known in the art and include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), beta-human chorionic gonadotropin, alpha-fetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-1a, p53, prostein, PSMA, Her2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I receptor, IGF-II receptor, IGF-I receptor, CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B 1, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3,

TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof. The tumor antigens disclosed herein are merely included by way of example. The list is not intended to be exclusive and further examples will be readily apparent to those of skill in the art.

In one embodiment, the tumor antigen comprises one or more antigenic cancer epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for an immune attack. These molecules include, but are not limited to, tissue-specific antigens such as MART-1, tyrosinase and GP 100 in melanoma and prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) in prostate cancer. Other target molecules belong to the group of transformation-related molecules such as the oncogene HER-2/Neu/ErbB-2. Yet another group of target antigens are onco-fetal antigens such as carcinoembryonic antigen (CEA). In B-cell lymphoma the tumor-specific idiotype immunoglobulin constitutes a truly tumor-specific immunoglobulin antigen that is unique to the individual tumor. B-cell differentiation antigens such as CD19, CD20, CD22, and CD37 are other candidates for target antigens in B-cell lymphoma. Some of these antigens (CEA, HER-2, CD19, CD20, CD22, idiotype) have been used as targets for passive immunotherapy with monoclonal antibodies with limited success.

The type of tumor antigen may also be a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA is not unique to a tumor cell and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development when the immune system is immature and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells, but which are expressed at much higher levels on tumor cells.

Non-limiting examples of TSAs or TAAs include the following: Differentiation antigens such as MART-1/MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multi-lineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER-2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-

PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

In a preferred embodiment, the antigen binding domain portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) targets an antigen that includes but is not limited to CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B l, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an F(ab')2 fragment, an Fv fragment, a nanobody, a VHH,

a ligand peptide, and a single chain Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof. In yet another embodiment, a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is provided herein comprising a Tag or anti-Tag binding domain.

Depending on the desired antigen to be targeted, the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can be engineered to include the appropriate antigen binding domain that is specific to the desired antigen target. For example, if CD19 is the desired antigen that is to be targeted, an antibody or the scFv subfragment thereof specific for CD19 can be used as the antigen bind domain incorporated into the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

In one exemplary embodiment, the antigen binding domain portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) targets CD19. Preferably, the antigen binding domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is anti-CD19 scFV, wherein the nucleic acid sequence of the anti-CD19 scFV comprises the sequence set forth in SEQ ID NO: 27. In one embodiment, the anti-CD19 scFV comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 28. In another embodiment, the anti-CD19 scFV portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises the amino acid sequence set forth in SEQ ID NO: 28. In a second exemplary embodiment, the antigen binding domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) targets CD20. Preferably, the antigen binding domains in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is anti-CD20 scFv, wherein the nucleic acid sequence of the anti-CD20 scFv comprises the sequence set forth in SEQ ID NO: 1. In another embodiment, the anti-CD20 scFV portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises the amino acid sequence set forth in SEQ ID NO: 2. In a third exemplary embodiment, the antigen binding domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) targets CD22. Preferably, the antigen binding domains in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is anti-CD22 scFv, wherein the nucleic acid sequence of the anti-CD22 scFv comprises the sequence set forth in SEQ ID NO: 7. In another embodiment, the anti-CD22 scFV portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises the amino acid sequence set forth in SEQ ID NO: 8.

In one aspect of the present invention, there is provided a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) capable of binding to a non-TSA or non-

TAA including, for example and not by way of limitation, an antigen derived from Retroviridae (*e.g.* human immunodeficiency viruses such as HIV-1 and HIV-LP), Picornaviridae (*e.g.* poliovirus, hepatitis A virus, enterovirus, human coxsackievirus, rhinovirus, and echovirus), rubella virus, coronavirus, vesicular stomatitis virus, rabies virus, ebola virus, parainfluenza virus, mumps virus, measles virus, respiratory syncytial virus, influenza virus, hepatitis B virus, parvovirus, Adenoviridae, Herpesviridae [*e.g.* type 1 and type 2 herpes simplex virus (HSV), varicella-zoster virus, cytomegalovirus (CMV), and herpes virus], Poxviridae (*e.g.* smallpox virus, vaccinia virus, and pox virus), or hepatitis C virus, or any combination thereof.

In another aspect of the present invention, there is provided a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) capable of binding to an antigen derived from a bacterial strain of Staphylococci, Streptococcus, Escherichia coli, Pseudomonas, or Salmonella. Particularly, there is provided a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) capable of binding to an antigen derived from an infectious bacterium, for example, Helicobacter pyloris, Legionella pneumophilia, a bacterial strain of Mycobacteria sps. (*e.g.* M. tuberculosis, M. avium, M. intracellulare, M. kansaii, or M. gordonea), Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitides, Listeria monocytogenes, Streptococcus pyogenes, Group A Streptococcus, Group B Streptococcus (Streptococcus agalactiae), Streptococcus pneumoniae, or Clostridium tetani, or a combination thereof.

2. Transmembrane Domain

In the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein, the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises one or more transmembrane domains fused to the extracellular domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded linker domain is derived from the extracellular domain of IgG1, IgG2, IgG3 or IgG4, CD8, TNFRSF19, or CD28, and is linked to the transmembrane domain.

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded linker domain is derived from the extracellular domain of the transmembrane domain and is linked to the transmembrane domain.

In some instances, the transmembrane domain can be selected or by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19, Fc epsilon R, or any combination thereof. Alternatively, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). A glycine-serine doublet or a triple alanine motif provides a particularly suitable linker.

In one embodiment, the transmembrane domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) of the invention is the CD8 transmembrane domain. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 11. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 12. In another embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 12.

In some instances, the transmembrane domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises the CD8.alpha.hinge domain. In one embodiment, the CD8 hinge domain comprises the nucleic acid sequence of SEQ ID NO: 13. In one embodiment, the CD8 hinge domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 14. In another embodiment, the CD8 hinge domain comprises the amino acid sequence of SEQ ID NO: 14.

Without being intended to limit to any particular mechanism of action, it is believed that possible reasons for the enhanced therapeutic function associated with the exemplary single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein of the invention include, for example, and not by way of limitation, a) improved lateral movement within the plasma membrane allowing for more efficient signal transduction, b) superior location within plasma membrane microdomains, such as lipid rafts, and greater ability to interact with transmembrane signaling cascades associated with T cell

activation, c) superior location within the plasma membrane by preferential movement away from dampening or down-modulatory interactions, such as less proximity to or interaction with phosphatases such as CD45, and d) superior assembly into T cell receptor signaling complexes (*i.e.* the immune synapse), or any combination thereof.

In one embodiment of the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein, non-limiting exemplary transmembrane domains for use in the single, tandem, DuoCAR, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein include the TNFRSF16 and TNFRSF19 transmembrane domains may be used to derive the TNFRSF transmembrane domains and/or linker or spacer domains as disclosed in Applicant's issued U.S. Patent No. 10,421,810, entitled CHIMERIC ANTIGEN RECEPTORS AND METHODS OF USE, as filed on October 9, 2015, and assigned Lentigen Technology, Inc. matter number LEN_015PRO, including, in particular, those other TNFRSF members listed within the tumor necrosis factor receptor superfamily as listed in Table I therein.

3. Spacer Domain

In the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein, a spacer domain can be arranged between the extracellular domain and the TNFRSF transmembrane domain, or between the intracellular domain and the TNFRSF transmembrane domain. The spacer domain means any oligopeptide or polypeptide that serves to link the TNFRSF transmembrane domain with the extracellular domain and/or the TNFRSF transmembrane domain with the intracellular domain. The spacer domain comprises up to 300 amino acids, preferably 10 to 100 amino acids, and most preferably 25 to 50 amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,5667, 498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference herein in its entirety.

The spacer domain preferably has a sequence that promotes binding of a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) with an antigen and enhances signaling into a cell. Examples of an amino acid that is expected to promote the binding include cysteine,

a charged amino acid, and serine and threonine in a potential glycosylation site, and these amino acids can be used as an amino acid constituting the spacer domain.

As the spacer domain, the entire or a part of amino acid numbers 137 to 206 (SEQ ID NO: 15) which includes the hinge region of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3), amino acid numbers 135 to 195 of CD8.beta. (GenBank: AAA35664.1), amino acid numbers 315 to 396 of CD4 (NCBI RefSeq: NP.sub.--000607.1), or amino acid numbers 137 to 152 of CD28 (NCBI RefSeq: NP.sub.--006130.1) can be used. Also, as the spacer domain, a part of a constant region of an antibody H chain or L chain (CH1 region or CL region, for example, a peptide having an amino acid sequence shown in SEQ ID NO: 16) can be used. Further, the spacer domain may be an artificially synthesized sequence.

Further, in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), a signal peptide sequence can be linked to the N-terminus. The signal peptide sequence exists at the N-terminus of many secretory proteins and membrane proteins, and has a length of 15 to 30 amino acids. Since many of the protein molecules mentioned above as the intracellular domain have signal peptide sequences, the signal peptides can be used as a signal peptide for the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). In one embodiment, the signal peptide comprises the nucleotide sequence of the leader (signal peptide) sequence shown in SEQ ID NO: 5. In one embodiment, the signal peptide comprises the amino acid sequence shown in SEQ ID NO: 6.

4. Intracellular Domain

The cytoplasmic domain or otherwise the intracellular signaling domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is responsible for activation of at least one of the normal effector functions of the immune cell in which the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

Preferred examples of intracellular signaling domains for use in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capacity.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the single, tandem, DuoCAR, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein include those derived from TCR zeta (CD3 Zeta), FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. Specific, non-limiting examples, of the ITAM include peptides having sequences of amino acid numbers 51 to 164 of CD3.zeta. (NCBI RefSeq: NP.sub.--932170.1), amino acid numbers 45 to 86 of Fc.epsilon.RI.gamma. (NCBI RefSeq: NP.sub.--004097.1), amino acid numbers 201 to 244 of Fc.epsilon.RI.beta. (NCBI RefSeq: NP.sub.--000130.1), amino acid numbers 139 to 182 of CD3.gamma. (NCBI RefSeq: NP.sub.--000064.1), amino acid numbers 128 to 171 of CD3 .delta. (NCBI RefSeq: NP.sub.--000723.1), amino acid numbers 153 to 207 of CD3.epsilon. (NCBI RefSeq: NP.sub.--000724.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 707 to 847 of 0022 (NCBI RefSeq: NP.sub.--001762.2), amino acid numbers 166 to 226 of CD79a (NCBI RefSeq: NP.sub.--001774.1), amino acid numbers 182 to 229 of CD79b (NCBI RefSeq: NP.sub.--000617.1), and amino acid numbers 177 to 252 of CD66d (NCBI RefSeq: NP.sub.--001806.2), and their variants having the same function as these peptides have. The amino acid number based on amino acid sequence information of NCBI RefSeq ID or GenBank described herein is numbered based on the full length of the precursor (comprising a signal peptide sequence etc.) of each protein. In one embodiment, the cytoplasmic signaling molecule in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprises a cytoplasmic signaling

sequence derived from CD3 zeta. In another embodiment one, two, or three of the ITAM motifs in CD3 zeta are attenuated by mutation or substitution of the tyrosine residue by another amino acid.

In a preferred embodiment, the intracellular domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). For example, the intracellular domain of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such costimulatory molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, 276/B7-H3, and a ligand that specifically binds with CD83, and the like. Specific, non-limiting examples, of such costimulatory molecules include peptides having sequences of amino acid numbers 236 to 351 of CD2 (NCBI RefSeq: NP.sub.--001758.2), amino acid numbers 421 to 458 of CD4 (NCBI RefSeq: NP.sub.--000607.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 207 to 235 of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3), amino acid numbers 196 to 210 of CD83 (GenBank: AAA35664.1), amino acid numbers 181 to 220 of CD28 (NCBI RefSeq: NP.sub.--006130.1), amino acid numbers 214 to 255 of CD137 (4-1BB, NCBI RefSeq: NP.sub.--001552.2), amino acid numbers 241 to 277 of CD134 (OX40, NCBI RefSeq: NP.sub.--003318.1), and amino acid numbers 166 to 199 of ICOS (NCBI RefSeq: NP.sub.--036224.1), and their variants having the same function as these peptides have. Thus, while the disclosure herein is exemplified primarily with 4-1BB as the co-stimulatory signaling element, other costimulatory elements are within the scope of the disclosure.

The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker.

In one embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment,

the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28 and 4-1BB.

In one embodiment, the intracellular domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the nucleic acid sequence set forth in SEQ ID NO: 17 and the signaling domain of CD3-zeta comprises the nucleic acid sequence set forth in SEQ ID NO: 19.

In one embodiment, the intracellular domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 18 and the signaling domain of CD3-zeta comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 20.

In one embodiment, the intracellular domain in the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO: 18 and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 20.

5. Additional Description of Single, Tandem, DuoCARs, Multiple-Targeting CARs (With or Without One or More Boosting Elements)

Also expressly included within the scope of the invention are functional portions of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) as disclosed herein. The term "functional portion" when used in reference to a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) refers to any part or fragment of one or more of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein, which part or fragment retains the biological activity of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) of which it is a part (the parent single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements)). Functional portions encompass, for example, those parts of a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements). In reference

to the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). Desirably, the additional amino acids do not interfere with the biological function of the functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

Included in the scope of the disclosure are functional variants of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein. The term "functional variant" as used herein refers to a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), polypeptide, or protein having substantial or significant sequence identity or similarity to a parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), which functional variant retains the biological activity of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) of which it is a variant. Functional variants encompass, for example, those variants of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) described herein (the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements)) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). In reference to the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

A functional variant can, for example, comprise the amino acid sequence of the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) with at least one conservative amino acid substitution. Alternatively, or additionally, the functional variants can comprise the amino acid sequence of the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the

biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements).

Amino acid substitutions of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (*e.g.*, Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (*e.g.*, Ala, Gly, Val, He, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (*e.g.* Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (*e.g.*, Asn, Gin, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (*e.g.*, He, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (*e.g.*, His, Phe, Trp, and Tyr), etc.

The single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, *e.g.*, other amino acids, do not materially change the biological activity of the functional variant.

The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (or functional portions or functional variants thereof) retain their biological activity, *e.g.*, the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known

in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, -amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, -aminocyclopentane carboxylic acid, a-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-norbornane)-carboxylic acid, γ-diaminobutyric acid, β-diaminopropionic acid, homophenylalanine, and a-tert-butylglycine.

The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (including functional portions and functional variants thereof) can be obtained by methods known in the art. The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) may be made by any suitable method of making polypeptides or proteins. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan *et al.*, Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood *et al.*, Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Patent 5,449,752. Methods of generating chimeric antigen receptors, T cells including such receptors, and their use (*e.g.*, for treatment of cancer) are known in the art and further described herein (see, *e.g.*, Brentjens *et al.*, 2010, Molecular Therapy, 18:4, 666-668; Morgan *et al.*, 2010, Molecular Therapy, published online February 23, 2010, pages 1 -9; Till *et al.*, 2008, Blood, 1 12:2261 -2271; Park *et al.*, Trends Biotechnol., 29:550-557, 2011; Grupp *et al.*, N Engl J Med., 368:1509-1518, 2013; Han *et al.*, J. Hematol Oncol., 6:47, 2013; Tumaini *et al.*, Cytotherapy, 15, 1406-1417, 2013; Haso *et al.*, (2013) Blood, 121, 1165-1174; PCT Pubs. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety). For example, a nucleic acid molecule encoding a disclosed chimeric antigen binding receptor can be included in an expression vector (such as a lentiviral vector) used to transduce a host cell, such as a T cell, to make the disclosed single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements). In some embodiments, methods of using the chimeric antigen receptor include isolating T cells from a subject, transducing the T cells with an expression vector (such as a lentiviral vector) encoding the chimeric antigen receptor, and administering the single, tandem, DuoCAR, or

multiple-targeting CAR (with or without one or more boosting elements)-expressing T cells to the subject for treatment, for example for treatment of a tumor in the subject.

6. Description of Boosting Elements (Boosters)

In addition to the aforementioned description provided *supra*, the booster elements of the single, tandem, DuoCARs, multiple-targeting CARs that may be used in the patient-specific autologous or allogeneic anti-tumor, anti-autoimmune, anti-alloimmune, or anti-autoaggressive-lymphocyte cell population(s) may additionally comprise functional percent identity variants thereof, as set forth below.

In one specific embodiment, also expressly included within the scope of the invention are functional boosting element portions of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous or allogeneic anti-tumor lymphocyte cell population(s) as disclosed herein. Boosting elements encompass, for example, additional therapeutic proteins or peptides expressed or secreted by the engineered T cell populations such as: i) one or more A-beta DPs (amyloid beta degrading proteases), ii) one or more matrix proteases (such as MMP-9 and MMP9), iii) one or more peptides or soluble antibody-like binders that interfere with plaque formation, iv) one or more cytokines (such as TGF-beta, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21), v) one or more armor elements so as to overcome immunosuppression in TME, vi) one or more digestive enzymes to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration, vii) one or more pro-inflammatory immune activators, and viii) one or more on-switches or off-switches, or any combination thereof, to control the expression of the CAR, wherein the boosted CARs achieve a high surface expression on transduced T cells, a multi-targeting activity to overcome antigen escape, a high degree of cytolysis and transduced T cell *in vivo* expansion and persistence to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer or autoimmune, alloimmune, or autoaggressive disease, or prevention or amelioration of relapse of cancer or autoimmune, alloimmune, or autoaggressive disease, or a combination thereof, in a patient-specific manner. In reference to the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs, the functional boosting element portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs.

The functional parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent one or

more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs.

Included in the scope of the disclosure are functional variants of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein. The term "functional variant" as used herein refers to a single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements), polypeptide, or protein having substantial or significant sequence identity or similarity to a parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs which functional variant retains the biological activity of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) of which it is a variant. Functional variants encompass, for example, those variants of the single, tandem, DuoCAR, or multiple-targeting CAR (with or without one or more boosting elements) described herein (the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs. In reference to the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs.

A functional variant can, for example, comprise the amino acid sequence of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs with at least one conservative amino acid substitution. Alternatively, or additionally, the functional variants can comprise the amino acid sequence of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs.

Amino acid substitutions of the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino

acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (*e.g.*, Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (*e.g.*, Ala, Gly, Val, He, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (*e.g.* Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (*e.g.*, Asn, Gin, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (*e.g.*, He, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (*e.g.*, His, Phe, Trp, and Tyr), etc.

The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, *e.g.*, other amino acids, do not materially change the biological activity of the functional variant.

The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs (or functional portions or functional variants thereof) retain their biological activity, *e.g.*, the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, -amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, -aminocyclopentane carboxylic acid, a-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic

acid, a-(2-amino-2-norbornane)-carboxylic acid, γ-diaminobutyric acid, β-diaminopropionic acid, homophenylalanine, and a-tert-butylglycine.

The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs (including functional portions and functional variants thereof) can be obtained by methods known in the art. The parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs may be made by any suitable method of making polypeptides or proteins. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan *et al.*, Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood *et al.*, Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Patent 5,449,752. Methods of generating chimeric antigen receptors, T cells including such receptors, and their use (*e.g.*, for treatment of cancer) are known in the art and further described herein (see, *e.g.*, Brentjens *et al.*, 2010, Molecular Therapy, 18:4, 666-668; Morgan *et al.*, 2010, Molecular Therapy, published online February 23, 2010, pages 1 -9; Till *et al.*, 2008, Blood, 1 12:2261 -2271; Park *et al.*, Trends Biotechnol., 29:550-557, 2011; Grupp *et al.*, N Engl J Med., 368:1509-1518, 2013; Han *et al.*, J. Hematol Oncol., 6:47, 2013; Tumaini *et al.*, Cytotherapy, 15, 1406-1417, 2013; Haso *et al.*, (2013) Blood, 121, 1165-1174; PCT Pubs. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety). For example, a nucleic acid molecule encoding a disclosed chimeric antigen binding receptor can be included in an expression vector (such as a lentiviral vector) used to transduce a host cell, such as a T cell, to make the disclosed parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs. In some embodiments, methods of using the chimeric antigen receptor include isolating T cells from a subject, transducing the T cells with an expression vector (such as a lentiviral vector) encoding the chimeric antigen receptor, and administering the parent one or more boosting elements of the single, tandem, DuoCARs, or multiple-targeting CARs-expressing T cells to the subject for treatment, for example for treatment of a tumor in the subject.

B. Antibodies and Antigen Binding Fragments

One embodiment further provides a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell

population(s) disclosed herein, a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding domain or portion thereof, which specifically binds to one or more of the antigens disclosed herein. As used herein, a "T cell expressing a single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements)," or a "single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) T cell" means a T cell expressing a single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), and has antigen specificity determined by, for example, the antibody-derived targeting domain of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements).

As used herein, and "antigen binding domain" can include an antibody and antigen binding fragments thereof. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), and antigen binding fragments thereof, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof known in the art that retain binding affinity for the antigen.

A "monoclonal antibody" is an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. In some examples, a monoclonal antibody is an antibody produced by a single clone of B lymphocytes or by a cell into which nucleic acid encoding the light and heavy variable regions of the antibody of a single antibody (or an antigen binding fragment thereof) have been transfected, or a progeny thereof. In some examples monoclonal antibodies are isolated from a subject. Monoclonal antibodies can have conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Exemplary methods of production of monoclonal antibodies are known, for example, see Harlow & Lane, Antibodies, A Laboratory Manual, 2nd ed. Cold Spring Harbor Publications, New York (2013).

Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable domain genes. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region (or constant domain) and a variable region (or variable domain; see, *e.g.*, Kindt *et al.* Kuby Immunology, 6.sup.th ed., W.H. Freeman and Co., page 91 (2007).) In several embodiments, the heavy and the light chain variable regions combine to specifically bind the antigen. In additional embodiments, only the heavy chain variable region is required. For example, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (see, *e.g.*, Hamers-Casterman *et al.*, Nature, 363:446-448, 1993; Sheriff *et al.*, Nat. Struct. Biol., 3:733-736, 1996). References to "VH" or "VH" refer to the variable region of an antibody heavy chain, including that of an antigen binding fragment, such as Fv, scFv, dsFv or Fab. References to "VL" or "VL" refer to the variable domain of an antibody light chain, including that of an Fv, scFv, dsFv or Fab.

Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs" (see, *e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* ("Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991; "Kabat" numbering scheme), Al-Lazikani *et al.*, (JMB 273,927-948, 1997; "Chothia" numbering scheme), and Lefranc *et al.* ("IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev. Comp. Immunol., 27:55-77, 2003; "IMGT" numbering scheme). The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a VH CDR3 is the CDR3 from the variable domain of the heavy chain of the antibody in which it is found, whereas a VL CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3. Heavy chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3.

An "antigen binding fragment" is a portion of a full length antibody that retains the ability to specifically recognize the cognate antigen, as well as various combinations of such portions. Non-limiting examples of antigen binding fragments include Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multi-specific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole

antibodies or those synthesized de novo using recombinant DNA methodologies (see, *e.g.*, Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

A single-chain antibody (scFv) is a genetically engineered molecule containing the VH and VL domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, Science, 242:423 426, 1988; Huston *et al.*, Proc. Natl. Acad. Sci., 85:5879 5883, 1988; Ahmad *et al.*, Clin. Dev. Immunol., 2012, doi:10.1155/2012/980250; Marbry, IDrugs, 13:543-549, 2010). The intramolecular orientation of the VH-domain and the VL-domain in a scFv, is typically not decisive for scFvs. Thus, scFvs with both possible arrangements (VH-domain-linker domain-VL-domain; VL-domain-linker domain-VH-domain) may be used.

In a dsFv the heavy and light chain variable chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. Diabodies also are included, which are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, Proc. Natl. Acad. Sci., 90:6444 6448, 1993; Poljak *et al.*, Structure, 2:1121 1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., Immunology, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly, or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse *et al.*, Science 246:1275-1281 (1989), which is incorporated herein by reference. These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies, are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward *et al.*, Nature 341:544-546 (1989); Harlow and Lane, supra, 1988; Hilyard *et al.*, Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference).

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. Antibody competition assays are known, and an exemplary competition assay is provided herein.

A "humanized" antibody or antigen binding fragment includes a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) antibody or antigen binding fragment. The non-human antibody or antigen binding fragment providing the CDRs is termed a "donor," and the human antibody or antigen binding fragment providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they can be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized antibody or antigen binding fragment, except possibly the CDRs, are substantially identical to corresponding parts of natural human antibody sequences.

A "chimeric antibody" is an antibody which includes sequences derived from two different antibodies, which typically are of different species. In some examples, a chimeric antibody includes one or more CDRs and/or framework regions from one human antibody and CDRs and/or framework regions from another human antibody.

A "fully human antibody" or "human antibody" is an antibody which includes sequences from (or derived from) the human genome, and does not include sequence from another species. In some embodiments, a human antibody includes CDRs, framework regions, and (if present) an Fc region from (or derived from) the human genome. Human antibodies can be identified and isolated using technologies for creating antibodies based on sequences derived from the human genome, for example by phage display or using transgenic animals (see, *e.g.*, Barbas *et al.* Phage display: A Laboratory Manuel. 1st Ed. New York: Cold Spring Harbor Laboratory Press, 2004. Print.; Lonberg, Nat. Biotech., 23: 1117-1125, 2005; Lonenberg, Curr. Opin. Immunol., 20:450-459, 2008).

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a bispecific or bifunctional antibody has two different binding sites.

Methods of testing antibodies for the ability to bind to any functional portion of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, *e.g.*, Janeway *et al.*, *infra*, U.S. Patent Application Publication No. 2002/0197266 Al, and U.S. Patent No. 7,338,929).

Also, a single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, can be to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (*e.g.*, fluorescein isothiocyanate (FITC),

phycoerythrin (PE)), an enzyme (*e.g.*, alkaline phosphatase, horseradish peroxidase), and element particles (*e.g.*, gold particles).

C. Conjugates

The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) disclosed herein, a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or monoclonal antibodies, or antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. Conjugates include, but are not limited to, molecules in which there is a covalent linkage of an effector molecule or a detectable marker to an antibody or antigen binding fragment that specifically binds one or more of the antigens disclosed herein. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) chemotherapeutic agents, anti-angiogenic agents, toxins, radioactive agents such as ^{125}I, ^{32}P, ^{14}C, ^{3}H and ^{35}S and other labels, target moieties and ligands, etc.

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as a tumor cell).

The procedure for attaching an effector molecule or detectable marker to an antibody or antigen binding fragment varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups, such as carboxylic acid (COOH), free amine ($-NH_2$) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule or detectable marker. Alternatively, the antibody or antigen binding fragment is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody or antigen binding fragment to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the antibody or antigen binding fragment and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody or antigen binding fragment and the effector molecule or detectable marker are polypeptides, the linkers may

be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,5667, 498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference herein in its entirety.

In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody or antigen binding fragment in the intracellular environment. In yet other embodiments, the linker is not cleavable, and the effector molecule or detectable marker is released, for example, by antibody degradation. In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptide linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptide linker is at least two amino acids long or at least three amino acids long. However, the linker can be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, such as 1-2, 1-3, 2-5, 3-10, 3-15, 1-5, 1-10, 1-15 amino acids long. Proteases can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, for example, Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). For example, a peptide linker that is cleavable by the thiol-dependent protease cathepsin-B, can be used (for example, a Phenylalanine -Leucine or a Glycine- Phenylalanine -Leucine-Glycine linker). Other examples of such linkers are described, for example, in U.S. Pat. No. 6,214,345, incorporated herein by reference. In a specific embodiment, the peptide linker cleavable by an intracellular protease is a Valine-Citruline linker or a Phenylalanine-Lysine linker (see, for example, U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the Valine-Citruline linker).

In other embodiments, the cleavable linker is pH-sensitive, *i.e.*, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, for example, U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville *et al.*, 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral

pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, for example, a thioether attached to the therapeutic agent via an acylhydrazone bond (see, for example, U.S. Pat. No. 5,622,929).

In other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, for example, Thorpe *et al.*, 1987, Cancer Res. 47:5924-5931; Wawrzynczak *et al.*, In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987); Phillips *et al.*, Cancer Res. 68:92809290, 2008). See also U.S. Pat. No. 4,880,935.)

In yet other specific embodiments, the linker is a malonate linker (Johnson *et al.*, 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau *et al.*, 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau *et al.*, 1995, Bioorg-Med-Chem. 3(10):1305-12).

In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety).

In several embodiments, the linker is resistant to cleavage in an extracellular environment. For example, no more than about 20%, no more than about 15%, no more than about 10%, no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of conjugate, are cleaved when the conjugate is present in an extracellular environment (for example, in plasma). Whether or not a linker is resistant to cleavage in an extracellular environment can be determined, for example, by incubating the conjugate containing the linker of interest with plasma for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free effector molecule or detectable marker present in the plasma. A variety of exemplary linkers that can be used in conjugates are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317, each of which is incorporated by reference herein in its entirety.

In several embodiments, conjugates of a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, auristatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are provided.

Maytansine compounds suitable for use as maytansinoid toxin moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu *et al* (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, each of which is incorporated herein by reference. Conjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference.

Additional toxins can be employed with a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof. Exemplary toxins include Pseudomonas exotoxin (PE), ricin, abrin, diphtheria toxin and subunits thereof, ribotoxin, ribonuclease, saporin, and calicheamicin, as well as botulinum toxins A through F. These toxins are well known in the art and many are readily available from commercial sources (for example, Sigma Chemical Company, St. Louis, MO). Contemplated toxins also include variants of the toxins (see, for example, see, U.S. Patent Nos. 5,079,163 and 4,689,401).

Saporin is a toxin derived from Saponaria officinalis that disrupts protein synthesis by inactivating the 60S portion of the ribosomal complex (Stirpe *et al.*, Bio/Technology, 10:405-412, 1992). However, the toxin has no mechanism for specific entry into cells, and therefore requires conjugation to an antibody or antigen binding fragment that recognizes a cell-surface protein that is internalized in order to be efficiently taken up by cells.

Diphtheria toxin is isolated from Corynebacterium diphtheriae. Typically, diphtheria toxin for use in immunotoxins is mutated to reduce or to eliminate non-specific toxicity. A mutant known as CRM107, which has full enzymatic activity but markedly reduced non-specific toxicity, has been known since the 1970's (Laird and Groman, J. Virol. 19:220, 1976), and has been used in human clinical trials. See, U.S. Patent No. 5,792,458 and U.S. Patent No. 5,208,021.

Ricin is the lectin RCA60 from Ricinus communis (Castor bean). For examples of ricin, see, U.S. Patent No. 5,079,163 and U.S. Patent No. 4,689,401. Ricinus communis agglutinin (RCA) occurs in two

forms designated RCA_{60} and RCA_{120} according to their molecular weights of approximately 65 and 120 kD, respectively (Nicholson & Blaustein, J. Biochim. Biophys. Acta 266:543, 1972). The A chain is responsible for inactivating protein synthesis and killing cells. The B chain binds ricin to cell-surface galactose residues and facilitates transport of the A chain into the cytosol (Olsnes *et al.*, Nature 249:627-631, 1974 and U.S. Patent No. 3,060,165).

Ribonucleases have also been conjugated to targeting molecules for use as immunotoxins (see Suzuki *et al.*, Nat. Biotech. 17:265-70, 1999). Exemplary ribotoxins such as α-sarcin and restrictocin are discussed in, for example Rathore *et al.*, Gene 190:31-5, 1997; and Goyal and Batra, Biochem. 345 Pt 2:247-54, 2000. Calicheamicins were first isolated from Micromonospora echinospora and are members of the enediyne antitumor antibiotic family that cause double strand breaks in DNA that lead to apoptosis (see, for example Lee *et al.*, J. Antibiot. 42:1070-87,1989). The drug is the toxic moiety of an immunotoxin in clinical trials (see, for example, Gillespie *et al.*, Ann. Oncol. 11:735-41, 2000).

Abrin includes toxic lectins from Abrus precatorius. The toxic principles, abrin a, b, c, and d, have a molecular weight of from about 63 and 67 kD and are composed of two disulfide-linked polypeptide chains A and B. The A chain inhibits protein synthesis; the B chain (abrin-b) binds to D-galactose residues (see, Funatsu *et al.*, Agr. Biol. Chem. 52:1095, 1988; and Olsnes, Methods Enzymol. 50:330-335, 1978).

The single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can also be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, can also be conjugated with enzymes that are

useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, may be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect one or more of the antigens disclosed herein and antigen expressing cells by x-ray, emission spectra, or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for treatment of tumors in a subject, for example for treatment of a neuroblastoma. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

D. Nucleotides, Expression, Vectors, and Host Cells

Further provided by an embodiment of the invention is a nucleic acid comprising a nucleotide sequence encoding any of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), an antibody, or antigen binding portion thereof, described herein (including functional portions and functional variants thereof). The nucleic acids of the invention may comprise a nucleotide sequence encoding any of the leader sequences, antigen binding domains, transmembrane domains, and/or intracellular T cell signaling domains described herein.

In one embodiment, an isolated nucleic acid molecule encoding a chimeric antigen receptor (single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements)) is provided comprising, from N-terminus to C-terminus, at least one extracellular antigen binding domain, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded extracellular antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to the antigen.

In another embodiment of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded extracellular antigen binding domain comprises at least one heavy chain variable region of an antibody that binds to the antigen.

In yet another embodiment of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) extracellular antigen binding domain comprises at least one lipocalin-based antigen binding antigen (anticalins) that binds to the antigen.

In one embodiment of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule is provided wherein the encoded extracellular antigen binding domain is connected to the transmembrane domain by a linker domain.

In another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded extracellular antigen binding domain is preceded by a sequence encoding a leader or signal peptide.

In yet another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded extracellular antigen binding domain targets an antigen that includes, but is not limited to, CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, GD2, GD3, GM2, Ley, polysialic acid, fucosyl GMl, GM3, Tn, STn, sLe(animal), GloboH, CD5, CD7, CD19, CD20, CD22, CD25, CD37, CD30, CD33, CD38, CD123, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, CD276/ B7-H3, B7-H4, B7-DC, HLA-DR carcinoembryonic antigen (CEA), TAG-72, EpCAM, folate-binding protein, folate receptor alpha (FOLR1), folate receptor beta (FOLR2), A33, G250, pro state- specific membrane antigen (PSMA), ferritin, CA-125, CA19-9, CD44v6, epidermal growth factor, pl85, IL-2 receptor, interleukin 1 receptor accessory protein (IL1RAP), EGFRvIII (de2-7), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, αvβ3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, p53 nonmutant, NY-ESO-1, MelanA/MART 1, Ras mutant, gp100, FGFR1, FGFR2, FGFR3, FGFR4, GPC1, GPC2, GPC3, p53 mutant, PR1, bcr-abl, tyrosinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint fusion protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B l, MYCN, RhoC, TRP-2, mesothelin, PSCA, MAGE Al, MAGE A3, CYP1B 1, PLAV1, BORIS, ETV6-AML, NY-BR-1, RGS5, SART3, Carbonic anhydrase IX, PAX5, OY-TES 1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 3, PAGE4, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, TRAIL 1, MUC1, MUC16/CA125, MAGE A4, MAGE C2, GAGE, EGFR, EGFR1, EGFR2/Her2, CMET, HER3, CA6, NAPI2B, TROP2, TEM1, TEM7, TEM8, FAP, LAP, CLDN3, CLDN6, CLDN8, CLDN16, CLDN18.2, RON, LY6E, DLL3, PTK7, UPK1B, STRA6, TMPRSS3, TMRRSS4, TMEM238, Clorfl86, LIV1, ROR1, ROR2, Fos-related antigen 1, VEGFR1, endoglin, CD90, CD326, CD70, SSEA4, CD318, CLA, TSPAN8, GPRC5D, EpCAM, Thy1, IL13Ra2, BDCA1, BDCA2, BDCA3, GD2, PSMA, FAP, CLL1, SLAMF7/CS1, CD147, DPPA5, GRP78, CD66c, VISTA, LRRC5, LRRC15, or any combinations thereof or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a fragment selected from the group consisting of an Fab fragment, an $F(ab')_2$ fragment, an Fv fragment, a nanobody, a VHH, a ligand peptide, and a single chain

Fv (ScFv), or a fragment of any of the preceding, or a molecule that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to any of the preceding, or any combination thereof.

In certain embodiments of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded extracellular antigen binding domain comprises an anti-CD19 scFV antigen binding domain, an anti-CD20 scFV antigen binding domain, an anti-CD22 scFV antigen binding domain, an anti-ROR1 scFV antigen binding domain, an anti-TSLPR scFV antigen binding domain, an anti-mesothelin scFV antigen binding domain, an anti-CD33/IL3Ra scFV antigen binding domain, an anti-CD38 scFV antigen binding domain, an anti-CD123 (IL3RA) scFV antigen binding domain, an anti-CD138 scFV antigen binding domain, an anti-BCMA (CD269) scFV antigen binding domain, an anti-GPC2 scFV antigen binding domain, an anti-GPC3 scFV antigen binding domain, an anti-FGFR4 scFV antigen binding domain, an anti-c-Met scFV antigen binding domain, an anti-PMSA scFV antigen binding domain, an anti-glycolipid F77 scFV antigen binding domain, an anti-EGFRvIII scFV antigen binding domain, an anti-GD-2 scFV antigen binding domain, an anti-NY-ESo-1 TCR scFV antigen binding domain, an anti-MAGE A3 TCR scFV antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one aspect of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) provided herein further comprise a linker domain.

In one embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the extracellular antigen binding domain, the intracellular signaling domain, or both are connected to the transmembrane domain by a linker domain.

In one embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded linker domain is derived from the extracellular domain of CD8, and is linked to the transmembrane domain.

In yet another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the nucleic acid sequence encoding the transmembrane domain comprises a nucleotide sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In one embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded transmembrane domain comprises an amino acid sequence comprising at least one but not more than 10 modifications, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) further comprises a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In yet another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded intracellular signaling domain further comprises a CD3 zeta intracellular domain.

In one embodiment of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) disclosed herein, an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded intracellular signaling domain is arranged on a C-terminal side relative to the CD3 zeta intracellular domain.

In another embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting

CAR (with or without one or more boosting elements) is provided wherein the encoded at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, or a combination thereof.

In further embodiments of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided wherein the encoded at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), CD2, OX40, or a combination thereof.

In one embodiment of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s), an isolated nucleic acid molecule encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) is provided that further contains a leader sequence or signal peptide sequence.

In some embodiments, the nucleotide sequence may be codon-modified. Without being bound to a particular theory, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

In an embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes the antigen binding domain of the inventive single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements). In another embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes any of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) described herein (including functional portions and functional variants thereof).

"Nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (*e.g.*, isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In some embodiments,

the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

A recombinant nucleic acid may be one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques, such as those described in Sambrook *et al.*, supra. The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra*. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1 -methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3- (3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Integrated DNA Technologies (Coralville, IA, USA).

The nucleic acid can comprise any isolated or purified nucleotide sequence which encodes any of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) or functional portions or functional variants thereof. Alternatively, the nucleotide sequence can comprise a nucleotide sequence which is degenerate to any of the sequences or a combination of degenerate sequences.

An embodiment also provides an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

The nucleotide sequence which hybridizes under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (*e.g.*, 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70°C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements). It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

Also provided is a nucleic acid comprising a nucleotide sequence that is at least about 70% or more, *e.g.*, about 80%, about 90%, about 91 %, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to any of the nucleic acids described herein.

In an embodiment, the nucleic acids can be incorporated into a recombinant expression vector. In this regard, an embodiment provides recombinant expression vectors comprising any of the nucleic acids. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors are not naturally-occurring as a whole.

However, parts of the vectors can be naturally-occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double- stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

In an embodiment, the recombinant expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences, Glen Burnie, MD), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA).

Bacteriophage vectors, such as λϋTIO, λϋTI 1, λZapII (Stratagene), EMBL4, and λNMI 149, also can be used. Examples of plant expression vectors include pBIOl, pBI101.2, pBHOl .3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, *e.g.*, a retroviral vector or a lentiviral vector. A lentiviral vector is a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone *et al.*, Mol. Ther. 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include, for example, and not by way of limitation, the LENTIVECTOR.RTM. gene delivery technology from Oxford BioMedica plc, the LENTIMAX.TM. vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

A number of transfection techniques are generally known in the art (see, *e.g.*, Graham *et al.*, Virology, 52: 456-467 (1973); Sambrook *et al.*, supra; Davis *et al.*, Basic Methods in Molecular Biology, Elsevier (1986); and Chu *et al.*, Gene, 13: 97 (1981).

Transfection methods include calcium phosphate co-precipitation (see, *e.g.*, Graham *et al.*, supra), direct micro injection into cultured cells (see, *e.g.*, Capecchi, Cell, 22: 479-488 (1980)), electroporation (see, *e.g.*, Shigekawa *et al.*, BioTechniques, 6: 742-751 (1988)), liposome mediated gene transfer (see, *e.g.*, Mannino *et al.*, BioTechniques, 6: 682-690 (1988)), lipid mediated transduction (see, *e.g.*, Feigner *et al.*, Proc. Natl. Acad. Sci. USA, 84: 7413-7417 (1987)), and nucleic acid delivery using high velocity microprojectiles (see, *e.g.*, Klein *et al.*, Nature, 327: 70-73 (1987)).

In an embodiment, the recombinant expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook *et al.*, supra, and Ausubel *et al.*, supra. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, *e.g.*, from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (*e.g.*, bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate, and taking into

consideration whether the vector is DNA- or RNA-based. The recombinant expression vector may comprise restriction sites to facilitate cloning.

The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. Marker genes include biocide resistance, *e.g.*, resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements). The selection of promoters, *e.g.*, strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, *e.g.*, a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus.

The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, *e.g.*, a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine daminase, purine nucleoside phosphorylase, and nitroreductase.

An embodiment further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, *e.g.*, plant, animal, fungi, or algae, or can be a prokaryotic cell, *e.g.*, bacteria or protozoa. The host cell can be a cultured cell or a primary cell, *i.e.*, isolated directly from an organism, *e.g.*, a human. The host cell can be an adherent cell or a suspended

cell, *i.e.*, a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5a E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, *e.g.*, a DH5a cell. For purposes of producing a recombinant single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). The host cell may be a T cell.

For purposes herein, the T cell can be any T cell, such as a cultured T cell, *e.g.*, a primary T cell, or a T cell from a cultured T cell line, *e.g.*, Jurkat, SupTl, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4+/CD8+ double positive T cells, CD4+ helper T cells, *e.g.*, Th1 and Th2 cells, CD8+ T cells (*e.g.*, cytotoxic T cells), tumor infiltrating cells, memory T cells, naive T cells, and the like. The T cell may be a CD8+ T cell or a CD4+ T cell.

In an embodiment, the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) as described herein can be used in suitable non-T cells. Such cells are those with an immune-effector function, such as, for example, NK cells, and T-like cells generated from pluripotent stem cells.

Also provided by an embodiment is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, *e.g.*, a host cell (*e.g.*, a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, *e.g.*, a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (*e.g.*, consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

Single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) (including functional portions and variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), can be isolated and/or purified. For example, a purified (or isolated) host cell preparation is one in which the host cell is more pure than cells in their natural environment within the body. Such host cells may be produced, for example, by standard purification techniques. In some embodiments, a preparation of a host cell is purified such that the host cell represents at least about 50%, for example at least about 70%, of the total cell content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

E. Methods of Treatment

It is contemplated that the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) used in the patient-specific autologous anti-tumor lymphocyte cell population(s) can be used in methods of treating or preventing a disease in a mammal. In this regard, an embodiment provides a method of treating or preventing cancer in a mammal, comprising administering to the mammal the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), the nucleic acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies and/or the antigen binding portions thereof, and/or the pharmaceutical compositions in an amount effective to treat or prevent cancer in the mammal. Additional methods of use of the aforementioned single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) have been disclosed *supra*.

An embodiment further comprises lymphodepleting the mammal prior to administering the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein. Examples of lymphodepletion include, but may not be limited to, nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy, total body irradiation, etc.

For purposes of the methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal. As used herein, allogeneic means any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically. As used herein, "autologous" means any material derived from the same individual to whom it is later to be re-introduced into the individual.

The mammal referred to herein can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). Preferably, the mammal is a human.

With respect to the methods, the cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bladder cancer (*e.g.*, bladder carcinoma), bone cancer, brain cancer (*e.g.*, medulloblastoma), breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, head and neck cancer (*e.g.*, head and neck squamous cell carcinoma), Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer (*e.g.*, non-small cell lung carcinoma and lung adenocarcinoma), lymphoma, mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, B-chronic lymphocytic leukemia (CLL), hairy cell leukemia, acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), and Burkitt's lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumors, synovial sarcoma, gastric cancer, testicular cancer, thyroid cancer, and ureter cancer.

The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods can provide any amount or any level of treatment or prevention of cancer in a mammal.

Furthermore, the treatment or prevention provided by the method can include treatment or prevention of one or more conditions or symptoms of the disease, *e.g.*, cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

Another embodiment provides a method of detecting the presence of cancer in a mammal, comprising: (a) contacting a sample comprising one or more cells from the mammal with the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), the nucleic

acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies, and/or the antigen binding portions thereof, or the pharmaceutical compositions, thereby forming a complex, (b) and detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the mammal.

The sample may be obtained by any suitable method, *e.g.*, biopsy or necropsy. A biopsy is the removal of tissue and/or cells from an individual. Such removal may be to collect tissue and/or cells from the individual in order to perform experimentation on the removed tissue and/or cells. This experimentation may include experiments to determine if the individual has and/or is suffering from a certain condition or disease-state. The condition or disease may be, *e.g.*, cancer.

With respect to an embodiment of the method of detecting the presence of a proliferative disorder, *e.g.*, cancer, in a mammal, the sample comprising cells of the mammal can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, *e.g.*, a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction. If the sample comprises whole cells, the cells can be any cells of the mammal, *e.g.*, the cells of any organ or tissue, including blood cells or endothelial cells.

The contacting can take place *in vitro* or *in vivo* with respect to the mammal. Preferably, the contacting is *in vitro*.

Also, detection of the complex can occur through any number of ways known in the art. For instance, the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (*e.g.*, alkaline phosphatase, horseradish peroxidase), and element particles (*e.g.*, gold particles) as disclosed *supra*.

Methods of testing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) for the ability to recognize target cells and for antigen specificity are known in the art. For instance, Clay *et al.*, J. Immunol, 163: 507-513 (1999), teaches methods of measuring the release of cytokines (*e.g.*, interferon-γ, granulocyte/monocyte colony stimulating factor (GM-CSF), tumor necrosis factor a (TNF-a) or interleukin 2 (IL-2)). In addition, single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) function can be evaluated by measurement of cellular cytotoxicity, as described in Zhao *et al.*, J. Immunol. 174: 4415-4423 (2005).

Another embodiment provides for the use of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), nucleic acids, recombinant expression vectors, host cells, populations of cells, antibodies, or antigen binding portions thereof, and/or pharmaceutical

compositions of the invention, for the treatment or prevention of a proliferative disorder, *e.g.*, cancer, in a mammal. The cancer may be any of the cancers described herein.

Any method of administration can be used for the disclosed therapeutic agents, including local and systemic administration. For example, topical, oral, intravascular such as intravenous, intramuscular, intraperitoneal, intranasal, intradermal, intrathecal and subcutaneous administration can be used. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (for example the subject, the disease, the disease state involved, and whether the treatment is prophylactic). In cases in which more than one agent or composition is being administered, one or more routes of administration may be used; for example, a chemotherapeutic agent may be administered orally and an antibody or antigen binding fragment or conjugate or composition may be administered intravenously. Methods of administration include injection for which the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) T Cell, conjugates, antibodies, antigen binding fragments, or compositions are provided in a nontoxic pharmaceutically acceptable carrier such as water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, fixed oils, ethyl oleate, or liposomes. In some embodiments, local administration of the disclosed compounds can be used, for instance by applying the antibody or antigen binding fragment to a region of tissue from which a tumor has been removed, or a region suspected of being prone to tumor development. In some embodiments, sustained intra-tumoral (or near-tumoral) release of the pharmaceutical preparation that includes a therapeutically effective amount of the antibody or antigen binding fragment may be beneficial. In other examples, the conjugate is applied as an eye drop topically to the cornea, or intravitreally into the eye.

The disclosed therapeutic agents can be formulated in unit dosage form suitable for individual administration of precise dosages. In addition, the disclosed therapeutic agents may be administered in a single dose or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of treatment may be with more than one separate dose, for instance 1-10 doses, followed by other doses given at subsequent time intervals as needed to maintain or reinforce the action of the compositions. Treatment can involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years. Thus, the dosage regime will also, at least in part, be determined based on the particular needs of the subject to be treated and will be dependent upon the judgment of the administering practitioner.

Typical dosages of the antibodies or conjugates can range from about 0.01 to about 30 mg/kg, such as from about 0.1 to about 10 mg/kg.

In particular examples, the subject is administered a therapeutic composition that includes one or more of the conjugates, antibodies, compositions, single, tandem, DuoCARs, multiple-targeting CARs

(with or without one or more boosting elements), single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) T cells or additional agents, on a multiple daily dosing schedule, such as at least two consecutive days, 10 consecutive days, and so forth, for example for a period of weeks, months, or years. In one example, the subject is administered the conjugates, antibodies, compositions or additional agents for a period of at least 30 days, such as at least 2 months, at least 4 months, at least 6 months, at least 12 months, at least 24 months, or at least 36 months.

In some embodiments, the disclosed methods include providing surgery, radiation therapy, and/or chemotherapeutics to the subject in combination with a disclosed antibody, antigen binding fragment, conjugate, single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) (for example, sequentially, substantially simultaneously, or simultaneously). Methods and therapeutic dosages of such agents and treatments are known to those skilled in the art, and can be determined by a skilled clinician. Preparation and dosing schedules for the additional agent may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, (1992) Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md.

In some embodiments, the combination therapy can include administration of a therapeutically effective amount of an additional cancer inhibitor to a subject. Non-limiting examples of additional therapeutic agents that can be used with the combination therapy include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and angiogenesis inhibitors. These agents (which are administered at a therapeutically effective amount) and treatments can be used alone or in combination. For example, any suitable anti-cancer or anti-angiogenic agent can be administered in combination with the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements)- T cells, antibodies, antigen binding fragment, or conjugates disclosed herein. Methods and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

Additional chemotherapeutic agents for combination immunotherapy include, but are not limited to alkylating agents, such as nitrogen mustards (for example, chlorambucil, chlormethine, cyclophosphamide, ifosfamide, and melphalan), nitrosoureas (for example, carmustine, fotemustine, lomustine, and streptozocin), platinum compounds (for example, carboplatin, cisplatin, oxaliplatin, and BBR3464), busulfan, dacarbazine, mechlorethamine, procarbazine, temozolomide, thiotepa, and uramustine; antimetabolites, such as folic acid (for example, methotrexate, pemetrexed, and raltitrexed), purine (for example, cladribine, clofarabine, fludarabine, mercaptopurine, and tioguanine), pyrimidine (for example,

capecitabine), cytarabine, fluorouracil, and gemcitabine; plant alkaloids, such as podophyllum (for example, etoposide, and teniposide), taxane (for example, docetaxel and paclitaxel), vinca (for example, vinblastine, vincristine, vindesine, and vinorelbine); cytotoxic/antitumor antibiotics, such as anthracycline family members (for example, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin), bleomycin, rifampicin, hydroxyurea, and mitomycin; topoisomerase inhibitors, such as topotecan and irinotecan; monoclonal antibodies, such as alemtuzumab, bevacizumab, cetuximab, gemtuzumab, rituximab, panitumumab, pertuzumab, and trastuzumab; photosensitizers, such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin; and other agents, such as alitretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase, axitinib, bexarotene, bevacizumab, bortezomib, celecoxib, denileukin diftitox, erlotinib, estramustine, gefitinib, hydroxycarbamide, imatinib, lapatinib, pazopanib, pentostatin, masoprocol, mitotane, pegaspargase, tamoxifen, sorafenib, sunitinib, vemurafinib, vandetanib, and tretinoin. Selection and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

In certain embodiments of the present invention, cells activated and expanded using the methods described herein, or other methods known in the art where T cells are expanded to therapeutic levels, are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu *et al.*, Cell 66:807-815, 1991; Henderson *et al.*, Immun 73:316-321, 1991; Bierer *et al.*, Curr. Opin. Immun 5:763-773, 1993). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the

present invention. In an additional embodiment, expanded cells are administered before or following surgery.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about 100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used.

The combination therapy may provide synergy and prove synergistic, that is, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation, a synergistic effect may be attained when the compounds are administered or delivered sequentially, for example by different injections in separate syringes. In general, during alternation, an effective dosage of each active ingredient is administered sequentially, *i.e.* serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

In one embodiment, an effective amount of an antibody or antigen binding fragment that specifically binds to one or more of the antigens disclosed herein or a conjugate thereof is administered to a subject having a tumor following anti-cancer treatment. After a sufficient amount of time has elapsed to allow for the administered antibody or antigen binding fragment or conjugate to form an immune complex with the antigen expressed on the respective cancer cell, the immune complex is detected. The presence (or absence) of the immune complex indicates the effectiveness of the treatment. For example, an increase in the immune complex compared to a control taken prior to the treatment indicates that the treatment is not effective, whereas a decrease in the immune complex compared to a control taken prior to the treatment indicates that the treatment is effective.

F. Biopharmaceutical Compositions

Biopharmaceutical or biologics compositions (hereinafter, "compositions") are provided herein for use in gene therapy, immunotherapy, adoptive immunotherapy, and/or cell therapy that include one or more of the disclosed single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibodies, antigen binding fragments, conjugates, single, tandem, DuoCARs,

multiple-targeting CARs (with or without one or more boosting elements), or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) that specifically bind to one or more antigens disclosed herein, in a carrier (such as a pharmaceutically acceptable carrier). The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating clinician to achieve the desired outcome. The compositions can be formulated for systemic (such as intravenous) or local (such as intra-tumor) administration. In one example, a disclosed single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibody, antigen binding fragment, conjugate, is formulated for parenteral administration, such as intravenous administration. Compositions including a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a conjugate, antibody or antigen binding fragment as disclosed herein are of use, for example, for the treatment and detection of a tumor, for example, and not by way of limitation, a neuroblastoma. In some examples, the compositions are useful for the treatment or detection of a carcinoma. The compositions including a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), a conjugate, antibody or antigen binding fragment as disclosed herein are also of use, for example, for the detection of pathological angiogenesis.

The compositions for administration can include a solution of the single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), conjugate, antibody or antigen binding fragment dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, adjuvant agents, and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibody or antigen binding fragment or conjugate in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs. Actual methods of preparing such

dosage forms for use in in gene therapy, immunotherapy and/or cell therapy are known, or will be apparent, to those skilled in the art.

A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995).

A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibodies, antigen binding fragments, or conjugates may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibody or antigen binding fragment or conjugate solution is then added to an infusion bag containing 0.9% sodium chloride, USP, and in some cases administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody or antigen binding fragment and conjugate drugs; for example, antibody drugs have been marketed in the U.S. since the approval of RITUXAN® in 1997. A single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibodies, antigen binding fragments and conjugates thereof can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg antibody or antigen binding fragment (or the corresponding dose of a conjugate including the antibody or antigen binding fragment) may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

Controlled release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules,

nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres, the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements), or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), antibody or antigen binding fragment or conjugate compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al., Pharm. Res.* 9:425-434, 1992; and Pec *et al., J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al., Int. J. Pharm.* 112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al., Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

G. Kits

In one aspect, Kits employing the single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) disclosed herein are also provided. For example, kits for treating a tumor in a subject, or making a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) T cell that expresses one or more of the single, tandem, DuoCARs, multiple-

targeting CARs (with or without one or more boosting elements) disclosed herein. The kits will typically include a disclosed antibody, antigen binding fragment, conjugate, nucleic acid molecule, single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) or T cell expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) as disclosed herein. More than one of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) can be included in the kit.

The kit can include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements). In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition.

The label or package insert typically will further include instructions for use of a disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, single, tandem, DuoCARs, multiple-targeting CARs (with or without one or more boosting elements) or T cells expressing a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements), for example, in a method of treating or preventing a tumor or of making a single, tandem, DuoCAR, multiple-targeting CAR (with or without one or more boosting elements) T cell. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

EXAMPLES

This invention is further illustrated by the examples of the CARs depicted within the accompanying Figures *infra* and the disclosure at pages 14-19, inclusive *supra*, which examples are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

While various details have been described in conjunction with the exemplary implementations outlined above, various alternatives, modifications, variations, improvements, and/or substantial equivalents, whether known or that are or may be presently unforeseen, may become apparent upon reviewing the foregoing disclosure.

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each issued patent; "application cited documents"), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference, and may be employed in the practice of the invention. More generally, documents or references are cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein cited references"), as well as each document or reference cited in each of the herein cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference.

The foregoing description of some specific embodiments provides sufficient information that others can, by applying current knowledge, readily modify or adapt for various applications such specific embodiments without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. In the drawings and the description, there have been disclosed exemplary embodiments and, although specific terms may have been employed, they are unless otherwise stated used in a generic and descriptive sense only and not for purposes of limitation, the scope of the claims therefore not being so limited. Moreover, one skilled in the art will appreciate that certain steps of the methods discussed herein may be sequenced in alternative order or steps may be combined. Therefore, it is intended that the appended claims not be limited to the particular embodiment disclosed herein. Those skilled in the art will recognize, or be able to ascertain using no more than routine

experimentation, many equivalents to the embodiments of the invention described herein. Such equivalents are encompassed by the following claims.

DESCRIPTION OF EXAMPLES

Two examples are provided whereby CAR T cells in a single, tandem or multi-cistronic DuoCAR format with or without boosting elements are described. Example 1 describes the generation and in vitro evaluation of boosted CAR T cells targeting MSLN and/or ROR1 antigen for the treatment solid tumors. Example 2 describes the evaluation of the anti-tumor function of the ROR1 and MSLN-targeting CAR T cells in a mouse tumor xenograft model.

EXAMPLE 1

Development of ROR1 and/or MSLN Targeting CAR Constructs With Boosting Elements

Materials and Methods

Cell lines

The ovarian cancer cell line OVCAR3, lung squamous cell carcinoma cell line NCI-H226, pancreatic cancer cell lines CAPAN-2 and AsPC-1, and leukemia cell line HL-60 were purchased from American Tissue Culture Collection (ATCC, Manassas, VA). The MEC-1 leukemia line was purchased from DSMZ (Leibniz Institute DSMZ, Braunschweig, Germany). NCI-H226 and AsPC-1 were cultivated in RPMI-1640 medium (Corning, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT). OVCAR-3 was cultured in RPMI-1640 medium(Corning, NY) supplemented with 20% heat-inactivated FBS and 10 μg/ml bovine insulin (Sigma, St Louis, MO). CAPAN-2 were propagated in McCoy-5a (ATCC, VA) supplemented with 10% heat-inactivated FBS. HL-60 was maintained in IMDM (Hyclone, Logan, UT) with 20% FBS. MEC-1 cell line and its derivatives were maintained in IMDM supplemented with 10% FBS.

OVCAR-3, NCI-H226 and HL60 luciferase expressing cell lines were generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase, followed by limiting dilution and selection of luciferase-positive clones. Capan-2 single clone of luciferase and GFP expressing cell line was generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase and GFP connected with 2A peptide (Lentigen Technology, Inc., Gaithersburg, MD), followed

by selection of luciferase-positive clones. MEC-1 ROR1^{hi} MSLN^{hi} cells were generated by stable transduction with lentivirus encoding ROR1 or MLSN gene, followed by microbeads selection and Tyto sorting (Miltenyi Biotec) for ROR1 or MSLN positivity.

Generation of CAR constructs and Lentiviral Vector production

The constructs of fully human anti-ROR1 and/or MSLN chimeric antigen receptor (CAR) with boosting elements were designed as CAR molecule and a booster molecule connected with P2A ribosomal skipping element sequence. CAR molecules included mono- and multi- targeting CAR. The various single chain variable fragment (ScFv) sequences targeting the extracellular domain of human ROR1 or MSLN were identified in house, the R12 ScFv targeting ROR1 was used as CAR ROR1 control. Mono CAR comprised of an antiROR1 or anti MSLN scFv, a IgG4 short hinge for ROR1 scFv, a CD8 hinge for MSLN scFv, connected to CD8 or OX40 transmembrane domain, costimulatory domain(s) derived from human ICOS,CD28, OX40 and 4-1BB, followed by CD3-ζ activating domain sequences. Multi-targeting CARs denoted tandem CARs and DuoCARs. Tandem CARs comprised of a MSLN targeting scFv connected with ROR1scFv9 via G4S linker, followed by IgG4 hinge, CD8 or CD28 transmembrane, 4-1BB or CD28_4-1BBcostimulatory domain(s), and CD3-ζ activating domain sequences. Bicistronic CARs contained a ROR1-targeting mono CAR, and a MSLN-targeting mono CAR, connected with P2A sequence. Boosting elements various from cytokines (membrane bound IL7), armors (TGFβRIIdn), suicide tag (tEGFR), extracellular matrix enzymes, chemokine receptors (CXCL8, CCL2), stroma targeting molecules (FAP), et al. ROR1 or MSLN mono CARs and MSLN_ROR1 tandem CARs without boosters were included as comparison.

CAR sequences were cloned into a Lentiviral Vector (LV) expression cassette under the control of the human EF-1α promoter or MND promoter (Lentigen Technology Inc., Gaithersburg, MD). Lentiviral particles were generated by transient transfection of HEK 293T cells, pelleted by centrifugation and stored at -80°C until transduction.

Primary T cell preparation and transduction

Healthy donor primary T cells were isolated from leukapheresis collections (AllCells, Alameda, CA) or from processed buffy coats (Oklahoma Blood Institute, Tulsa, OK), with donors' written consent. The CD4-positive and CD8-positive human T cells were purified via positive selection using a 1:1 mixture of CD4 and CD8 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Purified T cells were activated with CD3/CD28 MACS® GMP T Cell TransAct reagent (Miltenyi Biotec), and cultured in serum free TexMACS medium supplemented with 30 IU/ml IL-2 at a density of 1x 10^6 cells/ml. Further, activated T cells were transduced on day 1 with lentiviral vector particles encoding

CAR constructs. On day 3, the transduced T cells were washed and resuspended to 0.5 x 10^6/ml to continue expansion. Every 2-3 days thereafter, cultures were supplemented with fresh TexMACS medium containing 30 IU/ml IL-2, until harvesting time on day 8-10.

Flow cytometric analysis of CAR surface expression

Half million CAR T cells were washed in cold AutoMACS buffer supplemented with 0.5% bovine serum albumin (Miltenyi Biotec, Bergisch Gladbach, Germany) and stained with CAR detection reagents. For ROR CAR, cells were stained with ROR1-Fc peptide (R&D System, Minneapolis, MN), followed by anti Fc-AF647 conjugate(Jackson ImmunoResearch, West Grove, PA). For MSLN CAR, cells were stained with MSLN-His (R&D System, Minneapolis, MN) or anti-His-APC (Miltenyi Biotec). The 7-Aminoactinomycin D staining (7-AAD, BD Biosciences, San Jose, CA) was added to exclude dead cells. CD4 antibody labeled with VioBlue fluorochrome or CD8 antibody labeled with VioGreen fluorochrome was used to separate CD4 and CD8 population. Non-transduced cells (UTD) were used as a negative control. Cells were washed twice, resuspended in 200 µl running buffer, and acquired by flow cytometry. Flow cytometric analysis was performed on a MACSQuant® 10 Analyzer (Miltenyi Biotec), and data plots were generated using FlowJo software (Ashland, OR).

CAR T cell cytotoxicity and cytokine assay

To assess CAR T cell mediated cytotoxicity, 5x10^3 tumor target cells stably transduced with firefly luciferase were combined with CAR T cells at the indicated effector to target ratios and incubated overnight at 37°C with 5% CO_2. SteadyGlo reagent (Promega, Madison WI) was added to each well and the resulting luminescence quantified as counts per second (sample CPS). Target only wells (max CPS) and target only wells plus 1% Tween-20 (min CPS) were used to determine assay range. Percent specific lysis was calculated as: (1-(sample CPS-min CPS)/(max CPS-min CPS)). For cytokine release analysis, supernatants from overnight co-cultures were collected and analyzed by ELISA (eBioscience, San Diego, CA) for IFNγ, TNFα and IL-2 concentration. Two technical replicates were performed for each condition, and each experiment was repeated using CAR T cells generated from different healthy donors as indicated.

CAR T cell mediated cytotoxicity were evaluated using xCelligence RTCA instrument (Agilent, Santa Clara, CA) following manufactory instruction. Briefly, 4x10^4 AsPC-1 tumor target cells were seeded in a 96/E-Plate, incubated in the cradle of xCelligence RTCA instrument at 37°C with 5% CO_2 for overnight. Effector CAR T cells were added into the plate at E T ratio 2:1 when target cells index reached or exceeded 1. Cell index was continuously monitored for desired assay time. Percentage of cytolysis was calculated by RTCA software as Cytolysis (sample) % = (1-normalized sample index/reference average normalized index)x100. The time required to reach 50 % of the maximal killing of tumor cells was reported

as KT50. In select experiments, TGFβ was spiked into the co-incubated cultures at the onset of experiment, at the concentrations indicated.

IL-2 withdrawal assay

Transduced CAR T cells were washed and seeded at density of 1e6 cells/ml in TexMACS medium without IL-2 supplement. Cell growth, viability and diameters were assessed weekly by Vicell counter, and fresh medium supplied as needed. Cell density was adjusted to 1e6/ml or as is. The end point of each construct was determined as no cell expansion detected and cell counts dropped continuously over 2-3 weeks.

Western blotting

Ten million CAR T cells were washed with cold PBS (Lonza, Walkersville, MD), then lysed in 100 µl cold RIPA buffer containing a protease inhibitor cocktail (Thermo-Fisher Scientific, Grand Island, NY). The lysate was incubated at 4 °C for 1hr, pelleted at 21000 g in a table top centrifuge at 4 °C for 15 min. Supernatants were collected and protein concentration was quantified using SBS standard following the Quick Start Bradford Protein Assay (Bio-Rad). Cell lysate were aliquoted and frozen at -80 °C. Samples were denatured at 90 °C in Laemmli sample buffer (Bio-Rad) with 50 mM DTT for 5 min and resolved on 4–12% gradient SDS-PAGE gel under reducing conditions in SDS running buffer Proteins were transferred to 0.45 µm nitrocellulose transfer membrane (BioRad, Hercules, CA) and blocked in the washing buffer containing 5% nonfat milk at room temperature for 1 hr. After blocking, membrane was probed with antibody against IL7 (Thermo-Fisher Scientific), and followed by goat anti mouse IgG HRP conjugated second antibody(Abcam, Cambridge, UK). Bands were developed using West Femto detection kit (ThermoFisher Scientific) according to manufacturer's protocol and bands were visualized and quantified on an Odyssey imaging system with Image Studio lite software (LI-COR, Lincoln, Nebraska). The blot with GAPDH antibody and goat anti rabbit IgG HRP conjugated second antibody (Abcam) was included as loading control.

HPSE ELISA

To confirm HPSE expression, CAR-T cells were seeded in the absence of IL-2 and supernatant was collected after 2 days and analyzed by ELISA (Abcam, Cambridge, UK). Two technical replicates were performed for each dilution.

Trans-well assay

To assess ECM degradation *in vitro*, CAR-T cells were seeded in Cultrex™ BME-coated transwell inserts (Corning Life Sciences), and migration into the reciprocal chamber was measured. Corning® BioCoat® control inserts (8.0 μm PET membrane) in 24-well plate formats were uncoated or coated with 100 μL Cultrex™ BME (R&D Systems, Minneapolis, MN) at 5 mg/mL diluted in 0.01M Tris-HCl pH 8.0, 0.7% NaCl. Coated transwell inserts were allowed to solidify for 2h at 37C. CAR-T cells were thawed and resuspended in TexMACS medium. 0.5E6 cells were seeded in each transwell insert (500 μL volume). TexMACS medium with 5% FBS was used as a chemoattractant in the bottom chamber (750 μL volume). Cells that had migrated into the bottom chamber after 24h were collected, washed and processed for flow cytometry. Cell counts were normalized to Absolute counting beads (Invitrogen, Waltham, MA).

Results

Example 1 describes the generation and *in vitro* evaluation of boosted CAR T cells targeting MSLN and/or ROR1 antigen for the treatment solid tumors.

Boosted CAR was designed to enhance the functionality of ROR1 and/or MSLN CAR. Schematic representations of the boosted CAR constructs are shown in Figure 1. Boosted CAR comprised of a CAR molecule, in frame to a boosting element linked by P2A ribosomal skipping element sequence. CAR molecule denoted to mono CAR, tandem CAR and DuoCAR structure. Fully human binders scFv4, scFv9 targeting ROR1 and anti MSLN scFv were developed in house, ROR1 R12 scFv was included as well. Mono CAR configured with a scFv targeting ROR1 or MSLN, in frame to IgG4 or CD8 hinge, CD8 or OX40 transmembrane, 41-BB, OX40, CD28, ICOS, costimulatory and a CD3ζ activation domain. Tandem CARs designed as a MSLN scFv connected with ROR1 scFv 9 through G4S linker, followed by IgG4 hinge, CD8 or CD28 transmembrane region, 4-1BB or CD28_4-1BB co-stimulatory domain and CD3ζ activation domain. DuoCAR constructs comprised of a mono ROR1 CAR and a mono MSLN CAR separated by P2A sequence. Booster elements in this example include a cytokine (membrane bound IL7), an armor (TGFβRIIdn), a suicide tag (tEGFR), and extracellular matrix enzymes.

Table 1 listed designated MSLN CAR and ROR1 CAR and booster CAR constructs.

Table1 ROR1 and/or MSLN CAR constructs

	Construct Number	Construct designation	Promoter
Single CAR	LTG2527	ROR1 R12 -IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	LTG2528	ROR1 scFv 4-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	LTG2529	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	D0181	MSLN-CD8H&TM-41BB-CD3ζ	EF1α
Single CAR with booster(s)	D0229	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_mIL7	EF1α
	D0245	MSLN-CD8H&TM-41BB-CD3ζ_2A_mIL7	EF1α
	D0284	MSLN-CD8H&TM-CD28-CD3ζ_2A_mIL7	EF1α
	D0228	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_ TGFβRII dn	EF1α
	D0211	MSLN-CD8H&TM-41BB-CD3ζ_2A_TGFβRII dn	EF1α
	D0231	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_ tEGFR	EF1α
	D0246	MSLN-CD8H&TM-41BB-CD3ζ_2A_mIL7_2A_tEGFR	EF1α
	D0347	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_HPSE	EF1α
	D0348	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_MMP2	EF1α
	D0349	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_PH20	EF1α
	D0344	MSLN-CD8H&TM-41BB-CD3ζ_2A_HPSE	EF1α
	D0345	MSLN-CD8H&TM-41BB-CD3ζ_2A_MMP2	EF1α
	D0346	MSLN-CD8H&TM-41BB-CD3ζ_2A_PH20	EF1α
Tandem CAR	D0233	MSLN-ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
Tandem CAR	D0358	MSLN-ROR1 scFv9-IgG4 hinge-CD28TM-CD28-41BB-CD3ζ	MND
Tandem CAR with booster(s)	D0279	MSLN-ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_mIL7	EF1α
	D0280	MSLN-ROR1 scFv9-IgG4 hinge-CD28TM-CD28-41BB-CD3ζ _ 2A_mIL7	EF1α
	D0281	MSLN-ROR1 scFv9-IgG4 hinge-CD8TM-CD28-41BB-CD3ζ _ 2A_mIL7	EF1α
DuoCAR with booster(s)	D0282	ROR1scFv9-IgG4H-OX40TM-OX40-CD3ζ_2A_MSLN-CD8H& TM-ICOS-CD3ζ_2A_mIL7	EF1α
	D0283	ROR1scFv9-IgG4H-CD8TM-41BB-CD3ζ_2A_MSLN-CD8H& TM-CD28-CD3ζ_2A_mIL7	EF1α
Tandem CAR with booster(s)	D0355	MSLN-ROR1 scFv9-IgG4 hinge-CD28TM-CD28-41BB-CD3ζ_ 2A_mIL7	MND
DuoCAR with booster(s)	D0356	ROR1scFv9-IgG4H-OX40TM-OX40-CD3ζ_2A_MSLN-CD8H& TM-ICOS-CD3ζ_2A_mIL7	MND
	D0357	ROR1scFv9-IgG4H-CD8TM-41BB-CD3ζ_2A_MSLN-CD8H& TM-CD28-CD3ζ_2A_mIL7	MND

To prove the concept of boosted CAR functionality, ROR1 or MSLN mono CARs, MSLN_ROR1 tandem CARs and MSLN/ROR1 DuoCARs with membrane bound IL7 (mIL7) as booster element were characterized *in vitro*. ROR1 and MSLN mono CARs and tandem CARs without mIL7 were included as control (Figure 2A). CAR sequences were further incorporated into a third-generation lentiviral vectors and transduced into human primary T cells at MOI 40, to generate the ROR1 and/or MSLN CAR T cells under the control of the mammalian EF-1α promoter. Un-transduced T cells derived from same donor (UTD) were used as negative control. Surface expression of CARs with ROR1 binder on transduced T cells was measured by flow cytometry using ROR1-Fc, followed by staining with anti-Fc Alexa Flour 647. CARs with MSLN scFv surface expression was detected by MSLN-His followed by anti-His APC. Different CAR

constructs exhibited reasonable surface expression than un-transduced T cells (n=2). Quantified CAR positive percentage and mean fluorescence intensity (MFI) of ROR1 binders (Figure 2B, 2D) and MSLN binders (Figure 2C, 2E) were plotted as bar figure. For this donor, ROR1 CAR LTG2529 and IL7 boosted ROR1 CAR D0229, MSLN CAR D0181 and booster MSLNCAR D0245 showed similar percentage of CAR positivity and MFI, suggested mIL7 has negative impact on CAR expression. Tandem CARs with and without booster and DuoCARs with booster exhibited robust expression (30% to 50%). These results demonstrate high transduction efficiency and CAR expression.

To evaluate the target-specific cytotoxicity of ROR1 and/or MSLN CARs *in vitro*, $ROR1^{+}MSLN^{+}$ ovarian cancer line OVCAR-3, lung cancer line NCI-H226, pancreatic cancer line Capan-2, and $ROR1^{-}$ $MSLN^{-}$ leukemic lines HL-60 were selected as target lines. CAR-T cells were co-incubated with target tumor cell lines at 10 different effector to target (ET) ratios. After overnight co-incubation, cytotoxicity of CARs was analyzed in a luminescence based *in vitro* killing assays. Percentage of specific lysis was plotted with ET ratio using non-linear curve fit. Complete killing curve of OVCAR-3 and HL60 were shown in Figure 3. CARs with ROR scFv showed similar or higher killing capacity, compared to mono ROR1 CAR LTG2529, at all ET ratio tested (Figure 3A). In contrast, tandem or DuoCARs with MSLN scFv outperformed mono MSLN CAR D0181 and mono MSLN booster CAR D0245 (Figure 3B). UTD non-specific killing was noticed at high ET, due to a greater sensitivity of these tumor lines toward human T cells. Furthermore, no killing or limited background killing of HL-60 cell line, which is negative for the expression of ROR1 and MSLN, was observed in CAR T or UTD control groups (Figure 3C), confirming that the cytotoxicity of ROR1 and MSLN CARs is target-specific.

Relative potency was calculated using EC50 function in GraphPad Prism. As normalized to ROR1 CAR LTG2529, the relative potencies of all tested constructs targeting OVCAR-3 (Figure 4A), NCI-H226 (Figure 4B), and Capan-2 (Figure 4C) are shown. All boosted CARs revealed similar or higher potency *in vitro* as compared to their non-boosted CAR counterparts, including ROR1 CAR LTG2529 vs boosted CAR D0229, MSLN CAR D0181 vs boosted CARs D0245, or D0284; non-boosted tandem CAR D0233 vs boosted CARs D0279, D0280 and D0281.

Production of the T cell homeostatic and pro-inflammatory cytokines IL-2, IFN-γ, and TNF-α by the fully human ROR1 and/or MSLN CARs, was examined by ELISA. Culture supernatants after overnight co-incubation of CAR T cells with NCI-H226 target line (Figure 5A-AC), were harvested for the measurement of cytokines elaboration by CAR T cells. Fully human ROR1 and/or MSLN CAR with or without boosting element exhibited CAR T cell dose dependent cytokine response when compared to UTD, indicating robust, target-specific CAR T cell cytokine responses. The intensity of cytokine release of duo ROR1/MSLN booster CAR constructs tend to be the highest for all three cytokines.

Western blotting was performed to further verify the expression of mIL7 in booster CARs. Human primary T cells were transduced with CAR constructs at MOI 10. Five million transduced T cells were harvested and lysed for western blot using IL7 antibody. GAPDH was included as loading control. Boosted CARs demonstrated mIL7 overexpression as compared to controls (Figure 6A). Boosted MSLN mono CARs D0245 and D0284 showed the strongest expression among the boosted CAR constructs. To further assess the functionality of mIL7 to support T cells growth, transduced T cells were cultured with TexMACS medium without IL-2 supplement at density of 1e6/ml. Cell expansion (Figure 6B) and cell size (Figure 6C) were monitored periodically. CAR T cells without mIL7, shown on the left in Figures 6B and 6C, did not expand and cell size dropped immediately. As contrast, mIL7 boosted CAR T cells, shown in the middle and right panels, remained in activated state and continued proliferating. At the last time point on day 82 after IL-2 withdrawal, all boosted CAR T cells populations contracted from the peak expansion and returned to quiescent state. To evaluate the boosted CAR T cell cytotoxicity after IL-2 withdrawal, the boosted MSLN CAR with D0245 and boosted Duo CAR D0282 were maintained in IL-2-depleted TexMACS medium for 67 days, then cocultured with pancreatic cancer cell line AsPC1. MSLN CAR D0181, booster CAR D0245 and D0282 without IL-2 withdrawal were included as comparison. Target cell killing was monitored for 3 days. The killing time reached 50% target cell lysis (KT50) and relative potency based on CAR D0181 was calculated (Figure 7A and 7B). Despite 67 days of IL-2 withdrawal, the boosted CARs D0245 and D0282 maintained their cytotoxic potency. Therefore, mIL7 in boosted CARs supported CAR T cell homeostasis and functionality in the absence of IL-2. Transforming growth factor beta (TGF-β) is a multifunction cytokine, which may play important role in the immunosuppressive tumor microenvironment (TME).

The dominant negative TGFβ receptor II (TGFβRIIdn) was designed as booster element to enhance CAR T cell functionality to against inhibitory TME. To evaluate the *in vitro* function of TGFβRIIdn , CAR MSLN D0181 and TGFβRIIdn armored CAR MSLN D0211 (Figure 8A) were transduced with primary human T cells, surface expression of CAR MSLN and TGFβRIIdn were examined by flow cytometry. Armored CAR D0211 effectively transduced and expressed on the primary T cell surface with 81.2% MSLN CAR positivity, and TGFβRII showed robust but weaker (32.3%) compared to CAR expression (Figure 8B). Target specific cytotoxicity of MSLN CAR without TGFβRIIdn was assessed with $MSLN^+$ cell lines NCI-H226, A431-MSLN, $MSLN^-$ cell line A431 was included as control. Both MSLN CAR D0181 and armed CAR D0211 exhibited effective killing potency during coculture with NCI-H226 and A431-MSLN (Figure 8C). Non-specific killing towards $MSLN^-$ A431 lines was noticed at very high E T ratios, possibly due to allo-reactivity, for both MSLN CAR constructs, while CAR D0211 showed less non-specific killing. The supernatant of overnight coculture of NCH-H226 and CAR T cells were used to exam T cell homeostatic and pro-inflammatory cytokines IFNγ, and TNFα. Armed CAR D0211 demonstrated

target specific cytokine release at a similar level as CAR D0181 (Figure 8D). The protective activity of the TGFBRIIdn element was investigated in the context of MLSLN and ROR1 CAR T cell constructs. For each target, a CAR alone (ROR1 LTG2529 and MSLN D0181), or the TGFBRIIdn armored CAR (ROR1 D0228, MSLN D0211) were included. CAR T cells were combined with AsPC-1 pancreatic tumor cells, which are MSLN-positive and ROR1-positive, for a kinetic co-culture assay (xCELLigence RTCA) either in the absence of TGFβ, or in the presence of TGFβ1 at concentration of 1 ng/ml, 3 ng/ml, or 9 ng/ml (Figure 8E). While the cytotoxic activity of the non-armored CARs was impeded by TGFβ in concentration-dependent manner, as indicated by KT50 (time to kill 50% of all tumor cells) and reduced relative potency, the armored CAR T cells sustained their cytotoxic function in the presence of TGFβ. These results underscore the functionality of the TGFβRIIdn boosting element in CAR T cells.

In an additional experiment, ROR CAR LTG2529 and TGFβRIIdn armed ROR CAR D0228 were also used for evaluating TGFβRIIdn effect *in vitro*. Primary human T cells were transduced by MOI40 and MOI 80, ROR1 scFv positivity was detected by flow. At both MOI, TGFβRIIdn armed ROR1 CAR D0228 demonstrated higher transduction efficiency compared to ROR1 CAR LTG2529 (Figure 9A). When co incubation with $ROR1^+$ target lines OVCAR3 (Figure9B), CAPAN-2 (Figure 9C), NCI_H226 (Figure 9D), boosted CAR D0228 exhibited comparable target specific cytolysis as ROR1 CAR LTG2529. The results suggested co-expression of TGFβRIIdn has no negative impact to CAR functionality.

One alternative approach to boost CAR-T therapy is by targeting the ECM components in solid tumors via co-expression of ECM enzymes. Schematic representations of the boosted CAR constructs are shown in Figure 10A. CAR + ECM enzymes comprised of MSLN/ROR1 CAR molecule, in frame to an extracellular matrix enzyme linked by P2A ribosomal skipping element sequence. Fully human binders scFv9 targeting ROR1 and anti MSLN scFv were developed in house. Mono CAR configured with a scFv targeting ROR1 or MSLN, in frame to IgG4 or CD8 hinge, CD8 transmembrane, 41-BB, costimulatory and a CD3ζ activation domain. ECM enzymes included in this set are heparanase (HPSE), matrix metalloproteinase-2 (MMP-2), or secreted hyaluronan (sPH-20 IgG1 Fc).

To test CAR-T functionality *in vitro*, ROR1 or MSLN mono CARs with HPSE, MMP-2 or sPH-20 as booster elements were characterized. ROR1 and MSLN CAR +/- ECM enzymes were further incorporated into a third-generation lentiviral vectors and transduced into human primary T cells at MOI 40, to generate the ROR1 and/or MSLN CAR T cells under the control of the mammalian EF-1α promoter. Un-transduced T cells derived from same donor (UTD) were used as negative control. Surface expression of CARs with ROR1 binder on transduced T cells was measured by flow cytometry using ROR1-Fc, followed by staining with anti-Fc Alexa Flour 647. CARs with MSLN scFv surface expression was detected by MSLN-His followed by anti-His APC. Different CAR constructs exhibited reasonable surface

expression than un-transduced T cells. Quantified CAR positive percentage of ROR1 binders and MSLN binders (Figure 10B) were plotted as a quadratic plot of CD4 vs. CAR. For this donor, CARs co-expressing HPSE or MMP-2 had similar CAR expression to that of CARs alone (62.5-84.3%). CARs co-expressing sPH-20 had reduced expression compared to that of CAR alone (39-44%). This could be due to the large payload size of the sPH-20. These results demonstrate effective transduction efficiency and CAR expression.

To evaluate the target specific cytotoxicity of ROR1 and/or MSLN CARs *in vitro*, MEC-1 overexpressing $ROR1^{+}MSLN^{+}$ B cell line, lung cancer line NCI-H226, and $ROR1^{-}MSLN^{-}$ leukemic lines HL-60 and MEC-1 were selected as target lines. CAR-T cells were co-incubated with target tumor cell lines at effector to target ratios 10, 5, 1.25:1. After overnight co-incubation, cytotoxicity of CARs was analyzed in a luminescence based *in vitro* killing assays. Percentage of specific lysis was plotted with E:T ratio using bar graphs (Figure 11 A-D). CARs with HPSE (D0344, D0347) showed similar killing capacity, compared to mono ROR1 or MSLN CAR, at all E:T ratio tested, suggesting that HPSE addition does not reduce CAR potency (Figure 11A, C). In contrast, CAR + MMP-2 (D0345, D0348) had slightly less killing and CAR + sPH-20 (D0346, D0349) killing was marginal compared to CAR alone (Figure 11A, C). Furthermore, no killing of ROR1 and MSLN negative HL-60 and MEC-1 cell lines by CAR T cells, as compared to the negative control UTD, was observed (Figure 11B, D), demonstrating the robust target-specific cytotoxic function of all ROR1 and/or MLSN CAR constructs designed.

Production of the HPSE enzyme by the fully human ROR1 and/or MSLN CARs (D0347, D0344), was examined by ELISA. CAR-T supernatants after 2d in culture without IL-2 was harvested for the measurement of specific cytokine release. Fully human ROR1 and/or MSLN CAR with HPSE was observed when supernatants were diluted 5 or 10 fold (Figure 12 A). In comparison, no HPSE was observed in UTD or mono CAR-T cells alone (D0181, D0290). These results suggest that CARs co-expressing HPSE have robust CAR and enzyme expression (Figures 10B and 12A).

As a measure of CAR-T migration through an ECM-rich environment *in vitro*, CAR-T were subjected to an invasion assay in transwell inserts coated with Cultrex™ BME. UTD, CAR alone, or CARs bicistronically expressing HPSE were thawed, counted, and seeded into uncoated or Cultrex™-coated transwell inserts for 24h (in the absence of IL-2). Medium in the bottom chamber was then collected, washed and processed via flow cytometry and normalized to Absolute counting beads. As shown in Figure 12B, ROR1 or MSLN CARs co-expressing HPSE (D0347 or D0344) had greater migration in 5mg/mL Cultrex™-coated transwell inserts than that of CARs alone (D0290, D0181). The results discussed here illustrate that CARs co-expressing HPSE can functionally by-pass an ECM better than CARs alone.

In summary, ROR1 and/or MSLN CARs constructs with mIL7 booster demonstrated reproducible and robust transduction efficiency, comparable cytotoxic function as their non-boosted CAR counterparts,

and specific cytokine induction *in vitro* during coculture with target cells. In the absence of IL-2, the expression of mIL7 from boosted CARs extended CAR T cell survival and preserved cytotoxic function.

EXAMPLE 2

Evaluation of the Anti-Tumor Function of ROR1 or MSLN Targeting CAR T Cells in an Ovarian Mouse Tumor Xenograft Model

Materials and methods

In vivo analysis of CAR T function in JeKo-1 and OVCAR3 xenograft model

Animal experiments were performed in compliance with the applicable laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of MI Bioresearch (Ann Arbor, MI) Animal Care and Use Committee.

In JeKo-1 xenograft model, the function of ROR1 or MSLN targeting CAR T cells was evaluated in NSG (NOD.Cg-Prkdcs^{cid}IL-2rg^{tm1Wjl}/SzJ) mice *in vivo* using OVCAR-3 ovarian cancer cells. Six to eight week old female NSG mice, 5 per group, were injected intraperitoneally with 5 x 10^5 Jeko-1 ROR1$^+$MSLN$^-$ mantel cell lymphoma cancer cells on day 0. Tumor burden was measured using IVIS bioluminescent imaging by IVIS-S5 instrument (Perkin Elmer, Waltham, MA). On day 7, mice were randomized into groups to achieve equal or similar overall mean tumor burden, and 5.0 × 10^6 CAR T$^+$ cells/mouse (normalized for transduction efficiency) were administered via tail vain at same day. Tumor regression was determined by bioluminescent imaging (BLI) at day13, 20, 27, 34, 41, and 48. Mouse weights were monitored three times/week.

In OVCAR-3 ovarian cancer model, six to eight week old female NSG mice, 5 per group, were injected intraperitoneally with 1 x 10^7 OVCAR-3 ROR1$^+$MSLN$^+$ ovarian cancer cells on day 0. Tumor burden was determined by IVIS bioluminescent imaging. On day 7, mice were randomized to groups based on equal or similar overall mean tumor burden, and 5.0 × 10^6 CAR T$^+$ cells/mouse (normalized for transduction efficiency) were administered via tail vain. Tumor regression was determined by bioluminescent imaging on days 10, 17, 24, 31, 38, 45, 52 using IVIS-S5. Animal body weights were recorded three times per week. Bioluminescent images were analyzed using Living Image, version 4.3, software (Perkin Elmer) and the bioluminescent signal flux for each mouse was expressed as average radiance.

Results

JeKo-1 mantle cell lymphoma NSG xenograft model was used to evaluate the *in vivo* tumor rejection functionality of the CAR ROR1 candidates LTG2527, LTG2528, and LTG2529. $ROR1^+$ $MSLN^-$ JeKo-1 cells were stably transduced with lentiviral vector encoding luciferase. Half a million JeKo-1 tumor cells were injected intravenously (i.v.) into each NSG mouse. At day 6, tumor burden was measured by IVIS imaging and mice were randomized into each group to achieve similar mean tumor burden. ROR1 CAR constructs, LTG2527, LTG2528, LTG2529, as well as non-related CAR MSLN D0181 were included in the study. Mice inoculated with un-transduced T cells (UTD) from same donor, and untreated mice groups served as controls. At day 7, 5×10^6 human CAR+ T cells or UTD cells were administered by i.v. injection. Tumor growth was measured and quantified by in vivo imaging system (IVIS) at the denoted time points (Figures 14A and 14B). ROR1 CAR constructs LTG2527 and LTG2529 showed robust tumor rejection starting at day 13, and the remission was maintained until the study termination. ROR1 CAR LTG2528 controlled tumor progression at day13. However, two mice relapsed on day27. In contrast, tumors progressed rapidly in tumor alone (TA), UTD and MSLN CAR D0181 control groups. All mice in the ROR1 CAR T treated group survived until day 50 without significant body weight loss (Figure 15), thus no ROR1 CAR-related toxicity was detected in this model.

To further assess the tumor rejection functionality of CAR constructs in solid tumor xenograft models, the ROR1-positive MSLN-positive OVCAR-3 ovarian cancer cell line was stably transduced with luciferase gene and intraperitoneally implanted into female NSG mice to establish the OVCAR-3 xenograft model. CAR MSLN D0181 and ROR1 CARs LTG2527, LTG2528, and LTG2529, were included in the study, whereas mice dosed with donor-matched UTD cells, and untreated mice served as control groups. Ten million OVCAR-3 tumor cells were injected into each NSG mouse. Mice were distributed into experimental groups based on similar tumor burden measured by IVIS imaging on day 6. Five million human CAR+ T cells or UTD cells were administrated by i.v. injection at day 7. Tumor growth kinetics was recorded weekly (Figure 15A and 15B). Only in ROR1 CAR LTG2529 treated group, tumor cells were strongly rejected, and this effect was maintained until the study termination on day 52. All other CAR constructs, including ROR1 CAR LTG2527, LTG2528 and MSLN CAR D0181, failed to control the OVCAR3 tumor growth, similarly to the untreated TA group. Two mice in UTD treated group showed tumor regression after study day 45, which may due to grafts vs tumor effect of donor T cells. Body weights of enrolled animals were monitored three times a week. As shown in Figure17, ROR1 CAR LTG2529 treated group did not lose weight throughout the study, while other groups showed lower body weight compared to the body weight of the study initiation. This results effective tumor rejection and lack of demonstrates overt toxicity of the ROR1 CAR LTG2529.

In summary, ROR1 CAR LTG2529 efficiently eliminated tumors in JeKo-1 and OVCAR-3 NSG

xenografts, representing the hematologic (MCL) and solid (ovarian) tumors, respectively. In contrast, ROR1 CAR LTG2527 was only effective in the hematologic tumor JeKo-1 model, while ROR1 CAR LTG2528 failed to clear tumors in both the hematologic and the solid tumor xenograft models in vivo. Therefore, CAR LTG2529 was identified as the leading candidate for CAR T therapy targeting ROR1^{+} tumor types.

EXAMPLE 3

ROR1 or FolR1 CARs Boosted with HPSE, MMP-2, MMP-9, or PH-20

Introduction:

Another approach for boosting CAR-T therapy for solid tumors is by targeting the ECM via co-expression of ECM degrading/remodeling enzymes. The development and characterization of mono CARs against FolR1 and ROR1 co-expressing ECM enzymes heparanase (HPSE), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), or membrane-anchored or secreted hyaluronan (PH-20 +/- GPI) is hereby described.

Materials and Methods

Cell lines

The ovarian cancer cell line OVCAR3, lung squamous cell carcinoma cell line NCI-H226, and leukemia cell line HL-60 were purchased from American Tissue Culture Collection (ATCC, Manassas, VA). The MEC-1 leukemia line was purchased from DSMZ (Leibniz Institute DSMZ, Braunschweig, Germany). NCI-H226 were cultivated in RPMI-1640 medium (Corning, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT). OVCAR-3 was cultured in RPMI-1640 medium (Corning, NY) supplemented with 20% heat-inactivated FBS and 10 μg/ml bovine insulin (Sigma, St Louis, MO). HL-60 was maintained in IMDM (Hyclone, Logan, UT) with 20% FBS. MEC-1 cell line and its derivatives were maintained in IMDM supplemented with 10% FBS. OVCAR-3, NCI-H226 and HL-60 luciferase expressing cell lines were generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase, followed by limiting dilution and selection of luciferase-positive clones. MEC-1 ROR1hi cells were generated by stable transduction with lentivirus encoding ROR1 gene, followed by microbeads selection for ROR1.

Generation of CAR constructs and Lentiviral Vector production

The constructs of fully human anti-ROR1 and FolR1 chimeric antigen receptor (CAR) with boosting elements were designed as CAR molecule and a booster molecule connected with P2A ribosomal skipping element sequence. CAR molecules included mono- targeting CAR. The various single chain variable fragment (ScFv) sequences targeting the extracellular domain of human ROR1 or FolR1 were identified in house. Mono CAR comprised of an anti-ROR1 or anti FolR1 scFv, a IgG4 short hinge for ROR1 scFv, a CD8 hinge for FolR1 scFv, connected to CD8 transmembrane domain, costimulatory domain derived from human 4-1BB, followed by CD3-ζ activating domain sequences. Bicistronic CARs contained a ROR1-targeting mono CAR, and a FolR1-targeting mono CAR, connected with P2A sequence. Boosting elements contained extracellular matrix enzymes heparanase (HPSE), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), or membrane-anchored or secreted hyaluronan (PH-20 +/- GPI). PH-20 was followed by a native or tPA signaling peptide in the presence, absence or retains 7 amino acids of the GPI anchor. ROR1 or FolR1 mono CARs without boosters were included as comparison.

CAR sequences were cloned into a Lentiviral Vector (LV) expression cassette under the control of the human EF-1a promoter (anti-ROR1 and some anti-FolR1 CARs) or PGK (some anti-FolR1 CARs) promoter (Lentigen Technology Inc., Gaithersburg, MD). Lentiviral particles were generated by transient transfection of HEK 293T cells, pelleted by centrifugation and stored at -80°C until transduction.

Primary T cell preparation and transduction

Healthy donor primary T cells were isolated from leukapheresis collections (AllCells, Alameda, CA) or from processed buffy coats (Oklahoma Blood Institute, Tulsa, OK), with donors' written consent. The CD4-positive and CD8-positive human T cells were purified via positive selection using a 1:1 mixture of CD4 and CD8 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Purified T cells were activated with CD3/CD28 MACS® GMP T Cell TransAct reagent (Miltenyi Biotec), and cultured in serum free TexMACS medium supplemented with 30 IU/ml IL-2 at a density of 1x 10^6 cells/ml. Furthermore, activated T cells were transduced on day 1 with lentiviral vector particles encoding CAR constructs. On day 3, the transduced T cells were washed and resuspended to 0.5 x 10^6/ml to continue expansion. Every 2-3 days thereafter, cultures were supplemented with fresh TexMACS medium containing 30 IU/ml IL-2, until harvesting time on day 8-10.

Flow cytometric analysis of CAR surface expression

0.32E6 CAR-T cells were washed in cold AutoMACS buffer supplemented with 0.5% bovine serum albumin (Miltenyi Biotec, Bergisch Gladbach, Germany) and stained with CAR detection reagents. For ROR CAR, cells were stained with ROR1-Fc peptide (R&D Systems, Minneapolis, MN) and FolR1 CAR were stained with FolR1-Fc peptide (Acro Biosystems, Newark, DE) followed by anti Fc-AF647 conjugate (Jackson ImmunoResearch, West Grove, PA). The 7-Aminoactinomycin D staining (7-AAD, BD Biosciences, San Jose, CA) was added to exclude dead cells. CD4 antibody labeled with VioBlue fluorochrome was used to separate CD4 and CD8 population. Processed mouse bone marrow and spleen were stained with human CD3 VioBlue and human CD45 FITC (Miltenyi Biotec) to determine the presence of CAR-T. To assess for memory phenotype, cells were stained with CD62L PE and CD45RA APC-Vio700 (Miltenyi Biotec). Non-transduced cells (UTD) were used as a negative control. Cells were washed twice, resuspended in 200 µl running buffer, and acquired by flow cytometry. Flow cytometric analysis was performed on a MACSQuant® 10 Analyzer (Miltenyi Biotec), and data plots were generated using FlowJo software (Ashland, OR).

MMP-9/HPSE ELISA

To confirm MMP-9 or HPSE expression, supernatant from CAR-T cells on day 10 of production was collected and analyzed by ELISA (MMP-9 ELISA- Invitrogen, Waltham, MA; HPSE ELISA- Abcam, Cambridge, UK). Supernatants were diluted 10-fold and two technical replicates were performed for each sample.

CAR-T cell cytotoxicity and cytokine assay

To assess CAR-T cell mediated cytotoxicity, $5x10^3$ tumor target cells stably transduced with firefly luciferase were combined with CAR-T cells at the indicated effector to target ratios and incubated overnight at 37°C with 5% CO2. SteadyGlo reagent (Promega, Madison WI) was added to each well and the resulting luminescence quantified as counts per second (sample CPS). Target only wells (max CPS) and target only wells plus 1% Tween-20 (min CPS) were used to determine assay range. Percent specific lysis was calculated as: (1-(sample CPS-min CPS)/(max CPS-min CPS)). For cytokine release analysis, supernatants from overnight co-cultures were collected and analyzed by ELISA (eBioscience, San Diego, CA) for IFNγ, TNFα and IL-2 concentration. Two technical replicates were performed for each condition, and each experiment was repeated using CAR-T cells generated from different healthy donors as indicated.

Transwell Migration

To assess ECM degradation *in vitro*, CAR-T cells were seeded in either Cultrex™ BME-coated transwell inserts (Corning Life Sciences) to test functionality of MMP-2, MMP-9 or HPSE or in hyaluronan-coated (Lifecore Biomedical LLC) transwell inserts to test functionality of PH-20 and migration into the reciprocal chamber was measured. Corning® BioCoat® control inserts (8.0 μm PET membrane) in 24-well plate formats were uncoated or coated with 100 μL Cultrex™ BME (R&D Systems, Minneapolis, MN) at 5 mg/mL diluted in 0.01M Tris-HCl pH 8.0, 0.7% NaCl or 500 μL 5mg/mL hyaluronan in TexMACS medium (Miltenyi Biotec). Cultrex™ coated transwell inserts were allowed to solidify for 2h at 37C and hyaluronan coated insets were used immediately upon coating. CAR-T cells were thawed and resuspended in TexMACS medium. 0.5E6 cells were seeded in each transwell insert (500μL volume for Cultrex™ coated inserts and 100μL volume for hyaluronan coated inserts). TexMACS medium + 5% FBS was used as a chemoattractant in the bottom chamber (750 μL volume). Cells that had migrated into the bottom chamber after 24h were collected, washed and processed for flow cytometry. Cell counts were normalized to Absolute counting beads (Invitrogen, Waltham, MA).

In vivo analysis of CAR-T function OVCAR3 xenograft model

Animal experiments were performed in compliance with the applicable laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of MI Bioresearch (Ann Arbor, MI) Animal Care and Use Committee.

In OVCAR-3 ovarian cancer model, six to eight week old female NSG mice, 4 per group, were injected intraperitoneally with 1 x 10^7 OVCAR-3 $ROR1^+FolR1^+$ ovarian cancer cells. Tumor burden was determined by IVIS bioluminescent imaging. On day 7, mice were randomized to groups based on equal or similar overall mean tumor burden, and 5.0 × 10^6 CAR-T+ cells/mouse (normalized for transduction efficiency) were administered via tail vain on day 8. Tumor regression was determined by bioluminescent imaging on days 11, 18, 25, 32, and 39 using IVIS-S5. Animal body weight was recorded three times weekly. All BLI Images were analyzed using Living Image, version 4.3, software (Perkin Elmer) and the bioluminescent signal flux for each mouse was expressed as average radiance. At the study termination day 41, ovary, pancreas, Peritoneal cavity wall, were harvested and fixed in 4% paraformaldehyde.

Results

Schematic representations of CAR-T co-expressing ECM enzymes are shown in Figure 17A. CAR + ECM enzymes are comprised of FolR1/ROR1 CAR in frame to an extracellular matrix enzyme linked by P2A ribosomal skipping element sequence. Fully human binders Farletuzumab (Farle) targeting FolR1 and ScFv9 targeting ROR1 hereby were developed in house. Mono CAR configured with a scFv targeting FolR1 or MSLN, in frame to CD8 or IgG4 hinge, CD8 transmembrane, 4-1BB, costimulatory and a CD3ζ activation domain. ECM enzymes included in this set are heparanase (HPSE), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), or membrane-anchored or secreted hyaluronan (PH-20 +/- GPI, or 7 amino acids of the GPI). ROR1 CARs are under the control of the EF1α promoter and the Farle CARs are under the control of either the EF1α or PGK promoter. Table 2 summarizes ROR1 CAR and FolR1 CAR constructs with boosters.

Table 2

	Construct Number	Construct designation	Promoter
Single CAR	D0290	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	LTG2529	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	D0351	Farle-CD8H&TM-41BB-CD3ζ	PGK
Single CAR with booster(s)	D0348	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_MMP-2	EF1α
	D0368	Farle-CD8H&TM-41BB-CD3ζ_2A_HPSE	EF1α
	D0369	Farle-CD8H&TM-41BB-CD3ζ_2A_HPSE	PGK
	D0373	MMP-9 2A ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
	D0422	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_tPA-SP PH-20 GPI	EF1α
	D0423	Farle-CD8H&TM-41BB-CD3ζ_2A_tPA-SP PH-20 GPI	EF1α
	D0424	Farle-CD8H&TM-41BB-CD3ζ_2A_ tPA-SP PH-20 GPI	PGK
	D0459	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_PH-20	EF1α
	D0460	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_tPA-SP PH-20 7 a.a. GPI	EF1α
	D0461	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_NSP PH-20 GPI	EF1α
	D0462	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_PH-20	EF1α
	D0463	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_NSP PH-20 7 a.a. GPI	EF1α

To test CAR-T functionality *in vitro*, ROR1 or FolR1 mono CARs with MMP-2, MMP-9, HPSE or PH-20 as booster elements were characterized. ROR1 and FolR1 CAR +/- ECM enzymes were further incorporated into a third-generation lentiviral vectors and transduced into human primary T cells at MOI 10 for the ROR1 CARs and MOI 20 for the FolR1 CARs, to generate the ROR1 or FolR1 CAR-T cells under the control of the mammalian EF1α or PGK promoter. Un-transduced T cells derived from same donor (UTD) were used as a negative control. Surface expression of CARs with ROR1 or FolR1 binder on transduced T cells was

measured by flow cytometry using ROR1-Fc or FolR1-Fc respectively, followed by staining with anti-Fc Alexa Flour 647. CAR constructs exhibited sustained T cell surface expression as compared to un-transduced T cells. Percentage of CAR positive T cells , based on flow cytometric detection of ROR1 binders and FolR1 binders (Figure 17B) were plotted as a quadratic plot of CD4 vs. CAR. For the donors shown in Figure 17B, CARs co-expressing MMP-2, MMP-9, HPSE or PH-20 had similar CAR expression to that of CARs alone (41-65% for the MMP-2, MMP-9 set; 40-70% for the ROR-1 CARs co-expressing PH-20; 80-93% for the Farle CARs co-expressing either HPSE or PH-20), with the exception of CAR construct D0424, which had ~45% expression, compared to 93.5% expression of the Farle CAR alone. These results overall demonstrate effective transduction efficiency and CAR expression of CARs combined with a digestive enzyme. However, the non-triviality of co-expressing CAR with a digestive element was exemplified by construct D0424.

To evaluate the target specific cytotoxicity of ROR1 CARs *in vitro*, MEC-1 overexpressing ROR1$^+$ B cell line, lung cancer line NCI-H226, and ROR1$^-$ leukemic line MEC-1 were selected as target lines. CAR-T cells were co-incubated with target tumor cell lines at effector to target ratios 10, 5, 1.25:1. After overnight co-incubation, cytotoxicity of CARs was analyzed in a luminescence based in vitro killing assays. Percentage of specific lysis was plotted with E:T ratio using bar graphs (Figure 18A, 18B). CARs with MMP-2 and MMP-9 (D0348, D0373) showed similar killing capacity as compared to mono ROR1 CAR, at all E:T ratios tested (Figure 18A). There was no background killing of ROR1 negative MEC-1 line, demonstrating the robust target-specific cytotoxic function of all ROR1 CAR constructs designed. Furthermore, ROR1 CAR with co-expression of PH-20 element (D0422, D0450-D0463) showed similar killing capacity, compared to mono ROR1 CAR, at all E:T ratios tested, demonstrating that addition of a PH-20 element does not interfere with CAR function.

The specific cytotoxicity of FolR1 CARs was evaluated using OVCAR3 FolR1$^+$ ovarian cancer cell line and HL-60 FolR1$^-$ leukemia cell line as target lines. CAR-T cells were co-incubated with target tumor cell lines at effector to target ratios 10, 2.5, 1.25:1. After overnight co-incubation, cytotoxicity of CARs was analyzed in a luminescence based in vitro killing assays. Percentage of specific lysis was plotted with E:T ratio using bar graphs (Figure 18C). All FolR1 CARs with HPSE or PH-20 (D0368, D0369, D0423, D0424) showed similar killing capacity, compared to mono FolR1, at all E:T ratios tested (Figure 18C). There was no background killing of FolR1 negative HL-60 line, demonstrating the robust target-specific cytotoxic function of all FolR1 CAR constructs tested.

Production of T cell pro-inflammatory cytokines IL-2, IFNγ, and TNFα by the fully human ROR1 and FolR1 CARs were examined by ELISA. Culture supernatants after overnight co-incubation of CAR-T cells with NCI-H226 (Figure 18D) and OVCAR3 (Figure 18E) target lines at all tested E:T ratios were harvested for the measurement of specific cytokine release. For three donors tested, fully human ROR1 CAR with or without boosting elements MMP-2 or MMP-9 exhibited CAR-T cell dose dependent cytokine response when compared to UTD (Figure 18D). Additionally, for three donors tested (one representative donor shown in Figure 18E), fully human FolR1 CAR with or without boosting elements HPSE or PH-20 exhibited CAR-T cell dose dependent cytokine response similar to that of mono-CAR. These results indicate robust, target-specific CAR-T cell cytokine responses.

Production of MMP-9 by the ROR1 CAR (D0373) and the HPSE enzyme by the FolR1 CARs (D0368, D0369), was examined by ELISA. CAR-T supernatants were collected on day 10 of CAR-T production when CAR-T were grown in TexMACS + IL-2. All supernatants were measured upon dilution of 10-fold (Figure 19A). There was a significant amount of MMP-9 secreted by ROR1 CAR co-expressing MMP-9 (D0373). In comparison, marginal MMP-9 expressed was measured for UTD or mono ROR1 CAR-T cells alone (D0290). Likewise, there was a substantial amount of HPSE secreted by FolR1 CARs D0368 and D0369 compared to negligible amounts by UTD or mono FolR1 CAR. CAR D0368 (EF1α promoter) secreted a considerable amount more HPSE than CAR D0369 (PGK promoter), demonstrating the non-obviousness of optimal promoter selection for CARs co-expressed with an ECM-digestive enzyme. These results suggest that CARs co-expressing MMP-9 or HPSE have robust CAR and enzyme expression (Figures 17B and 19A).

As a measure of CAR-T migration through an ECM-rich environment *in vitro*, CAR-T were subjected to an invasion assay in transwell inserts coated with Cultrex™ BME for MMP-2, MMP-9, and HPSE or hyaluronan for PH-20 activity. UTD, CAR alone, or CARs bicistronically expressing ECM enzymes were thawed, counted, and seeded into uncoated, Cultrex™-coated, or hyaluronan-coated transwell inserts for 24h (in the absence of IL-2). Medium in the bottom chamber was then collected, washed and processed via flow cytometry and normalized to Absolute counting beads. As shown in Figure 19B, ROR1 CARs co-expressing MMP-2 or MMP-9 (D0348 or D0373) had greater migration in 5mg/mL Cultrex™-coated transwell inserts than that of CARs alone in 3 separate donors tested (D0290). Out of three distinct donors tested, FolR1 CARs co-expressing HPSE (D0368, D0369) had pronounced migration compared to mono-FolR1 CAR and UTD (Figure 19C). In Figure 19D, FolR1 CARs tested for migration through a hyaluronan coated insert shown greater migration

when co-expressing PH-20 (D0423, D0424) The results discussed here illustrate that CARs co-expressing ECM enzymes can functionally by-pass an ECM better than CARs alone.

In conclusion, ROR1 and FolR1 CARs co-expressing various ECM enzymes were shown to have robust CAR-T cytolysis and ECM targeting functionality *in vitro*. The next direction is to test these top candidates in ECM-rich *in vivo* model(s).

OVCAR3 ovarian cancer cell line stably transduced with luciferase with was used to evaluate the *in vivo* tumor rejection functionality of the FolR1 CAR candidates D0351, D0368, D0369, D0423, and D0424. CAR construct LTG2529, the fully human ROR1 CAR expressed alone, was previously characterized in same in vivo model, and was used as a positive control for OVCAR3 tumor regression. Mice cohorts Tumor Alone (TA) and UTD (tumor-bearing mice treated with same donor non-transduced T cells) were added as negative controls. $ROR1^{+}FolR1^{+}$ OVCAR3 ovarian cancer cells were intraperitoneally implanted into NSG mice to establish OVCAR-3 xenograft model. Ten million OVCAR-3 tumor cells were injected into each NSG mouse. Mice were distributed into experimental groups based on similar tumor burden measured by IVIS imaging on day 7. Five million human CAR^{+} T cells or UTD cells were administrated by i.v. injection at day 8. Tumor growth kinetics was recorded weekly (Figure 20B). ROR1 CAR alone, LTG2529, mediated rapid tumor regression, followed by CAR D0424 (PGK Farle 2A PH-20) which achieved similar tumor regression to LTG2529 by the end of the study. CAR D0369 (PGK Farle 2A HPSE) mediated moderate tumor regression response. All other CAR constructs, including mono-FolR1 D0351, EF1α Farle + HPSE D0368 and EF1α Farle + PH-20 CARs, failed to control the OVCAR3 tumor cell growth, and tumor burden in these groups remained high, similarly to the negative controls UTD and TA (Figure 20A, 20B). These results suggest that PH-20 and HPSE elements improved the function of FolR1 CAR in the disseminated OVCAR3 in vivo model, as compared to mono-FolR1 D0351 (FolR1-taregting CAR without ECM element). Body weights of enrolled animals were monitored three times a week. As shown in Figure 20C, there were no specific FolR1 or ROR1 CAR treated groups that lost more weight over others throughout the study, however some mice needed to be euthanized near the end of the study due to significant body weight loss believed to be due to GVHD. These results suggested no CAR-mediated overall toxicity and effective tumor rejection of ROR1 CAR LTG2529, FolR1 CAR co-expressing PH-20 under the PGK promoter (D0424), and partial response by FolR1 CAR co-expressing HPSE under the PGK promoter (D0369). Therefore, optimal combinations of CAR and ECM enzymes, and selection of suitable promoter needs to be determined empirically and is not trivial.

To assess the fitness and phenotype of CAR-T cells in vivo, bone marrow and spleens from mice were processed at the study end. These tissues were harvested, minced, filtered and processed for flow cytometry to evaluate the presence of human CAR-T cells (Figure 21). Groups D0368 (EF1α Farle 2A HPSE) and LTG2529 (mono-ROR1 CAR) did not have enough mice per group to quantify end of life CAR-T cell phenotype, and were excluded from analysis. Notably, there was no significant difference in spleen weight amongst the different groups. All groups had significant amounts of CAR-T in both the bone marrow and spleen compared to tumor alone group. Percent CAR expression in the bone marrow and spleen were similar to CAR expression in T cell products prior to implant (Figure 21A, 21B).

Next, CAR-T cells from bone marrow and spleen fractions were evaluated for memory phenotype (Figure 21B). Samples were stained with CD62L and CD45RA to distinguish CAR-T cells' naïve, central memory, effector memory and effector phenotypes. In both bone marrow and spleen fractions, PGK Farle 2A PH-20 D0424 had a greater effector population than the other groups (significance is measured for the effector populations). This proportion was more distinct in the CD4 fraction in the bone marrow and the CD8 fraction in the spleen.

In summary, FolR1 CAR D0424 co-expressing PH-20 efficiently eliminated tumors in OVCAR3 NSG xenografts, similar to LTG2529 but with slower kinetics. FolR1 CAR D0369 co-expressing HPSE mediated slower, but detectable tumor regression. In contrast, D0351 mono-Farle CAR and D0369 and D0423 (under the EF1α promoter) failed to clear tumors in the OVCAR3 xenograft model. Therefore, CAR D0424 and D0369 were identified as leading candidates for CAR-T boosted therapy targeting FolR1^{+} tumor types.

EXAMPLE 4

Development of ROR1 targeting CAR constructs with anti CD276 chimeric costimulatory receptor (CCR) as a boosting element

Materials and Methods

Cell lines

The ovarian cancer cell line OVCAR3, lung squamous cell carcinoma cell line NCI-H226, pancreatic cancer cell lines AsPC-1, and acute lymphoblastic leukemia ALL cell line RS4;11 were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA). OVCAR-3 was cultured in RPMI-1640 medium (Corning, NY) supplemented with 20% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT) and 10 μg/ml bovine insulin

(Sigma, St Louis, MO). NCI-H226, AsPC-1 and RS4;11 cells were cultivated in RPMI-1640 medium supplemented with 10% heat-inactivated FBS.

OVCAR-3 and NCI-H226 luciferase expressing cell lines were generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase, followed by limiting dilution and selection of luciferase-positive clones. AsPC-1 and RS4;11 clones of luciferase and GFP expressing cell line was generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase and GFP connected with 2A peptide (Lentigen Technology, Inc., Gaithersburg, MD), followed by selection of luciferase-positive clones. RS4;11 Luc GFP cell line was then transduced with lentiviral vectors encoding ROR1 or CD276 proteins, in order to create target overexpressing cell lines for testing the cognate CAR T cell killing function, named RS4;11-ROR1 and RS4;11-CD276, respectively. Target-positive RS4;11 cells were selected by ROR1 or CD276 magnetic microbeads, expanded, and utilized in luciferase-based overnight killing assays.

Generation of CAR constructs and production of Lentiviral Vectors

The constructs of fully human anti-ROR1 CAR with anti-CD276 CCR (Chimeric Co-stimulatory Receptor) were comprised of a ROR1-CAR molecule in frame to anti-CD276 CCR booster molecule connected with P2A ribosomal skip element. Mono ROR1 CAR and CD276 CARs were included as controls. Mono CARs were comprised of antiROR1 or anti CD276 scFv, a IgG4 short hinge for ROR1 scFv, a CD8 hinge for CD276 scFv, connected to CD8 transmembrane domain, 4-1BB costimulatory domain, followed by CD3-ζ activating domain. Anti-CD276 CCRs were comprised of CD276 targeting scFv followed with CD8 hinge and transmembrane, and CD28 costimulatory domain, without CD3-ζ activating domain sequence.

CAR sequences were cloned into a Lentiviral Vector (LV) expression cassette under the control of the human EF-1α promoter (Lentigen Technology Inc., Gaithersburg, MD). Lentiviral particles were generated by transient transfection of HEK 293T cells, pelleted by centrifugation and stored at -80°C until transduction.

Flow cytometric analysis of CAR surface expression

Half million CAR T cells were washed in cold AutoMACS buffer supplemented with 0.5% bovine serum albumin (Miltenyi Biotec, Bergisch Gladbach, Germany) and stained with CAR detection reagents. For ROR1 CAR, cells were stained with ROR1-Fc peptide (R&D System, Minneapolis, MN), followed by anti Fc-AF647 conjugate(Jackson ImmunoResearch,

West Grove, PA). For CD276 CAR or CCR, cells were stained with CD276-His (Acro biosystems, Newark, De), followed by anti-His PE (Miltenyi Biotech). The 7-Aminoactinomycin D staining (7-AAD, BD Biosciences, San Jose, CA) was added to exclude dead cells. CD4 antibody labeled with VioBlue was used to separate CD4 and CD8 population. Non-transduced cells (UTD) were used as a negative control. Cells were washed twice, resuspended in 200 µl running buffer, and acquired by flow cytometry. Flow cytometric analysis was performed on a MACSQuant® 10 Analyzer (Miltenyi Biotec), and data plots were generated using FlowJo software (Ashland, OR).

CAR T cell cytotoxicity and cytokine assay

To assess CAR T cell mediated cytotoxicity, $5x10^3$ tumor target cells stably transduced with firefly luciferase were combined with CAR T cells at the indicated effector to target ratios and incubated overnight at 37°C with 5% CO2. SteadyGlo reagent (Promega, Madison WI) was added to each well and the resulting luminescence quantified as counts per second (sample CPS). Target only wells (max CPS) and target only wells plus 1% Tween-20 (min CPS) were used to determine assay range. Percent specific lysis was calculated as: (1-(sample CPS-min CPS)/(max CPS-min CPS)). GraphPad Prism software, nonlinear EC50 shift, where x is log of concentration, was used for curve fit and relative potency calculation.

Results

In order to enhance ROR1 CAR functionality, ROR1 CAR was bicistronically co-expressed with a chimeric co-stimulatory receptor (CCR) targeting a second tumor associated antigen, CD276, providing an additional co-stimulatory signal to CAR. ROR1 CAR LTG 2529 and CD276-targeting CCR were co-expressed by lentiviral transduction in primary human T cells for functional evaluation. CAR LTG2529 is comprised of ROR1 targeting scFv9, in frame to IgG4 hinge, CD8 transmembrane, 41-BB costimulatory and a CD3ζ activation domain. The co-expressed CD276 CCR boosters comprised of in-house developed CD276 targeting binders, CD276-22 or CD276-30, followed CD8 hinge and transmembrane domain and CD 28 costimulatory domain, but without CD3ζ activation domain. Mono-targeting CD276 CARs based on different targeting scFv domains, CD8 hinge and transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ activation domain were included for comparison. Previously published CD276-specific scFv 376.96 was included as positive control. Table 3 lists CD276 and ROR1 mono-targeting CARs constructs, and constructs co-expressing ROR1 CAR with CD276 CCR boosters.

Table 3 CD276 CAR and ROR1 CAR armored with CD276 CCR constructs

	Construct Number	Construct designation	Promoter
Single CARs	D0426	CD276 -22 CD8 hinge-CD8TM-41BB-CD3	EF1α
	D0427	CD276-30 CD8 hinge-CD8TM-41BB-CD3ζ	EF1α
	D0480	CD276 376.96 CD8 hinge-CD8TM-41BB-CD3ζ	EF1α
	LTG2529	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ	EF1α
Single CAR with booster(s)	D0432	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_ CD276 -22 CD8 hinge-CD8TM-CD28	EF1α
	D0433	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_ CD276 -30 CD8 hinge-CD8TM-CD28	EF1α
	D0397	ROR1 scFv9-IgG4 hinge-CD8TM-41BB-CD3ζ_2A_ CD276 -376.96 CD8 hinge-CD8TM-CD28	EF1α

CD276 specific targeting-scFvs were first evaluated in CAR context *in vitro*, ROR1 CAR was included as control. The ROR1 and CD276 mono CARs (Figure 22A) sequences were incorporated into third-generation lentiviral vectors and transduced into human primary T cells at MOI 20, to generate the ROR1 or CD276 CAR T cells under the control of the mammalian EF-1α promoter. Surface expression of CD276 CARs with ROR1 binder on transduced T cells was measured by flow cytometry using CD276-His, followed by staining with anti-His PE. ROR1 CAR expression was determined as previously described. Representative flow plots from one donor are shown in Figure 22 B. CAR surface expression, as measured by flow cytometry, was robust: CD276 CARs, 276-22 CAR D0426 and 276-30 CAR, as well as 276-96 CD276 CAR D0480, were expressed at 60%-80%, while ROR1 CAR LTG2529 transduction of T cells from same donors ranged 45%-65% (Figure 22C). The target specific cytotoxicity of CD276 and/or ROR1 CARs was measured by luciferase based overnight killing assy. $ROR1^{+}CD276^{+}$ ovarian cancer line OVCAR3, pancreatic cancer line AsPC-1, lung cancer line NCI-H226 were used as target lines, and co-incubated with CAR T cells at 10 different effector to target (ET) ratios. Untransduced (UTD) T cells from same donors were included as negative control. Percentage of specific lysis was plotted with ET ratio using non-linear curve fit, and is shown in Figure 23. The CD276 CARs, D0426, D0427 and D0480 demonstrated comparable killing potency as compared to mono ROR1 CAR LTG2529, at all ET ratios tested (Figure 23). UTD cells showed no appreciable target-specific killing, further demonstrating binder specificity of CD276 scFvs.

ROR1 CAR with CD276 CCR booster constructs (Figure 24A) were evaluated for transduction efficiency and *in vitro* cytotoxicity. Human primary T cells were transduced with lentiviral vector bicistronically encoding ROR1CAR/CD276CCR constructs at MOI 20. As compared to UTD T cells from same donor, CAR/CCR construct D0432 with 276-22 CCR and

D0397 with CD276 binder 376.96, showed effective ROR1 binder and CD276 binder co-expression (Figure 24B), 50% of all T cells were double positive for the CAR and the CCR in three experiments using T cells from three unrelated donors (Figure 24C). CAR/CCR construct D0433 with CD276 CCR comprising the 276-30 binder, had less CD276 binder expression as compared to ROR1 binder, with 30% of all T cells staining double positive for the ROR1 CAR and the CCR (Figure 24B, 24C). As expected, the mono ROR1 CAR LTG 2529 transduced T cells showed no CD276 binder expression. The *in vitro* cytotoxicity of ROR1 CAR/CD276 CCR constructs was measured at 10 different ET ratios with $ROR1^{+}CD276^{+}$ ovarian cancer line OVCAR3, $ROR1^{-}CD276^{-}$ ALL cell line RS4;11, single antigen ROR1 overexpressing line RS4;11-ROR1 and single antigen CD276 overexpressing cell line RS4;11-CD276 (Figure 25A-D). For ROR1+ target tumor cells, OVCAR3 or RS4;11-ROR1, all CAR/CCR constructs demonstrated high killing efficiency (Figure 25A, 25C). After normalization for percentage ROR1 CAR expression, the three ROR1CAR/CD276 CCR T constructs exhibited similar killing potency to that of the mono-targeting ROR1 CAR LTG2529 (Figure 26A, 26B). Notably, when ROR1CAR/CD276 CCR transduced T cells were co-cultured with CD276+ target cell line RS4;11-CD276, which is ROR1-negative, all constructs with CD276 CCRs revealed robust cytotoxicity (Figure 25D). Therefore, the activation of the CCR by CD276 expressed on target cells triggered target cell killing, even though the CCR is lacking the CD3ζ activation domain. This effect is believed to be mediated by engagement of the CAR molecule via the CCR, which enables signaling through CD3ζ on the ROR1 CAR. No cytotoxicity was observed when ROR1CAR/CD276 CCR T cells were co-incubated with $ROR1^{-}CD276^{-}$ RS4;11 cells confirming the target specificity of ROR1 and CD276 binders.

In summary, ROR1 CARs with CD276 CCR boosters demonstrated high transduction efficiency, and had comparable cytotoxic function to the mono ROR1 CAR. Engagement of CD276 antigen alone mediated cytotoxicity, comparable to the engagement of ROR1 CAR via ROR1 antigen alone. Therefore, a logic [OR] CAR gate was created relying on one CD3ζ domain only for targeting both CD276 and ROR1 antigens.

EXAMPLE 5

ROR1-CAR LTG2529 cleared both hematologic and solid tumors, and attenuated TGFβ-rich tumor microenvironment when armored with TGFβRIIdn element

Introduction

Autologous chimeric antigen receptor (CAR) T cell therapy has revolutionized treatment for patients with B-cell leukemia, lymphoma and multiple myeloma. Over a third of all CAR T cell patients treated to date with commercial CAR T cells products targeting CD19 or BCMA, respectively, achieve complete and durable remissions(2). However, despite wide-scale efforts to tackle solid tumors, which account for 90% of all cancer types, they have yet to demonstrate high therapeutic efficacy, similar to that observed in hematologic malignancies. The immunosuppressive tumor microenvironment (TME) has been identified as one major challenge to the success of solid tumor CAR T cell therapies in solid tumors(3), and will need to be addressed.

Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an attractive target for immunotherapy of solid and hematologic tumors. ROR1 plays an important role during early embryonic development but remains absent from vital adult human tissues, except for expression in a subset of immature B-cell precursors in adult bone marrow, and low-level expression in adipocytes(4, 5). By contract, ROR1 is overexpressed on the surface of a large array of hematologic tumors, including B-ALL, B-CLL, MCL, FL, MZL, DLBCL, and a subset of solid tumors, including ovarian, pancreatic, lung, skin, breast, and colon cancers (6-8). Zilovertamab Vedotin, a novel antibody-drug conjugate comprising the humanized monoclonal antibody zilovertamam (or Cirtuzumab) and a linker-monomethyl auristatin E (Vedotin), is an antibody drug conjugate targeting ROR1 holds a promise for success in lymphoid cancers, and has demonstrated safety and anti-tumor effects in mantle cell lymphoma (MCL) and Diffuse large B cell lymphoma (DBLCL). However, Srivastava et al (1) faced the challenge in solid tumor as their ROR1 CAR-T cells with scFv-R12 binder infiltrated tumors poorly and became dysfunctional in patients with ROR1+TNBC and NSCLC.

Transforming growth factor beta (TGFβ) is a master regulator of TME, is known to be secreted by tumor cells, stromal fibroblasts, and other cells in many solid cancers, including pancreatic cancer, creating an immunosuppressive environment, inhibiting T cell effector function, cytokine response, proliferation, persistence and memory formation, promoting

neoangiogenesis and metastasis (9). There are 3 isoforms of TGFβ1 in mammals, i.e. TGFβ1, 2, and 3. TGFβ signals by binding to TGFβRI and II on cell surface, leading to phosphorylation and activation of transcription factor Smad2/3, which in turn activates responsive genes that inhibit T cell proliferation and differentiation into helper T cells and CTLs(10). Overcoming the immunosuppressive effects of TGFβ in TME may therefore offer a unique opportunity to simultaneously improve multiple CAR T cell attributes. Modulating the anti-tumor inhibitory effect of TGFβ has been studied by other groups and ours, including armoring CAR T with dominant-negative TGFβRII targeting PSMA in prostate cancer (11) and BCMA in Multiple Myeloma models (12), or knocking out TGFβRII in CAR T (13). Clinical trial employing PSMA-CAR T armored with a dominant negative form of TGFβRII showed promising results in patients with prostate cancer when administered at a safe dose (14).

Here, a novel, fully human ROR1-LTG2529 CAR employing scFv9 targeting domain is reported, which effectively eliminated hematologic tumors in Jeko-1 MCL xenografts, as well as solid tumors in OVCAR-3 ovarian cancer and AsPC-1 pancreatic cancer xenograft models. ROR1-LTG2529 elaborated greater cytokines and rejected solid tumors more effectively than a comparator LTG2527 based on the scFv-R12 binder, in agreement with the reported efficacy profile of R12-based CAR T cells in solid tumors (1). Furthermore, LTG2529 CAR T cells armored with TGFβRIIdn overcame the inhibitory effect of TGFβ in vivo in a pancreatic xenograft model AsPC1 overexpressing TGFβ1. These findings support the application of TGFβRIIdn armor as a tool to ameliorate immunosuppressive TME for better treatment of patients with both hematologic and solid malignancies.

Methods

Generation of CAR constructs, lentiviral vector production and titration

The constructs of fully human anti-ROR1 chimeric antigen receptor (CAR) either alone or with a booster element dominant negative (DN) TGFβRII were designed as CAR molecule and a booster molecule connected with P2A ribosomal skipping element sequence. The single chain variable fragment (ScFv) sequence scFv9 targeting the extracellular domain of human ROR1 was identified in house, the R12 ScFv targeting ROR1 was used as CAR ROR1 control. Mono CAR comprised of a anti-ROR1 scFv, a IgG4 short hinge for ROR1 scFv, a CD8 hinge, connected to CD8 transmembrane domain, costimulatory domain(s) derived from human 4-1BB, followed by CD3-ζ activating domain sequence.

CAR sequences were cloned into a Lentiviral Vector (LV) expression cassette under the control of the human EF-1a promoter (Lentigen Technology Inc., Gaithersburg, MD). Lentiviral particles were generated by transient transfection of HEK 293T cells, pelleted by centrifugation and stored at -80°C until transduction. LV titers were determined by the serial transduction of SUP-T1 cell line and qPCR analysis of GAG and POL expression.

Preparation of human T-cells

Whole blood was collected from healthy volunteers at Oklahoma Blood Institute (OBI) with donors' written consent. Processed buffy coats were purchased from OBI (Oklahoma City, OK). The CD4-positive and CD8- positive human T cells were purified from buffy coats via positive selection using a 1:1 mixture of CD4- and CD8-MicroBeads (Miltenyi Biotec) according to manufacturer's protocol.

Preparation of tumor cell lines

The mantle cell lymphoma (MCL) Jeko-1, Plasmacytoma B lymphocyte RPMI 822, Acute T cell Leukemia T lymphoblast, Chronic Myelogenous Leukemia line K562, Acute Lymphocytic Leukemia line Reh, Acute Promyelocytic Leukemia promyeloblast HL-60, Lymphoblastic Lymphoma T lymphoblast SUP-T1, Ovary Adenocarcinoma epithelial OVCAR-3, Pancreas Adenocarcinoma Capan-2 and AsPC-1, and Lung Squamous Cell Carcinoma NCI-H226 cell lines and culture reagents were purchased from American Tissue Culture Collection (ATCC; Manassas, VA, USA), unless otherwise noted. All cell lines were cultured following the manufacturer's instructions. Single-cell clones of luciferase-expressing cell lines were generated by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase
(Lentigen Technology, Inc., Gaithersburg, MD), followed by cloning and selection of luciferase-positive clones. AsPC-1 cell line overexpressing human TGFβ1 was generated in-house.

Primary T cell transduction

Selected CD4+ and CD8+ human primary T cells from normal donors were cultivated in TexMACS medium (serum-free) supplemented with 40 IU/ml IL-2 at a density of 1e6 cells/ml, activated with CD3/CD28 MACS® GMP TransAct reagent (Miltenyi Biotec) on day 0 and transduced on day 1 with lentiviral vectors encoding CAR constructs, and media

exchanged on day 3. Cultures were propagated on day 6 until harvest on days 9-10 for co-incubation analysis. Extra CAR-T cells were cryopreserved using 10% DMSO (Amresco), 70% FBS (HyClone, Logan, UT, USA), and 20% TexMACS in a controlled-rate freezer (Mr. Frosty; Nalgene) and then stored at Liquid nitrogen (−160°C) until re-culture.

Luciferase-based cytotoxicity assay & CAR T potency calculation

Cytotoxicity assay was performed as previously described (*15*). Briefly, 5,000 target cells stably transduced with firefly luciferase were combined with CAR T cells at various effector to target ratios and incubated for 18 hrs. SteadyGlo reagent (Promega, Madison, WI) was added to each well and the resulting luminescence was analyzed on an GloMax microplate reader (Promega, Madison, WI) and recorded as counts per second (sample CPS). Target only wells (max CPS) and target only wells plus 1% Tween-20 (min CPS) were used to determine assay range. Percent specific lysis was calculated as: (1-(sample CPS-min CPS)/(max CPS-min CPS)). Absolute potency (EC_{50}) and relative potency of effector T-cells were calculated using Prism software with 4-parameter parallel-line analysis approach.

Impedance-based cytotoxicity assay

The assay was performed employing xCELLigence RTCA MP analyzer (Agilent Technologies, Santa Clara, CA, U.S.) following the manufacturer's instructions. Briefly, 40,000 AsPC-1 cells were co-cultured with 80,000 effector cells (i.e. E:T ratio = 2:1) and the cytolysis was monitored for 3 days. The data was analyzed by RTCA Software Pro.

Quantification of cytokine release from the co-culture of target cells and effector cells

Supernatant harvested from the co-culture of effector & target cells at the end of the assay was analyzed for IFN-γ, TNF-α, and IL-2 by ELISA (ThermoFisher Scientific, Inc., Waltham, MA) following manufacturer's instructions.

Quantification of TGF β1 produced by tumor cell lines in culture

Cell lines of interest were seeded in 6-well plates and cultured overnight in the appropriate medium, followed by exchange with fresh medium, and further cultured for 24 hrs. Then, 100 uL of supernatant was collected and subject to analysis for human TGFβ1 employing Duoset ELISA for human TGFβ1 (R&D Systems) following the manufacturer's instructions. Notes: No HCl treatment of samples was performed for detection of active TGFβ1 form.

Quantification of human cytokines in mouse serum

At the time points of interest during in vivo studies, 50 uL of blood from each mouse was collected and subject to analysis of human cytokines (GM-CSF, IFN-γ, TNF-α, IL-6, IL-2, TGF-β1) employing MSD U-plex assays (Mesoscale Discovery). To detect active form of TGF-β1, no HCL treatment of samples was performed.

Flow cytometric analysis

Flow cytometric analysis was performed as previously described (*12, 15, 16*). All cell staining reagents for flow cytometry were from Miltenyi Biotec, unless otherwise noted. These include anti-ROR1.AF647, mouse IgG1 control. APC, anti-CD45.PE, anti-CD8.Viogreen, anti-CD3.VioBlue, anti-CD45.VioBright FITC, anti-CD45RA.APC-Vio770, anti-CD62L.PE, anti-PD1.PE-Vio770, Streptavidin.PE (Miltenyi Biotec). Cell viability solution (7-AAD), BD Pharm Lysing buffer were purchased from BD Biosciences. Anti-ROR1.AF647 was from R&D systems. ROR1.Fc was from Sino Biologicals. Anti-human Fc.AF647 was from Jackson ImmunoResearch. Countbright absolute counting beads were from ThermoFisher Scientific. Anti-TGFβRII. Biotin was from Biolegend. Stained cells were analyzed using the MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec).

Antigen density quantification assay

Target cells of interest were stained with Anti-ROR1.PE or mouse IgG2b control.PE, and Cell viability solution (7-AAD). Antigen density was calculated based on BD QuantiBrite beads. All reagents were purchased from BD Biosciences, and the assay was performed following the manufacturer's instructions.

In vivo analysis of CAR-T activity

All animal studies were approved by Jackson Laboratory Animal Care and Use Committee (Sacramento, CA). Female 7 to 8-week old NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*$^{tm1Wjl/}$SzJ), Jackson Laboratory (Bar Harbor, ME) were utilized.

Mantle Cell Lymphoma (MCL) Jeko-1 xenograft model: Mice (6 mice/group) were intravenously (i.v.) implanted with Jeko-1 cells (0.5e6 cells/mouse). On day 6 following Jeko-1 injection, tumor engraftment was measured by i.p. injection of 150 mg/kg luciferin and imaging 10 min later for 40 s on a Xenogen IVIS-200 instrument (Caliper Biosciences, now Perkin Elmer, Shelton, Connecticut). Images were analyzed using Living Image, version 4.1,

software (Perkin Elmer) and the bioluminescent signal flux for each mouse was expressed as average radiance (photons per second per cm2 per steradian). Mice were distributed equally to study groups (staging) on day 6 based on tumor burden. CAR T cells were administered to mice via tail vein injection on Day 7 at the dose of 3e6 total CAR^+T cells/mouse . Un-transduced T cells from the same donor (UTD) and Tumor alone group served as controls. The amount of injected UTD T cells was adjusted to the number of total T cells in the CAR groups with the highest total cell count. Imaging was performed on days 6, 13, 20, 27, 34, and 41 following injection to establish the kinetics of tumor growth and eradication by CAR T cells. Body weight was monitored 3 times/week.

Ovarian Adenocarcinoma OVCAR-3 xenograft model: the study was perform as described in the MCL Jeko-1 model above with some modifications as following: Mice (5 mice/group) were intraperitoneally (i.p.) implanted with OVCAR-3 cells (10e6 cells/mouse). CAR T cells were administered to mice via tail vein injection on Day 7 at the dose of 5e6 total CAR^+T cells/mouse. Imaging was performed on days 3, 10, 17, 24, 31, 38, and 47 following injection.

Pancreas Adenocarcinoma AsPC-1 xenograft model: Mice (5 mice/group) were subcutaneously (s.c.) implanted with AsPC-1 cells (1e6 cells/mouse) in the right flank. Once tumors reached approx. 100 mm^3 as measured with a caliper, mice were stagged and CAR T cells were administered to mice via tail vein injection on Day 17 at the dose of 5e6 total CAR^+T cells/mouse. Tumor volume was measured by caliper 5 times per week for the first 2 weeks, followed by 3 times per week until study termination time point; the same schedule was applied to body weight monitoring. All mice untreated or treated with UTD T cells were sacrificed at day 52 post T cell infusion. Mice treated with either armored or non-armored CAR Ts and showed complete tumor clearance (i.e. tumor volume = 0 mm^3) were re-challenged with AsPC-1 (1e6 cells/mouse) by s.c. injecting the tumor cells in the left flank at day 73 post T cell dosing (or 90 days after the first tumor implantation). Notably, 13 days before the re-challenge, one mouse from the armored CAR T treated group was sacrificed due to body weight dropped beyond 20%, therefore, there were 4 mice in the non-armored CAR and 3 mice in the armored one entered the re-challenge study; 4 age-matched mice were used as controls. Tumor volume on both flanks were measured by caliper 5 times per week for the first 2 weeks, and 3 times per week for the following weeks; body weight was monitored in the same schedule.

Pancreas Adenocarcinoma AsPC-1/TGFβ xenograft model: The study was performed as described in the AsPC-1 xenograft model above except that CAR T cells were injected after 15 days of tumor implantation and the study was ended at day 49 post T-cell infusion.

Histology & Immunohistochemistry staining of tumor tissues

After 7 days of CAR T infusion, tumor tissues from 1 mouse per group (in AsPC-1 or AsPC-1/TGFβ xenograft model) were collected, fixed with 4%PFA buffer for 24 hrs, then stored in 70% EtOH before embedded in Paraffin. The sectioned tissues were then subject to H&E, Masson Trichrome staining, and immunohistochemistry staining with anti-CD3 antibody (Cell Signaling), anti-TGF-β antibody (abcam), or rabbit isotype control (Cell Signaling).

Graphs and statistical analysis

All statistical analyses were performed using Prism 9.3.1 software (GraphPad, San Diego, CA, USA). Technical replicates represent repeated measurements of same donor-derived population of cells, and biological replicates indicated 2 or more donor-derived cellular populations or separate mice. Bioluminescence, cytotoxicity of target cells, and expansion of T cells data were log transformed prior to analysis using parametric tests. Statistical significance was determined by one- or two-way ANOVA, followed by Tukey's multiple comparison test. Survival was evaluated by Kaplan-Meier test. p values were reported as the following: ns (non-significant), $p > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Error bars represent standard error of the mean.

Results

The novel fully-human ROR1-LTG2529 exerted comparable cytotoxic activity against hematologic tumor cell lines positive for ROR1 vs comparator CAR R12-ROR1 LTG2527 in vitro, but elaborated greater levels of cytokines

ROR1 is a 12-kDa protein containing extracellular immunoglobulin-like, Frizzled, and Kringle domains. Hudecek M et al (17) developed a second generation CAR specific to ROR1 with rabbit R12-scFv binder, short Hinge IgG4-Fc spacer, 4-1BB co-stimulating domain, and CD3z signaling domain which has recently been reported to poorly perform in phase 1 clinical trial on patients with ROR1+TNBC and NSCLC (3). In this study, similar CAR constructs were generated with either a new fully-human scFv binder (namely scFv9) specific to ROR1

and named it LTG2529, or the control R12-scFv binder (namely scFvR12) and named it LTG2527, a schematic diagram of which is shown in Figure 27A. Lentiviral transduction of human primary T cells with LTG2529 or LTG2527 revealed higher cell surface density for LTG2529 compared to LTG2527 (i.e. approx. 4 fold), although transduction efficiency (%CAR^+T-cells/total T-cells) were comparable for LTG2529 vs LTG2527 (Figure 27B). Next, cytotoxicity of ROR1 CARs was evaluated in vitro in MCL Jeko-1 cells.

Overnight co-culture of LTG2529-transduced T cells with Jeko-1 cell line, exhibiting high ROR1 density (Figure 27C), showed comparable cytotoxic potency between LTG2529 and LTG2527 (Figure 27D). Further, Jeko-1 cells elicited significantly higher cytokine production from LTG2529 as compared to LTG2527 (Figure 27E) suggesting that LTG2529 CAR mediated greater activation of T cells.

LTG2529 and LTG2527 were equally effective in eradicating hematologic Jeko-1 MCL xenograft in vivo.

Next whether the similar anti-tumor activity of the 2 CARs in vitro would translate to in vivo was examined. To address this question, Jeko-1 MCL xenograft model was used (Figure 27F). Comparable tumor regression and improved survival were observed in Jeko-1-implanted immunodeficient NSG mice treated with LTG2529 or LTG2527, whereas 5 out of 6 untreated mice reached euthanasia criteria after 34 days of inoculation with Jeko-1 tumor (Figures 27G and 27H). No significant weight loss was observed in mice treated with either CAR during this study (Figure 27I).

Blood from the mice was sampled and observe only a few Jeko-1 cells were observed in mice treated with either CAR, whereas the number of Jeko-1 cell was elevated 1000-fold in untreated mice after 20 days of tumor inoculation (Figure 27J), which correlated with tumor progression, as measured by luciferase activity (BLI) (Figures 27G and 27H). Furthermore, LTG2529 exerted a faster expansion of total CAR-positive T cells as compared to LTG2527 CAR within 6 days after administration, due to higher expansion of both $CD8^+$ and $CD4^+$T-cell populations (Figure 27K).

LTG2529 showed enhanced cytokine response and comparable or greater cytotoxicity against OVCAR-3, Capan-2 and NCI-H226 ROR1$^+$ solid tumor cell lines in vitro as compared to LTG2527.

Furthermore, anti-tumor reactivity of LTG2529 vs LTG2527-transduced T cells against solid cancer cell lines in vitro was investigated. Quantification of ROR1 surface density on solid tumor cell lines, including NCI-H226, Capan-2, and OVCAR-3 (lung, pancreatic, ovarian, respectively) revealed broad range of ROR1 expression (Figure 28A). T cells were transduced with either LTG2529 or LTG2527, and co-cultured with the target cells overnight. LTG2529-transduced T cells, as compared to LTG2527, exhibited comparable cytotoxic potency against OVCAR-3 and NCI-226 tumor lines, but higher potency against Capan-2, which may reflect overcoming the intrinsic resistance of pancreatic tumors to T cell therapy by LTG2529, but not LTG2527 (Figure 28B). Additionally, greater amounts of IFNγ, TNFα, and IL2 were produced by LTG2529 vs LTG2527 T cells in OVCAR-3 (Figure 28C), consistently with greater elaboration of cytokines by LTG2529 in response to hematologic tumor lines (Figure 27E), suggesting a universal heightened cytokine response of LTG2529, irrespective of tumor type.

Only LTG2529, but not LTG2527, mediated tumor regression in *in vivo* ovarian cancer model.

In order to investigate the anti-tumor response of CARs against solid tumor in in vivo, OVCAR-3 ovarian cancer model was chosen. Although both CARs showed similar cytotoxic activity against OVCAR-3 *in vitro* as shown in the above data, only LTG2529 mediated tumor regression *in vivo* (Figures 28D-29F). Additionally, mice administered LTG2529 T cells did not show significant weight loss as compared to mice administered either LTG2527, un-transduced T-cells, or untreated mice (Figure 28G). Rapid expansion of CD8$^+$T and memory T cells after CAR T cell administration predicts positive clinical outcomes. Analysis of blood samples from mice treated with either LTG2529 or LTG2527 T cells revealed rapid expansion of LTG2529 group's CD8$^+$ and CD4$^+$T-cells within the first 10 days after T cell administration as compared to LTG2527 (Figure 28H). Additionally, from day 3 to day 10 post administration, LTG2529-transduced T cells showed a rapid expansion of T_{EM} cells in both CD8 (43 fold, from 0.53% to 23%) and CD4 (4 fold, from 4.2% to 17.6%), indicative of prompt effector CAR T cell activity, as compared to LTG2527 (3.4 fold, from 5% to 17% for CD8, and no increase in % of CD4 T_{EM} cells); a similar increase was observed in the fraction of T_{CM} cells transduced

with LTG2529, with faster expansion in CD8 (7 folds; i.e. from 6.6% to 46%) and CD4 (about 3 folds; i.e. from 17% to 49.7%) as compared to LTG2527 (2 folds for CD8 and 1.8 fold for CD4) (Figure 28I), suggesting effective formation of immune memory and reserve for durable LTG2529 T cell function. In summary, our data demonstrate that LTG2529 exhibited anti-tumor efficacy in *in vivo* xenograft model of solid tumor, particularly ovarian cancer, which is attributed to timely expansion of CAR^+T cells, and enrichment for T_{EM} and T_{CM} phenotypes in both CD8 and CD4 T cell populations; whereas the suboptimal anti-tumor response of LTG2527 T cells was reflected in delayed CAR T cell expansion and emergence of the effector and central memory phenotypes.

TGFβRIIdn-armored LTG2529 attenuated the inhibitory effect of TGF-β1 on CAR T-cell cytotoxic activity in vitro

Having demonstrated high in vitro and in vivo potency of ROR-1 CAR T cells, protecting LTG2529 T cells from the inhibitory effects of TGFβ was proceeded. Lentiviral vector co-expressing ROR1 CAR and the TGFβRIIDN armor element was constructed, separated by ribosomal skip site, to facilitate co-expression of the two polypeptides, namely D0228 (Figure 29A). TGFβRIIDN is a truncated form of TGFβ receptor II, capable of TGFβ binding, but devoid of intracellular signaling activity (*12*), thus attenuating the TGFβ-induced suppression of T cells. The armored ROR1 CAR was expressed robustly on healthy donor T cells with comparable enriched CAR^+T_N and T_{CM} phenotypes in both the CD8 and CD4 T cell fraction, similarly ROR-1 CAR alone (Figure 29B). The overexpression of TGFβRIIdn element on the surface of armored LTG2529 T cells was visualized by flow cytometry using an anti-TGFβRII antibody (Figure 29C). TGFβ signals through TGFβRII on cell surface, leading to phosphorylation of transcription factor Smad2/3. A reduction of pSmad2/3 in TGFβRIIdn -armored LTG2529 T cells treated with TGF-β1 compared to non-armored LTG2529 Ts was observed (Figure 29D), which indicates the functional effect of the dominant negative TGFβRII on TGF-β1 signal transduction.

TGFβ is known for its negative effect cytotoxic T cells, including inhibiting the expression of multiple effector molecules (granzyme A, granzyme B, perforin, IFNγ and TNFα) (*18*). To demonstrate the functional effect of TGFβRIIdn on anti-tumor activity of CAR-transduced T cells in vitro, CAR T cells were co-cultured with pancreatic adenocarcinoma AsPC-1 cells (which highly expresses ROR1 (Figure 29E) in the presence of

TGF-β1. TGF-β1 reduced cytotoxic activity of LTG2529 T cells, decreased production of IFNγ and TNFα in the co-culture supernatant, which were restored in the armored LTG2529 T cells (Figures 29F and 29G). AsPC-1 cells express low level of latent (i.e. inactive) form of TGF-β1 in cell culture, which was detected upon activation by acidic treatment (Figure 29H). AsPC-1 cell overexpressing TGF-β1 (namely AsPC-1/TGFβ) were then generated to investigate the effect of the armor in vitro and in vivo; as shown in Figure 29I, this cell line produced high amount of both active (approx. 16,000 pg/mL) and latent (approx.. 90,000 pg/mL) forms of TGF-β1 when cultured overnight. Cytotoxicity of LTG2529 T as well as its' production of cytokines (i.e. IFN-γ, TNF-α) were dramatically reduced when co-cultured with AsPC-1/TGFβ in comparison to AsPC-1 control cell; however, this effect was attenuated for LTG2529 T armored with TGFβRIIdn (Figures 29J and 29K). Thus, our data demonstrated that the dominant negative TGFbRII lessened the inhibitory effect of TGFβ1 on cytotoxicity of CAR T cells in vitro.

TGFβRIIdn-armored LTG2529 (D0228) showed an increase in CAR+T-cell population in pancreatic cancer AsPC-1 xenograft model with low TGFβ1 expression.

TGFβ is known to be produced by various cell types (i.e. tumor, stomal, and immune cells) and exists as latent or inactive form in tumor microenvironment (TME), which is then activated by various enzymes in the extracellular matrix, including matrix metalloproteinases (MMPs) and acidic condition in TME in various cancers, including PDAC, providing a tumor protective environment. Whether the dominant negative TGFβRII would help T cell overcome this effect is to be determined . As mentioned above, pancreatic cancer AsPC-1 cell produces low amount of latent TGF-β1, mice were implanted with these cells subcutaneously (Figure 30A). As shown in Figure 30B, both LTG2529 with our without armor caused tumor volume reduction within 10 days after administration; interestingly, the one with armor started to show beneficial effect on day 17 post T cell dosing and the tumor was cleared in all mice started at day 24 whereas all mice with the non-armored CAR were cleared from tumor started at day 33. None of the CARs caused significant drop of body weight during the study course (Figure 30C). Analysis of T cells in blood from these mice revealed a high frequency $CD8^+CAR^+$T cell population in D0228 vs LTG2529 across all tested time points (Figure 30D).

To determine if the CAR T cells were still functional at the end of in vivo time course, same mice were re-challenged with AsPC-1 cells on the left flank (please note that the first

implantation was on the right flank)(Figure 30E). As shown in Figure 30F, no tumor was observed in mice treated with CAR T cells with or without armor whereas the tumor freely progressed in age-matching control mice; besides, no tumor was observed on the right flank in mice treated with both CAR constructs suggesting complete remission from the first tumor implantation. It's also worthy to note that 1 mouse from the armored CAR treated group was dead at day 60 post T cell dosing in the challenge study before re-challenge study which started at day 73 post T-cell dosing, which were most probably due to GvHD. Additionally, there was a benefit in survival for mice treated with the armored CAR (Figure 30G). Analysis of T cells from bloods revealed predominant effector memory and terminal effector T cells in both groups of mice (Figure 30H). CAR staining at day 23 and 42 post re-challenge revealed an increase in T cells positive for CAR which was much higher in the armored one (Figure 30I). T cells isolated from spleen and bone marrow harvested at the end of life shared the same features of CAR positivity as observed in blood (Figure 30J).

The Dominant negative (dn) TGFβ receptor II overcame the inhibitory effect of TGFβ on T cells in the AsPC-1 overexpressing TGF-β1 xenograft model.

To better investigate the effect of the dn element on anti-tumor activity of ROR1-CAR T-cells in the TGFβ-rich tumor microenvironment, NSG mice were implanted subcutaneously with AsPC-1/TGFβ cells (Figure 31A). The armored CAR cleared tumor after 33 days of T-cell infusion, whereas the non-armored CAR showed partial tumor regression only (Figure 31B). Importantly, analysis of cytokines in serum of these mice showed a significant reduction of TGFβ1 active form for both armored and non-armored CARs at day 5 post infusion compared to non-treated or UTD treated mice; this was probably due cytotoxic activity of CAR Ts against AsPC-1/TGFβ (Figure 31C, left panel); however, this effect didn't last long for non-armored CAR as the TGFβ1 amount from this group of mice reached the same level as of non-treated and UTD treated mice at day 15 post infusion, whereas the ones treated with the armored CAR remained low. Quantification of other cytokines (IFNγ, GM-CSF) suggesting the onset of activity of CAR-Ts around day 5 and reduced by day 15 post T cell infusion (Figure 31C, center & right panel).

Analysis of T-cells from blood of the mice revealed a 6-fold increase on day 12 vs day 2 post T cell infusion in the group treated with armored CAR whereas less than 2 fold increase was observed in the non-armored CAR groups (Figure 31D). The number of CAR^{+}T-cells in

both CD8 and CD4 populations declined after 12 day post infusion (Figure 31E) which correlated with tumor volume reduction as shown in Figure 31B. An increase in T-cells after day 29 post infusion was also observed (Figure 31D), which is possibly due to GvHD. Analysis of T-cells isolated from spleen and bone marrow at the end of the study showed higher frequency of CAR+T cells for the armored CAR (Figure 31F), and similar pattern of memory phenotype for both armored and non-armored CARs (Figure 31G).

Taken together, these results demonstrate the advantages of the TGFβRIIdn -armored ROR1 CAR in the treatment of solid tumors.

References

1. S. Srivastava, S. N. Furlan, C. A. Jaeger-Ruckstuhl, M. Sarvothama, C. Berger, K. S. Smythe, S. M. Garrison, J. M. Specht, S. M. Lee, R. A. Amezquita, V. Voillet, V. Muhunthan, S. Yechan-Gunja, S. P. S. Pillai, C. Rader, A. M. Houghton, R. H. Pierce, R. Gottardo, D. G. Maloney, S. R. Riddell, Immunogenic Chemotherapy Enhances Recruitment of CAR-T Cells to Lung Tumors and Improves Antitumor Efficacy when Combined with Checkpoint Blockade. *Cancer cell* **39**, 193-208 e110 (2021); published online EpubFeb 8 (10.1016/j.ccell.2020.11.005).
2. R. C. Sterner, R. M. Sterner, CAR-T cell therapy: current limitations and potential strategies. *Blood cancer journal* **11**, 69 (2021); published online EpubApr 6 (10.1038/s41408-021-00459-7).
3. N. M. Anderson, M. C. Simon, The tumor microenvironment. *Current biology : CB* **30**, R921-R925 (2020); published online EpubAug 17 (10.1016/j.cub.2020.06.081).
4. T. J. Kipps, ROR1: an orphan becomes apparent. *Blood* **140**, 1583-1591 (2022); published online EpubOct 6 (10.1182/blood.2021014760).
5. S. Baskar, K. Y. Kwong, T. Hofer, J. M. Levy, M. G. Kennedy, E. Lee, L. M. Staudt, W. H. Wilson, A. Wiestner, C. Rader, Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia. *Clinical cancer research : an official journal of the American Association for Cancer Research* **14**, 396-404 (2008); published online EpubJan 15 (10.1158/1078-0432.ccr-07-1823).
6. S. Zhang, L. Chen, J. Wang-Rodriguez, L. Zhang, B. Cui, W. Frankel, R. Wu, T. J. Kipps, The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. *The American journal of pathology* **181**, 1903-1910 (2012); published online EpubDec (10.1016/j.ajpath.2012.08.024).
7. A. H. Daneshmanesh, A. Porwit, M. Hojjat-Farsangi, M. Jeddi-Tehrani, K. P. Tamm, D. Grandér, S. Lehmann, S. Norin, F. Shokri, H. Rabbani, H. Mellstedt, A. Österborg, Orphan receptor tyrosine kinases ROR1 and ROR2 in hematological malignancies. *Leukemia & lymphoma* **54**, 843-850 (2013); published online EpubApr (10.3109/10428194.2012.731599).
8. S. Zhang, L. Chen, B. Cui, H. Y. Chuang, J. Yu, J. Wang-Rodriguez, L. Tang, G. Chen, G. W. Basak, T. J. Kipps, ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. *PloS one* **7**, e31127 (2012)10.1371/journal.pone.0031127).

9. R. Derynck, S. J. Turley, R. J. Akhurst, TGFbeta biology in cancer progression and immunotherapy. *Nature reviews. Clinical oncology* **18**, 9-34 (2021); published online EpubJan (10.1038/s41571-020-0403-1).
10. K. Tzavlaki, A. Moustakas, TGF-beta Signaling. *Biomolecules* **10**, (2020); published online EpubMar 23 (10.3390/biom10030487).
11. C. C. Kloss, J. Lee, A. Zhang, F. Chen, J. J. Melenhorst, S. F. Lacey, M. V. Maus, J. A. Fraietta, Y. Zhao, C. H. June, Dominant-Negative TGF-beta Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation And Augments Prostate Cancer Eradication. *Molecular therapy : the journal of the American Society of Gene Therapy* **26**, 1855-1866 (2018); published online EpubJul 5 (10.1016/j.ymthe.2018.05.003).
12. L. M. Alabanza, Y. Xiong, B. Vu, B. Webster, D. Wu, P. Hu, Z. Zhu, B. Dropulic, P. Dash, D. Schneider, Armored BCMA CAR T Cells Eliminate Multiple Myeloma and Are Resistant to the Suppressive Effects of TGF-beta. *Frontiers in immunology* **13**, 832645 (2022)10.3389/fimmu.2022.832645).
13. N. Tang, C. Cheng, X. Zhang, M. Qiao, N. Li, W. Mu, X. F. Wei, W. Han, H. Wang, TGF-beta inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors. *JCI insight* **5**, (2020); published online EpubFeb 27 (10.1172/jci.insight.133977).
14. V. Narayan, J. S. Barber-Rotenberg, I. Y. Jung, S. F. Lacey, A. J. Rech, M. M. Davis, W. T. Hwang, P. Lal, E. L. Carpenter, S. L. Maude, G. Plesa, N. Vapiwala, A. Chew, M. Moniak, R. A. Sebro, M. D. Farwell, A. Marshall, J. Gilmore, L. Lledo, K. Dengel, S. E. Church, T. D. Hether, J. Xu, M. Gohil, T. H. Buckingham, S. S. Yee, V. E. Gonzalez, I. Kulikovskaya, F. Chen, L. Tian, K. Tien, W. Gladney, C. L. Nobles, H. E. Raymond, I. Prostate Cancer Cellular Therapy Program, E. O. Hexner, D. L. Siegel, F. D. Bushman, C. H. June, J. A. Fraietta, N. B. Haas, PSMA-targeting TGFbeta-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nature medicine* **28**, 724-734 (2022); published online EpubApr (10.1038/s41591-022-01726-1).
15. D. Schneider, Y. Xiong, D. Wu, V. Nölle, S. Schmitz, W. Haso, A. Kaiser, B. Dropulic, R. J. Orentas, A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. *Journal for immunotherapy of cancer* **5**, 42 (2017)10.1186/s40425-017-0246-1).
16. D. Schneider, Y. Xiong, D. Wu, P. Hu, L. Alabanza, B. Steimle, H. Mahmud, K. Anthony-Gonda, W. Krueger, Z. Zhu, D. S. Dimitrov, R. J. Orentas, B. Dropulic, Trispecific CD19-CD20-CD22-targeting duoCAR-T cells eliminate antigen-heterogeneous B cell tumors in preclinical models. *Science translational medicine* **13**, (2021); published online EpubMar 24 (10.1126/scitranslmed.abc6401).
17. M. Hudecek, M. T. Lupo-Stanghellini, P. L. Kosasih, D. Sommermeyer, M. C. Jensen, C. Rader, S. R. Riddell, Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* **19**, 3153-3164 (2013); published online EpubJun 15 (10.1158/1078-0432.ccr-13-0330).
18. E. Batlle, J. Massague, Transforming Growth Factor-beta Signaling in Immunity and Cancer. *Immunity* **50**, 924-940 (2019); published online EpubApr 16 (10.1016/j.immuni.2019.03.024).

REFERENCE TO THE SEQUENCE LISTING

This application contains a Sequence Listing electronically to be submitted to the United States Patent and Trademark Receiving Office via a PDF file entitled "Sequence Listing". The Sequence Listing is incorporated by reference.

SEQUENCES OF THE DISCLOSURE

The nucleic and amino acid sequences listed below are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO: 1 nucleotide sequence of CD20-reactive scFv binding domain (LTG1495):

GAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCAGCGTG
AAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCACTGGG
TGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATCTACCCCGGGA
ATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACCCTGACCGCCG
ACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCTCCGAGGACTC
CGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTACTGGTTCTTC
GATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAGGATCC
GGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCG
GCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCG
TCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCGTCACCCAAG
CCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGCGGTTCAGCG
GGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGAGGCTGAGGA
CGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTTGGAGGC
GGTACTAAGCTGGAGATCAAA

SEQ ID NO: 2 amino acid sequence of CD20-reactive scFv binding domain (LTG1495):

EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGN
GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDV
WGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNY
MDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQ
QWSFNPPTFGGGTKLEIK

SEQ ID NO: 3 nucleotide sequence of CAR LTG1495 (LP-1495-CD8 TM-41BB-CD3zeta):

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC

ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGCGGCCGCAACTACCACCCCTGCC
CCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCC
CCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACT
TTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCT
GCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTAC
ATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGA
TGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAG
TTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACA
ACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGC
GGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGG
ACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGG
GATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGAC
TGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACC
CCGG

SEQ ID NO: 4 amino acid sequence of CAR LTG1495 (LP-1495-CD8 TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKAAATTTPAPRPPTPAPTIA
SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR
KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 5 nucleotide sequence of leader/signal peptide sequence:

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCG

SEQ ID NO: 6 amino acid sequence of leader/signal peptide sequence:

MLLLVTSLLLCELPHPAFLLIP

SEQ ID NO: 7 nucleotide sequence of CD22-reactive scFv binding domain LTG2200):

CAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAGCCAGACGCTG
TCCCTGACTTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGCGCGGCGTGGA
ATTGGATTCGACAATCTCCTTCCCGAGGGTTGGAATGGCTTGGACGAACATATTA
CAGATCCAAATGGTATAACGACTATGCGGTATCAGTAAAGTCAAGAATAACCAT
TAACCCCGACACAAGCAAGAACCAATTCTCTTTGCAGCTTAACTCTGTCACGCCA
GAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGACCTGGAAGAC
GCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTCAGGGGGCGGT
GGGAGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTCAGATGACCCA
GTCCCCTTCATCCTTGTCTGCCTCCGTCGGTGACAGGGTGACAATAACATGCAGA
GCAAGCCAAACAATCTGGAGCTATCTCAACTGGTACCAGCAGCGACCAGGAAAA
GCGCCAAACCTGCTGATTTACGCTGCTTCCTCCCTCCAATCAGGCGTGCCTAGTA
GATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTATAAGCTCTCTTCA
AGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTATACCTCAGACT
TTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCA

SEQ ID NO: 8 amino acid sequence of CD22-reactive scFv binding domain (LTG2200):

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRS
KWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREVTGDLEDAFDIW
GQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQTIWSY
LNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGSGTDFTLTISSLQAEDFATYYCQQ
SYSIPQTFGQGTKLEIKAAA

SEQ ID NO: 9 nucleotide sequence of the CAR LTG2200 (LP-2200-CD8 TM-41BB-CD3zeta):

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCT
GCTTATTCCCCAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAG
CCAGACGCTGTCCCTGACTTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGC
GCGGCGTGGAATTGGATTCGACAATCTCCTTCCCGAGGGTTGGAATGGCTTGGAC
GAACATATTACAGATCCAAATGGTATAACGACTATGCGGTATCAGTAAAGTCAA
GAATAACCATTAACCCCGACACAAGCAAGAACCAATTCTCTTTGCAGCTTAACTC
TGTCACGCCAGAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGA
CCTGGAAGACGCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTC
AGGGGGCGGTGGGAGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTC
AGATGACCCAGTCCCCTTCATCCTTGTCTGCCTCCGTCGGTGACAGGGTGACAAT
AACATGCAGAGCAAGCCAAACAATCTGGAGCTATCTCAACTGGTACCAGCAGCG
ACCAGGAAAAGCGCCAAACCTGCTGATTTACGCTGCTTCCTCCCTCCAATCAGGC
GTGCCTAGTAGATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTATAA
GCTCTCTTCAAGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTAT
ACCTCAGACTTTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCAACTAC
CACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTC
TCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGG
GGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCG
GCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAA
GCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAA
GAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACT
GCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAA

TCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGA
CAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACC
CTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACT
CAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTG
TACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAA
GCACTCCCACCCCGG

SEQ ID NO: 10 amino acid sequence of CAR LTG2200(LP-2200-CD8 TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAREVTGDLEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
LSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGS
GTDFTLTISSLQAEDFATYYCQQSYSIPQTFGQGTKLEIKAAATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 11 nucleotide sequence of DNA CD8 transmembrane domain:

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGG
TTATCACCCTTTACTGC

SEQ ID NO: 12 amino acid sequence of CD8 transmembrane domain:

IWAPLAGTCGVLLLSLVITLYC

SEQ ID NO: 13 nucleotide sequence of DNA CD8 hinge domain:

ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAG
CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCAC
ACGAGGGGGCTGGACTTTGCCTGCGATATCTAC
SEQ ID NO: 14 amino acid sequence of CD8 hinge domain:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY

SEQ ID NO: 15 amino acid sequence of amino acid numbers 137 to 206 of the hinge and transmembrane region of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3):

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV
LLLSLVITLYC

SEQ ID NO: 16 amino acid sequence of Human IgG CL sequence:

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT
PSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 17 nucleotide sequence of DNA signaling domain of 4-1BB:

AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCA
GTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAA
GAAGGAGGATGTGAACTG

SEQ ID NO: 18 amino acid sequence of signaling domain of 4-1BB:

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 19 nucleotide sequence of DNA signaling domain of CD3-zeta:

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAA
CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGA
CAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACA
GTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTT
TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG
GCCCTGCCCCCTCGC

SEQ ID NO: 20 amino acid sequence of CD3zeta:

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

SEQ ID NO: 21 nucleotide sequence of CAR LTG1562 (LP-CD19binder-CD8linker-CD4tm-4-1BB-CD3-zeta):

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGATATTCAGATGACCCAGACCACCAGCAGCCTGAGCGCGAGCC
TGGGCGATCGCGTGACCATTAGCTGCCGCGCGAGCCAGGATATTAGCAAATATC
TGAACTGGTATCAGCAGAAACCGGATGGCACCGTGAAACTGCTGATTTATCATA
CCAGCCGCCTGCATAGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCA
CCGATTATAGCCTGACCATTAGCAACCTGGAACAGGAAGATATTGCGACCTATTT
TTGCCAGCAGGGCAACACCCTGCCGTATACCTTTGGCGGCGGCACCAAACTGGA
AATTACCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCG
AAGTGAAACTGCAGGAAAGCGGCCCGGGCCTGGTGGCGCCGAGCCAGAGCCTG
AGCGTGACCTGCACCGTGAGCGGCGTGAGCCTGCCGGATTATGGCGTGAGCTGG
ATTCGCCAGCCGCCGCGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGGGCAGC
GAAACCACCTATTATAACAGCGCGCTGAAAAGCCGCCTGACCATTATTAAAGAT
AACAGCAAAAGCCAGGTGTTTCTGAAAATGAACAGCCTGCAGACCGATGATACC
GCGATTTATTATTGCGCGAAACATTATTATTATGGCGGCAGCTATGCGATGGATT
ATTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGCGCGGCGGCGCCGGCGCCGC
GCCCGCCGACCCCGGCGCCGACCATTGCGAGCCAGCCGCTGAGCCTGCGCCCGG
AAGCGTGCCGCCCGGCGGCGGGCGGCGCGGTGCATACCCGCGGCCTGGATTTTG
TGCAGCCGATGGCGCTGATTGTGCTGGGCGGCGTGGCGGGCCTGCTGCTGTTTAT
TGGCCTGGGCATTTTTTTTTGCGTGCGCTGCCGCCCGCGCCGCAAAAAACTGCTG
TATATTTTTAAACAGCCGTTTATGCGCCCGGTGCAGACCACCCAGGAAGAAGATG
GCTGCAGCTGCCGCTTTCCGGAAGAAGAAGAAGGCGGCTGCGAACTGCGCGTGA

AATTTAGCCGCAGCGCGGATGCGCCGGCGTATCAGCAGGGCCAGAACCAGCTGT
ATAACGAACTGAACCTGGGCCGCCGCGAAGAATATGATGTGCTGGATAAACGCC
GCGGCCGCGATCCGGAAATGGGCGGCAAACCGCGCCGCAAAAACCCGCAGGAA
GGCCTGTATAACGAACTGCAGAAAGATAAAATGGCGGAAGCGTATAGCGAAATT
GGCATGAAAGGCGAACGCCGCCGCGGCAAAGGCCATGATGGCCTGTATCAGGGC
CTGAGCACCGCGACCAAAGATACCTATGATGCGCTGCATATGCAGGCGCTGCCG
CCGCGC

SEQ ID NO: 22 amino acid sequence of CAR LTG1562 (LP-CD19binder-CD8link-CD4tm-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD
YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTD
DTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSAAAPAPRPPTPAPTIASQPLSLRPE
ACRPAAGGAVHTRGLDFVQPMALIVLGGVAGLLLFIGLGIFFCVRCRPRRKKLLYIFK
QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL
GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 23 nucleotide sequence of CD20_19-reactive scFv binding domain (LTG1497 dual specific binder):

GAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCAGCGTG
AAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCACTGGG
TGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATCTACCCCGGGA
ATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACCCTGACCGCCG
ACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCTCCGAGGACTC
CGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTACTGGTTCTTC
GATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAGGATCC
GGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCG
GCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCG
TCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCGTCACCCAAG
CCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGCGGTTCAGCG
GGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGAGGCTGAGGA
CGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTTGGAGGC
GGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGGAGGGTCCGG
AGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCGACATTCAGAT
GACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGTGACCATCTCA
TGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCAGCAGAAGCCC
GACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCACAGCGGAGTG
CCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTTACTATTTCCA
ACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAAACACCCTGC
CGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCACATCCGGTT
CCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCAAGCTGCAG
GAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGACTTGTACTG
TGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCAGCCACCTCG
GAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCACCTATTACAA
CTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAGTCACAAGT

GTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTACTATTGCGCC
AAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCCAGGGGACC
AGCGTGACCGTGTCATCCGCGGCCGCA

SEQ ID NO: 24 amino acid sequence of CD20_19-reactive scFv binding domain (LTG1497 dual specific binder):

EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGN
GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDV
WGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNY
MDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQ
QWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQTTSSLSAS
LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYS
LTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQ
ESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSA
LKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTV
SSAAA

SEQ ID NO: 25 nucleotide sequence of CAR LTG1497 (LP-LTG1497-CD8 TM-41BB-CD3zeta) or (LP-CD20 VH-$(GGGGS)_3$-CD20 VL-$(GGGGS)_5$-CD19VL-Whitlow linker-CD19 VH-CD8 hinge+TM-41BB-CD3zeta):

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAG
GAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGACTTGTACTG
TGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCAGCCACCTCG
GAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCACCTATTACAA
CTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAGTCACAAGT

GTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTACTATTGCGCC
AAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCCAGGGGACC
AGCGTGACCGTGTCATCCGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGA
CTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCG
CCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATAT
CTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTC
ATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGC
CGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGAT
TCCCT
GAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGAC
GCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGA
AGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGAT
GGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCC
AGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGG
AGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAA
GGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 26 amino acid sequence of CAR LTG1497 (LP-LTG1497-CD8 TM-41BB-CD3zeta) or (LP-CD20 VH $(GGGGS)_3$-CD20 VL-$(GGGGS)_5$-CD19 VL-Whitlow linker-CD19 VH-CD8 hinge+TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 27 nucleotide sequence of scFV for CD19:

GACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAG
TCACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCA
GCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATACATCAAGATTACA
CTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTC
ACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTA
ATACGCTTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAGATCACAGGTGGCG
GTGGCTCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCTGAGGTGAAACTGCAGG
AGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACTGT
CTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGA
AAGGGTCTGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAAT
TCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACTCCAAGAGCCAAGTTT
TCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCAA

ACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGAACCTCA
GTCACCGTCTCCTCA

SEQ ID NO: 28 amino acid sequence of scFV for CD19:

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV
PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGG
SGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVI
WGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAM
DYWGQGTSVTVSS

SEQ ID NO: 29 nucleotide sequence of CAR LTG 1494 (LP-CD19binder-CD8link-CD8tm-41BB-CD3zeta):

ATGCTTCTCCTGGTCACCTCCCTGCTCCTCTGCGAACTGCCTCACCCTGCCTTCCT
TCTGATTCCTGACACTGACATTCAGATGACTCAGACCACCTCTTCCTTGTCCGCGT
CACTGGGAGACAGAGTGACCATCTCGTGTCGCGCAAGCCAGGATATCTCCAAGT
ACCTGAACTGGTACCAACAGAAGCCCGACGGGACTGTGAAGCTGCTGATCTACC
ACACCTCACGCCTGCACAGCGGAGTGCCAAGCAGATTCTCCGGCTCCGGCTCGG
GAACCGATTACTCGCTTACCATTAGCAACCTCGAGCAGGAGGACATCGCTACCTA
CTTCTGCCAGCAAGGAAATACCCTGCCCTACACCTTCGGCGGAGGAACCAAATT
GGAAATCACCGGCTCCACGAGCGGCTCCGGGAAGCCTGGTTCCGGGGAAGGCTC
CACTAAGGGTGAAGTGAAGCTCCAGGAGTCCGGCCCCGGCCTGGTGGCGCCGTC
GCAATCACTCTCTGTGACCTGTACCGTGTCGGGAGTGTCCCTGCCTGATTACGGC
GTGAGCTGGATTCGGCAGCCGCCGCGGAAGGGCCTGGAATGGCTGGGTGTCATC
TGGGGATCCGAGACTACCTACTACAACTCGGCCCTGAAGTCCCGCCTGACTATCA
TCAAAGACAACTCGAAGTCCCAGGTCTTTCTGAAGATGAACTCCCTGCAAACTGA
CGACACCGCCATCTATTACTGTGCTAAGCACTACTACTACGGTGGAAGCTATGCT
ATGGACTACTGGGGCCAGGGGACATCCGTGACAGTCAGCTCCGCGGCCGCAACT
ACCACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCC
TCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCG
GGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGC
GGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGA
AGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGA
AGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAAC
TGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGA
ATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGG
ACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAAC
CCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTAC
TCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCT
GTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCA
AGCACTCCCACCCCGG

SEQ ID NO: 30 amino acid sequence of CAR LTG1494 (LP-CD19binder-CD8link-CD8tm-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPDTDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT
LPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMN

SLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 31 nucleotide sequence of CAR LTG1538 (LP-CD19binder-CD8link-CD8tm-signals (LTI re-engineered CD19 CAR):

ATGCTTCTCCTGGTCACCTCCCTGCTCCTCTGCGAACTGCCTCACCCTGCCTTCCT
TCTGATTCCTGACATTCAGATGACTCAGACCACCTCTTCCTTGTCCGCGTCACTGG
GAGACAGAGTGACCATCTCGTGTCGCGCAAGCCAGGATATCTCCAAGTACCTGA
ACTGGTACCAACAGAAGCCCGACGGGACTGTGAAGCTGCTGATCTACCACACCT
CACGCCTGCACAGCGGAGTGCCAAGCAGATTCTCCGGCTCCGGCTCGGGAACCG
ATTACTCGCTTACCATTAGCAACCTCGAGCAGGAGGACATCGCTACCTACTTCTG
CCAGCAAGGAAATACCCTGCCCTACACCTTCGGCGGAGGAACCAAATTGGAAAT
CACCGGCGGAGGAGGCTCCGGGGGAGGAGGTTCCGGGGGCGGGGGTTCCGAAG
TGAAGCTCCAGGAGTCCGGCCCCGGCCTGGTGGCGCCGTCGCAATCACTCTCTGT
GACCTGTACCGTGTCGGGAGTGTCCCTGCCTGATTACGGCGTGAGCTGGATTCGG
CAGCCGCCGCGGAAGGGCCTGGAATGGCTGGGTGTCATCTGGGGATCCGAGACT
ACCTACTACAACTCGGCCCTGAAGTCCCGCCTGACTATCATCAAAGACAACTCGA
AGTCC
CAGGTCTTTCTGAAGATGAACTCCCTGCAAACTGACGACACCGCCATCTATTACT
GTGCTAAGCACTACTACTACGGTGGAAGCTATGCTATGGACTACTGGGGGCAAG
GCACTTCGGTGACTGTGTCAAGCGCGGCCGCAACTACCACCCCTGCCCCTCGGCC
GCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCT
TGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGC
GATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGC
TGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAA
GCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTG
CAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACG
GTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCT
GAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCG
ACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTAC
AACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGAA
GGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGCA
CCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 32 amino acid sequence of CAR LTG1538 (LP-CD19binder-CD8link-CD8tm-signals (LTI re-engineered CD19 CAR):

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD
YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTD
DTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSL
RPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 33 nucleotide sequence of CD19_20-reactive scFv binding domain (LTG1496):

GACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCG
TGACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGGAGGCGGCGGCAGCGGCGGGGGAGGG
TCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCGAGGT
GCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCAGCGTGAAGAT
GAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCACTGGGTGAAA
CAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATCTACCCCGGGAATGGC
GATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACCCTGACCGCCGACAAG
AGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCTCCGAGGACTCCGCCG
ACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTACTGGTTCTTCGATGT
CTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAGGATCCGGTGG
AGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCGGCAAT
CCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCGTCCAG
CGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCGTCACCCAAGCCTTG
GATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGCGGTTCAGCGGGTCC
GGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGAGGCTGAGGACGCC
GCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTTGGAGGCGGTA
CTAAGCTGGAGATCAAAGCGGCCGCA

SEQ ID NO: 34 amino acid sequence of CD19_20-reactive scFv binding domain (LTG1496):

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV
PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPG
SGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA
MDYWGQGTSVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLQQSGAELVKPG
ASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATL
TADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGG
GSGGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKP
WIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTK
LEIKAAA

SEQ ID NO: 35 nucleotide sequence of CAR LTG1496 (LP-LTG1496-CD8 TM-41BB-CD3zeta) or (LP-CD19 VL-Whitlow linker-CD19 VH $(GGGGS)_5$ CD20 VH $(GGGGS)_3$-CD20 VL CD8 hinge+TM-41BB-CD3zeta):

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTG
GGCGACCGCGTGACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTC
AACTGGTACCAGCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACC
TCCCGGCTGCACAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTG
ACTACTCCCTTACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTG
CCAACAAGGAAACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAAT
CACTGGCAGCACATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAA
GGGGGAAGTCAAGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTC
ACTGTCCGTGACTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCC
TGGATCAGGCAGCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGT
TCCGAAACCACCTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGG
ATAACTCCAAGTCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACA
CGGCGATCTACTATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGA
CTACTGGGGCCAGGGGACCAGCGTGACCGTGTCATCCGGAGGCGGCGGCAGCGG
CGGGGGAGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTG
GCAGCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCA
GCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCA
CTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATCTACCC
CGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACCCTGAC
CGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCTCCGAG
GACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTACTGGT
TCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAG
GATCCGGT
GGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCGGCA
ATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCGTCC
AGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCGTCACCCAAGCCT
TGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGCGGTTCAGCGGGT
CCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGAGGCTGAGGACGC
CGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTTGGAGGCGGT
ACTAAGCTGGAGATCAAAGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCG
ACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCC
GCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATA
TCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGT
CATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAG
CCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGA
TTCCCT
GAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGAC
GCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGA
AGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGAT
GGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCC
AGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGG
AGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAA
GGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 36 amino acid sequence of CAR LTG1496 (LP-LTG1496-CD8 TM-41BB-CD3zeta) or (LP-CD19 VL-Whitlow linker-CD19 VH-$(GGGGS)_5$-CD20 VH $(GGGGS)_3$-CD20 VL-CD8 hinge+TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSL
PDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQ
TDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSGGGGSGGGGSGGGGSGGGGS
GGGGSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGA
IYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSY
WFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRA
SSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDA
ATYYCQQWSFNPPTFGGGTKLEIKAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAA
GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 37 nucleotide sequence of mesothelin-reactive scFv binding domain (LTG1904):

GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGG
TCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATA
GTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAG
ACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACA
CGGCCTTGTATTACTGTGCAAAAGATTTATCGTCAGTGGCTGGACCCTTTAACTA
CTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTGGTGG
AGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGGACCCTGCTGTG
TCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAGA
AGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTC
ATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCA
GCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAGGATGAGG
CTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATCTGGTATTCGGCGG
AGGCACCCAGCTGACCGTCCTCGGT

SEQ ID NO: 38 amino acid sequence of mesothelin-reactive scFv binding domain (LTG1904):

EVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNS
GSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDLSSVAGPFNYWG
QGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSR
DSSGNHLVFGGGTQLTVLG

SEQ ID NO: 39 nucleotide sequence of CAR LTG1904 (LP-LTG1904-CD8 TM-41BB-CD3zeta):

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTG
GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGC
CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTAT
TAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCAC
CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG
AGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATTTATCGTCAGTGGCTGGA
CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
GGTCTGGTGGAGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGG
ACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAG
ACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCC
CTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
CTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCG
GAGGATGAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATCTG
GTATTCGGCGGAGGCACCCAGCTGACCGTCCTCGGTGCGGCCGCAACTACCACC
CCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCT
TGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGC
TGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGT
GCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTG
CTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAG
GACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGC
GTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAG
CTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAA
GCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTC
AGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCA
GAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTA
CCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGC
ACTCCCACCCCGG

SEQ ID NO: 40 amino acid sequence of CAR LTG1904 (LP-LTG1904-CD8 TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
WVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAED
TALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAV
SVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSG
NTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGAAATTTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 41 nucleotide sequence of CD33-reactive single chain binding domain VH-4 (LTG1906):

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGAGCTGGG
TCCGCCAGGCTCCAAGACAAGGGCTTGAGTGGGTGGCCAACATAAAGCAAGATG
GAAGTGAGAAATACTATGCGGACTCAGTGAAGGGCCGATTCACCATCTCCAGAG
ACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CAGCCACGTATTACTGTGCGAAAGAAAATGTGGACTGGGGCCAGGGCACCCTGG
TCACCGTCTCCTCA

SEQ ID NO: 42 amino acid sequence of CD33-reactive single chain binding domain VH-4 (LTG1906):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPRQGLEWVANIKQDGS
EKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTATYYCAKENVDWGQGTLVTV
SS

SEQ ID NO: 43 nucleotide sequence of CAR LTG1906 (LP-VH4-CD8 TM-41BB-CD3zeta):

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTG
GAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGG
CATGAGCTGGGTCCGCCAGGCTCCAAGACAAGGGCTTGAGTGGGTGGCCAACAT
AAAGCAAGATGGAAGTGAGAAATACTATGCGGACTCAGTGAAGGGCCGATTCAC
CATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAG
AGCCGAGGACACAGCCACGTATTACTGTGCGAAAGAAAATGTGGACTGGGGCCA
GGGCACCCTGGTCACCGTCTCCTCAGCGGCCGCAACTACCACCCCTGCCCCTCGG
CCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAG
CTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTG
CGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCG
CTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCA
AGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGT
GCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCAC
GGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGC
TGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGC
GACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTA
CAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGA
AGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGC
ACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 44 amino acid sequence of CAR LTG1906 (LP-VH4-CD8 TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSW
VRQAPRQGLEWVANIKQDGSEKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA
TYYCAKENVDWGQGTLVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG
AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT
TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL

DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 45 nucleotide sequence of TSLPR-reactive scFv binding domain (LTG1789):

ATGGCACTGCCCGTGACCGCCCTGCTTCTGCCGCTTGCACTTCTGCTGCACGCCG
CTAGGCCCCAAGTCACCCTCAAAGAGTCAGGGCCAGGAATCCTCAAGCCCTCAC
AGACTCTGTCTCTTACTTGCTCATTCAGCGGATTCAGCCTTTCCACCTCTGGTATG
GGCGTGGGGTGGATTAGGCAACCTAGCGGAAAGGGGCTTGAATGGCTGGCCCAC
ATCTGGTGGGACGACGACAAGTACTACAACCCCTCACTGAAGTCCCAGCTCACT
ATTTCCAAAGATACTTCCCGGAATCAGGTGTTCCTCAAGATTACCTCTGTCGACA
CCGCTGATACCGCCACTTACTATTGTTCACGCAGACCGAGAGGTACCATGGACGC
AATGGACTACTGGGGACAGGGCACCAGCGTGACCGTGTCATCTGGCGGTGGAGG
GTCAGGAGGTGGAGGTAGCGGAGGCGGTGGGTCCGACATTGTCATGACCCAGGC
CGCCAGCAGCCTGAGCGCTTCACTGGGCGACAGGGTGACCATCAGCTGTCGCGC
ATCACAAGATATCTCTAAGTATCTTAATTGGTACCAGCAAAAGCCGGATGGAAC
CGTGAAGCTGCTGATCTACTACACCTCACGGCTGCATTCTGGAGTGCCTAGCCGC
TTTAGCGGATCTGGGTCCGGTACTGACTACAGCCTCACCATTAGAAACCTTGAAC
AGGAGGACATCGCAACTTATTTCTGCCAACAGGTCTATACTCTGCCGTGGACCTT
CGGCGGAGGTACCAAACTGGAGATTAAGTCCGG

SEQ ID NO: 46 amino acid sequence of TSLPR-reactive scFv binding domain (LTG1789):

MALPVTALLLPLALLLHAARPQVTLKESGPGILKPSQTLSLTCSFSGFSLSTSGMGVG
WIRQPSGKGLEWLAHIWWDDDKYYNPSLKSQLTISKDTSRNQVFLKITSVDTADTAT
YYCSRRPRGTMDAMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVMTQAASSLSA
SLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDY
SLTIRNLEQEDIATYFCQQVYTLPWTFGGGTKLEIKS

SEQ ID NO: 47 nucleotide sequence of CAR LTG1789 (LP-3G11-CD8 TM-41BB-CD3zeta):

ATGGCACTGCCCGTGACCGCCCTGCTTCTGCCGCTTGCACTTCTGCTGCACGCCG
CTAGGCCCCAAGTCACCCTCAAAGAGTCAGGGCCAGGAATCCTCAAGCCCTCAC
AGACTCTGTCTCTTACTTGCTCATTCAGCGGATTCAGCCTTTCCACCTCTGGTATG
GGCGTGGGGTGGATTAGGCAACCTAGCGGAAAGGGGCTTGAATGGCTGGCCCAC
ATCTGGTGGGACGACGACAAGTACTACAACCCCTCACTGAAGTCCCAGCTCACT
ATTTCCAAAGATACTTCCCGGAATCAGGTGTTCCTCAAGATTACCTCTGTCGACA
CCGCTGATACCGCCACTTACTATTGTTCACGCAGACCGAGAGGTACCATGGACGC
AATGGACTACTGGGGACAGGGCACCAGCGTGACCGTGTCATCTGGCGGTGGAGG
GTCAGGAGGTGGAGGTAGCGGAGGCGGTGGGTCCGACATTGTCATGACCCAGGC
CGCCAGCAGCCTGAGCGCTTCACTGGGCGACAGGGTGACCATCAGCTGTCGCGC
ATCACAAGATATCTCTAAGTATCTTAATTGGTACCAGCAAAAGCCGGATGGAAC
CGTGAAGCTGCTGATCTACTACACCTCACGGCTGCATTCTGGAGTGCCTAGCCGC
TTTAGC
GGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGG
GCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGAC
GACTCAGGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGG
GATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACA
GGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACG

ACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGG
CGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGC
GGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTC
ACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCT
TGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 48 amino acid sequence of CAR LTG1789 (LP-3G11-CD8 TM-41BB-CD3zeta):

MALPVTALLLPLALLLHAARPQVTLKESGPGILKPSQTLSLTCSFSGFSLSTSGMGVG
WIRQPSGKGLEWLAHIWWDDDKYYNPSLKSQLTISKDTSRNQVFLKITSVDTADTAT
YYCSRRPRGTMDAMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVMTQAASSLSA
SLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDY
SLTIRNLEQEDIATYFCQQVYTLPWTFGGGTKLEIKAAATTTPAPRPPTPAPTIASQPL
SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNE
LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 49 nucleotide sequence of CAR LTG1563 (LP-CD19-TNFRSF19TM-41BB-CD3zeta):

ATGCTGCTGCTGGTCACCAGCCTGCTGCTGTGCGAGCTCCCTCACCCCGCCTTTCT
GCTTATCCCGGACATTCAGATGACACAGACCACCTCGAGCTTGTCCGCGTCGCTG
GGCGATCGCGTGACCATCTCCTGCCGGGCCTCCCAAGACATTTCAAAGTATCTCA
ACTGGTACCAGCAGAAGCCGGACGGAACCGTGAAACTGCTGATCTACCATACCA
GCCGCCTGCACTCCGGCGTGCCGTCCCGCTTCTCCGGATCGGGTTCCGGAACTGA
CTACTCACTGACTATCTCCAACTTGGAACAAGAGGACATCGCCACTTACTTCTGT
CAACAAGGAAATACCCTTCCCTACACCTTCGGGGGGGGTACCAAGCTGGAGATC
ACTGGGGGCGGAGGCTCCGGTGGAGGCGGATCCGGCGGTGGAGGGAGCGAAGT
CAAGCTGCAGGAATCAGGACCAGGACTCGTGGCGCCATCCCAGTCCCTGTCGGT
GACCTGTACTGTCTCCGGAGTCAGCCTCCCCGATTACGGAGTGTCATGGATTAGG
CAACCCCCAAGAAAAGGGCTGGAATGGCTCGGAGTGATCTGGGGCTCCGAAACC
ACCTACTACAACTCGGCGCTGAAGTCCCGGCTGACCATCATCAAGGACAACTCC
AAGAGCCAAGTGTTCTTGAAGATGAACAGCTTGCAGACCGACGATACCGCAATC
TACTACTGTGCCAAGCACTATTACTACGGGGGGTCTTACGCCATGGACTACTGGG
GACAGGGCACCTCCGTGACTGTGTCGTCCGCGGCCGCGCCCGCCCCTCGGCCCCC
GACTCCTGCCCCGACGATCGCTTCCCAACCTCTCTCGCTGCGCCCGGAAGCATGC
CGGCCCGCCGCCGGTGGCGCTGTCCACACTCGCGGACTGGACTTTGATACCGCAC
TGGCGGCCGTGATCTGTAGCGCCCTGGCCACCGTGCTGCTGGCGCTGCTCATCCT
TTGCGTGATCTACTGCAAGCGGCAGCCTAGGCGAAAGAAGCTCCTCTACATTTTC
AAGCAACCCTTCATGCGCCCCGTGCAAACCACCCAGGAGGAGGATGGATGCTCA
TGCCGGTTCCCTGAGGAAGAAGAGGGCGGTTGCGAGCTCAGAGTGAAATTCAGC
CGGTCGGCTGACGCCCCGGCGTACCAGCAGGGCCAGAACCAGCTGTACAATGAG
CTCAACCTGGGGCGCCGCGAAGAGTACGACGTGCTGGACAAGAGGAGAGGCAG
AGATCCGGAAATGGGCGGAAAGCCAAGGCGGAAGAACCCGCAGGAAGGTCTTT
ACAACGAACTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGATTGGGATGA
AGGGAGAAAGACGGAGGGGAAAGGGACATGACGGACTTTACCAGGGCCTGAGC
ACTGCCACGAAGGACACCTATGATGCCCTGCACATGCAGGCGCTGCCGCCTCGG

SEQ ID NO: 50 amino acid sequence of CAR LTG1563 (LP-CD19-TNFRSF19TM-41BB-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQ
QKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD
YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTD
DTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSAAAPAPRPPTPAPTIASQPLSLRPE
ACRPAAGGAVHTRGLDFDTALAAVICSALATVLLALLILCVIYCKRQPRRKKLLYIFK
QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL
GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 51 nucleotide acid sequence of CAR LTG2228 (LP-CD20_CD19-CD8TM-CD28-CD3zeta):

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGCACTCCG

ACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACTACCAGC
CGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCAGAGTGAAGTTCAGCCG
CTCAGCCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCT
CAACCTGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAG
ATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTAC
AACGAACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAA
GGGAGAACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAA
CTGCCACTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGG

SEQ ID NO: 52 amino acid sequence of CAR LTG2228 (LP-CD20_CD19-CD8TM-CD28-CD3zeta):

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRP
GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 53 nucleotide sequence of D0043:

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA

ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGCACTCCG
ACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACTACCAGC
CGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCAGAGTGAAGTTCAGCCG
CTCAGCCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCT
CAACCTGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAG
ATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTAC
AACGAACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAA
GGGAGAACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAA
CTGCCACTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGGCG
CGCGAAACGCGGCAGCGGCGCGACCAACTTTAGCCTGCTGAAACAGGCGGGCGA
TGTGGAAGAAAACCCGGGCCCGCGAGCAAAGAGGAATATTATGCTTCTATTAGT
GACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCTGCTTATTCCCCAGG
TACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAGCCAGACGCTGTCCC
TGACTTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGCGCGGCGTGGAATTG
GATTCGACAATCTCCTTCCCGAGGGTTGGAATGGCTTGGACGAACATATTACAGA
TCCAAATGGTATAACGACTATGCGGTATCAGTAAAGTCAAGAATAACCATTAAC
CCCGACACAAGCAAGAACCAATTCTCTTTGCAGCTTAACTCTGTCACGCCAGAAG
ACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGACCTGGAAGACGCTTT
TGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTCAGGGGGCGGTGGGAG
TGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTCAGATGACCCAGTCCC
CTTCATCCTTGTCTGCCTCCGTCGGTGACAGGGTGACAATAACATGCAGAGCAAG
CCAAACAATCTGGAGCTATCTCAACTGGTACCAGCAGCGACCAGGAAAAGCGCC
AAACCTGCTGATTTACGCTGCTTCCTCCCTCCAATCAGGCGTGCCTAGTAGATTT
AGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTATAAGCTCTCTTCAAGCAG
AAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTATACCTCAGACTTTCGG
ACAGGGTACCAAGTTGGAGATTAAGGCTAGCGCAACCACTACGCCTGCTCCGCG
GCCTCCAACGCCCGCGCCCACGATAGCTAGTCAGCCGTTGTCTCTCCGACCAGAG
GCGTGTAGACCGGCCGCTGGCGGAGCCGTACATACTCGCGGACTCGACTTCGCTT
GCGACATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTGTTGCTGTC
CTTGGTTATTACGTTGTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTC
AAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCG
TGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGAGAGTCAAATTTTCC
AGGTCCGCAGATGCCCCCGCGTACCAGCAAGGCCAGAACCAACTTTACAACGAA
CTGAACCTGGGTCGCCGGGAGGAATATGATGTGCTGGATAAACGAAGGGGGAGG
GACCCTGAGATGGGAGGGAAACCTCGCAGGAAAAACCCGCAGGAAGGTTTGTAC
AACGAGTTGCAGAAGGATAAGATGGCTGAGGCTTACTCTGAAATAGGGATGAAG
GGAGAGAGACGGAGAGGAAAAGGCCATGATGGCCTTTACCAGGGCTTGAGCAC
AGCAACAAAGGATACTTACGACGCTCTTCACATGCAAGCTCTGCCACCACGG

SEQ ID NO: 54 amino acid sequence of D0043:

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRP
GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIM
LLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN
WIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDT
AVYYCAREVTGDLEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSL
SASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGSG
TDFTLTISSLQAEDFATYYCQQSYSIPQTFGQGTKLEIKASATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 55 nucleotide sequence of D0044:

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA

ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGCACTCCG
ACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACTACCAGC
CGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCAGAGTGAAGTTCAGCCG
CTCAGCCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCT
CAACCTGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAG
ATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTAC
AACGAACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAA
GGGAGAACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAA
CTGCCACTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGGCG
CGCGAAACGCGGCAGCGGCGCGACCAACTTTAGCCTGCTGAAACAGGCGGGCGA
TGTGGAAGAAAACCCGGGCCCGCGAGCAAAGAGGAATATTATGTTGCTGCTCGT
GACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCTGCTCATCCCTCAAG
TGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTGAGCCT
GACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGGCGGCCTGGAACTGG
ATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACTACCGG
TCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCGGATCACCATTAACC
CCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCCCGAGG
ATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTTCGACA
TTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGAGGAGGTTCCGGAG
GCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACCCAGAGCCCCTCCT
CGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTAGAGCGTCCCAGG
ACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCTTGGCTCCTCAACT
GCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATCACGCTTCTCCGGA
TCCGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTCCAGCCTGAGGACT
TCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATACCTTCGGAAGAGG
CACTAAGCTGGAAATCAAGGCTAGCGCAACCACTACGCCTGCTCCGCGGCCTCC
AACGCCCGCGCCCACGATAGCTAGTCAGCCGTTGTCTCTCCGACCAGAGGCGTGT
AGACCGGCCGCTGGCGGAGCCGTACATACTCGCGGACTCGACTTCGCTTGCGAC
ATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTGTTGCTGTCCTTGG
TTATTACGTTGTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCA
GCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAG
ATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGAGAGTCAAATTTTCCAGGTC
CGCAGATGCCCCCGCGTACCAGCAAGGCCAGAACCAACTTTACAACGAACTGAA
CCTGGGTCGCCGGGAGGAATATGATGTGCTGGATAAACGAAGGGGGAGGGACCC
TGAGATGGGAGGGAAACCTCGCAGGAAAAACCCGCAGGAAGGTTTGTACAACG
AGTTGCAGAAGGATAAGATGGCTGAGGCTTACTCTGAAATAGGGATGAAGGGAG
AGAGACGGAGAGGAAAAGGCCATGATGGCCTTTACCAGGGCTTGAGCACAGCA
ACAAAGGATACTTACGACGCTCTTCACATGCAAGCTCTGCCACCACGG

SEQ ID NO: 56 amino acid sequence of D0044:

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRP
GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIM
LLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN
WIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPEDT
AVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKASATTTPAPRPPTPAPTIASQP
LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 59 nucleotide sequence of D0046

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCT
GCTTATTCCCCAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAG
CCAGACGCTGTCCCTGACTTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGC
GCGGCGTGGAATTGGATTCGACAATCTCCTTCCCGAGGGTTGGAATGGCTTGGAC
GAACATATTACAGATCCAAATGGTATAACGACTATGCGGTATCAGTAAAGTCAA
GAATAACCATTAACCCCGACACAAGCAAGAACCAATTCTCTTTGCAGCTTAACTC
TGTCACGCCAGAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGA
CCTGGAAGACGCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTC
AGGGGGCGGTGGGAGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTC
AGATGACCCAGTCCCCTTCATCCTTGTCTGCCTCCGTCGGTGACAGGGTGACAAT
AACATGCAGAGCAAGCCAAACAATCTGGAGCTATCTCAACTGGTACCAGCAGCG
ACCAGGAAAAGCGCCAAACCTGCTGATTTACGCTGCTTCCTCCCTCCAATCAGGC
GTGCCTAGTAGATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTATAA
GCTCTCTTCAAGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTAT
ACCTCAGACTTTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCTACCAC
AACCCCTGCGCCCCGGCCTCCTACCCCCGCACCCACGATTGCTTCTCAACCTCTTT
CACTCCGACCTGAGGCTTGTAGACCTGCAGCCGGGGGTGCCGTCCACACACGGG
GACTCGACTTCGCTTGTGATATATATATTTGGGCGCCCCTGGCCGGCACTTGTGG
AGTTCTTTTGCTCTCTCTTGTTATCACATTGTACTGCAAGCGAGGTAGGAAGAAA
TTGCTTTACATTTTTAAGCAGCCGTTCATGCGACCAGTACAGACTACTCAAGAAG
AAGATGGGTGCTCTTGTCGGTTCCCGGAAGAAGAAGAGGGTGGTTGCGAGTTGA
GGGTGAAGTTCTCCCGCTCTGCCGACGCACCGGCATATCAGCAGGGACAAAACC

AGCTCTACAACGAATTGAACCTGGGTCGGCGGGAAGAATATGACGTGCTCGATA
AGCGGCGGGGTCGCGACCCAGAAATGGGAGGCAAACCGCGCAGGAAAAATCCA
CAGGAGGGACTTTATAACGAACTTCAAAAGGATAAGATGGCAGAGGCATACAGC
GAAATCGGGATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTTA
CCAGGGGCTTTCTACCGCGACGAAGGATACCTACGATGCTCTCCATATGCAAGCA
CTTCCTCCTAGACGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGA
AGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAGTAGACA
TGGCCCTGCCCGTCACTGCGCTGCTTCTTCCACTTGCGCTTCTGCTGCACGCAGCG
CGCCCGGAAGTCCAGCTCCAGCAAAGCGGAGCCGAACTCGTGAAGCCGGGGGCC
TCCGTGAAGATGAGCTGCAAGGCATCCGGCTACACCTTCACTAGCTACAACATGC
ACTGGGTGAAGCAGACTCCGGGTCAAGGGCTGGAGTGGATTGGGGCGATCTACC
CGGGCAACGGCGACACCTCCTACAACCAAAAGTTCAAGGGGAAGGCTACTCTTA
CGGCGGACAAGTCGTCCAGCACCGCATACATGCAACTCTCCTCCCTGACCTCCGA
GGACTCGGCGGACTACTACTGCGCCCGGAGCAACTACTACGGTTCCTCCTACTGG
TTCTTCGACGTGTGGGGTGCCGGAACTACTGTGACTGTGTCCTCCGGTGGTGGCG
GATCAGGCGGCGGGGGATCCGGCGGTGGAGGATCCGACATTGTGCTGACTCAGT
CCCCCGCAATCCTTTCGGCCTCCCCCGGAGAGAAGGTCACGATGACTTGCAGGGC
TTCGTCCTCCGTGAACTACATGGATTGGTACCAAAAGAAGCCCGGGTCGTCGCCT
AAGCCGTGGATCTACGCTACCTCAAACCTGGCTTCCGGCGTCCCTGCGCGGTTCA
GCGGCTCGGGGAGCGGTACCTCATACTCACTCACCATCTCCCGGGTGGAGGCCG
AAGATGCGGCCACCTATTATTGCCAACAGTGGTCCTTCAATCCGCCCACCTTCGG
GGGGGGAACCAAGCTCGAGATCAAGGGGGGTGGCGGCTCAGGGGGAGGCGGAA
GCGGAGGGGGTGGCTCGGGCGGCGGCGGTTCCGGCGGCGGAGGGTCCGATATCC
AAATGACCCAGACTACTAGCTCGTTGAGCGCCTCGCTCGGCGACAGAGTGACCA
TTAGCTGCAGGGCATCCCAGGACATTTCAAAGTACCTGAACTGGTACCAACAGA
AGCCCGACGGAACTGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCACTCCGG
AGTCCCGTCGAGATTTTCCGGCTCCGGAAGCGGAACCGATTATTCGCTCACCATT
TCTAACCTGGAACAGGAGGACATTGCCACTTACTTCTGTCAACAAGGAAACACTC
TGCCTTACACCTTTGGTGGCGGAACCAAGTTGGAAATTACCGGCTCCACCTCCGG
ATCCGGAAAGCCTGGATCCGGAGAGGGATCAACCAAGGGAGAAGTGAAGCTGC
AGGAGAGCGGGCCCGGCCTTGTCGCCCCGAGCCAGTCCTTGTCCGTGACCTGTAC
TGTCTCCGGAGTCAGCCTGCCGGACTACGGGGTGTCCTGGATCCGCCAGCCGCCT
CGCAAGGGCCTGGAGTGGCTCGGCGTGATCTGGGGATCCGAAACGACTTACTAC
AACTCGGCCCTCAAGTCGAGGCTCACTATTATCAAGGACAACTCGAAGTCCCAG
GTGTTCCTCAAGATGAACTCGCTGCAAACCGACGACACAGCGATCTACTACTGTG
CAAAGCATTACTACTACGGAGGCAGCTACGCAATGGACTACTGGGGACAGGGAA
CCTCCGTGACTGTCTCTAGCGCTAGCGCGACCACTACGCCCGCCCCCCGCCCACC
TACCCCCGCCCCGACCATTGCGAGCCAACCGTTGTCACTCCGCCCGGAAGCCTGC
CGCCCCGCCGCTGGCGGAGCCGTGCACACCCGGGGACTGGACTTCGCATGCGAC
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GAACATGACTCCTCGGCGGCCGGGGCCGACTCGGAAGCACTACCAGCCTTACGC
ACCCCCGAGAGATTTCGCGGCCTACCGCTCCCGGGTCAAGTTTTCCCGGTCTGCC
GACGCTCCGGCGTACCAGCAGGGGCAGAACCAGCTCTACAATGAGCTGAATCTG
GGTCGGAGAGAAGAGTACGATGTGCTGGATAAGCGGAGAGGCAGAGATCCAGA
AATGGGAGGAAAGCCTCGGAGAAAGAACCCACAGGAGGGACTGTATAATGAGC
TGCAGAAGGACAAAATGGCCGAAGCCTACAGCGAGATCGGCATGAAGGGAGAG
CGGCGCAGAGGGAAGGGACATGACGGCCTGTACCAGGGTCTGAGCACCGCGACT
AAGGACACCTACGATGCCCTTCATATGCAAGCACTCCCTCCGCGC

SEQ ID NO: 60 amino acid sequence of D0046:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAREVTGDLEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
LSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGS
GTDFTLTISSLQAEDFATYYCQQSYSIPQTFGQGTKLEIKAAATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAG
DVEENPGPRAKRVDMALPVTALLLPLALLLHAARPEVQLQQSGAELVKPGASVKMS
CKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSS
TAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGS
GGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSN
LASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGG
GSGGGGSGGGGSGGGGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNW
YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT
LPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMN
SLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSASATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSR
LLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 61 nucleotide sequence of D0047:

ATGTTGCTGCTCGTGACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCT
GCTCATCCCTCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCC
CAGACTCTGAGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGG
CGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGC
GCACTTACTACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCG
GATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGC
GTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAG
GACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGA
GGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACC
CAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTA
GAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCT
TGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATC
ACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTC
CAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATA
CCTTCGGAAGAGGCACTAAGCTGGAAATCAAGGCGGCCGCTACCACAACCCCTG
CGCCCCGGCCTCCTACCCCCGCACCCACGATTGCTTCTCAACCTCTTTCACTCCGA
CCTGAGGCTTGTAGACCTGCAGCCGGGGGTGCCGTCCACACACGGGGACTCGAC
TTCGCTTGTGATATATATATTTGGGCGCCCCTGGCCGGCACTTGTGGAGTTCTTTT
GCTCTCTCTTGTTATCACATTGTACTGCAAGCGAGGTAGGAAGAAATTGCTTTAC
ATTTTTAAGCAGCCGTTCATGCGACCAGTACAGACTACTCAAGAAGAAGATGGG
TGCTCTTGTCGGTTCCCGGAAGAAGAAGAGGGTGGTTGCGAGTTGAGGGTGAAG
TTCTCCCGCTCTGCCGACGCACCGGCATATCAGCAGGGACAAAACCAGCTCTACA

ACGAATTGAACCTGGGTCGGCGGGAAGAATATGACGTGCTCGATAAGCGGCGGG
GTCGCGACCCAGAAATGGGAGGCAAACCGCGCAGGAAAAATCCACAGGAGGGA
CTTTATAACGAACTTCAAAAGGATAAGATGGCAGAGGCATACAGCGAAATCGGG
ATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTTACCAGGGGCT
TTCTACCGCGACGAAGGATACCTACGATGCTCTCCATATGCAAGCACTTCCTCCT
AGACGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGAAGCAGGCC
GGGGATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAGTAGACATGGCCCTG
CCCGTCACTGCGCTGCTTCTTCCACTTGCGCTTCTGCTGCACGCAGCGCGCCCGG
AAGTCCAGCTCCAGCAAAGCGGAGCCGAACTCGTGAAGCCGGGGGCCTCCGTGA
AGATGAGCTGCAAGGCATCCGGCTACACCTTCACTAGCTACAACATGCACTGGG
TGAAGCAGACTCCGGGTCAAGGGCTGGAGTGGATTGGGGCGATCTACCCGGGCA
ACGGCGACACCTCCTACAACCAAAAGTTCAAGGGGAAGGCTACTCTTACGGCGG
ACAAGTCGTCCAGCACCGCATACATGCAACTCTCCTCCCTGACCTCCGAGGACTC
GGCGGACTACTACTGCGCCCGGAGCAACTACTACGGTTCCTCCTACTGGTTCTTC
GACGTGTGGGGTGCCGGAACTACTGTGACTGTGTCCTCCGGTGGTGGCGGATCA
GGCGGCGGGGGATCCGGCGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCC
GCAATCCTTTCGGCCTCCCCCGGAGAGAAGGTCACGATGACTTGCAGGGCTTCGT
CCTCCGTGAACTACATGGATTGGTACCAAAAGAAGCCCGGGTCGTCGCCTAAGC
CGTGGATCTACGCTACCTCAAACCTGGCTTCCGGCGTCCCTGCGCGGTTCAGCGG
CTCGGGGAGCGGTACCTCATACTCACTCACCATCTCCCGGGTGGAGGCCGAAGA
TGCGGCCACCTATTATTGCCAACAGTGGTCCTTCAATCCGCCCACCTTCGGGGGG
GGAACCAAGCTCGAGATCAAGGGGGGTGGCGGCTCAGGGGGAGGCGGAAGCGG
AGGGGGTGGCTCGGGCGGCGGCGGTTCCGGCGGCGGAGGGTCCGATATCCAAAT
GACCCAGACTACTAGCTCGTTGAGCGCCTCGCTCGGCGACAGAGTGACCATTAG
CTGCAGGGCATCCCAGGACATTTCAAAGTACCTGAACTGGTACCAACAGAAGCC
CGACGGAACTGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCACTCCGGAGTC
CCGTCGAGATTTTCCGGCTCCGGAAGCGGAACCGATTATTCGCTCACCATTTCTA
ACCTGGAACAGGAGGACATTGCCACTTACTTCTGTCAACAAGGAAACACTCTGC
CTTACACCTTTGGTGGCGGAACCAAGTTGGAAATTACCGGCTCCACCTCCGGATC
CGGAAAGCCTGGATCCGGAGAGGGATCAACCAAGGGAGAAGTGAAGCTGCAGG
AGAGCGGGCCCGGCCTTGTCGCCCCGAGCCAGTCCTTGTCCGTGACCTGTACTGT
CTCCGGAGTCAGCCTGCCGGACTACGGGGTGTCCTGGATCCGCCAGCCGCCTCGC
AAGGGCCTGGAGTGGCTCGGCGTGATCTGGGGATCCGAAACGACTTACTACAAC
TCGGCCCTCAAGTCGAGGCTCACTATTATCAAGGACAACTCGAAGTCCCAGGTGT
TCCTCAAGATGAACTCGCTGCAAACCGACGACACAGCGATCTACTACTGTGCAA
AGCATTACTACTACGGAGGCAGCTACGCAATGGACTACTGGGGACAGGGAACCT
CCGTGACTGTCTCTAGCGCTAGCGCGACCACTACGCCCGCCCCCCGCCCACCTAC
CCCCGCCCCGACCATTGCGAGCCAACCGTTGTCACTCCGCCCGGAAGCCTGCCGC
CCCGCCGCTGGCGGAGCCGTGCACACCCGGGGACTGGACTTCGCATGCGACATC
TACATTTGGGCCCCGCTGGCTGGAACCTGTGGAGTCCTGCTGCTCTCCCTCGTGA
TCACTCTGTACTGCCGGTCGAAGCGCTCAAGACTGCTGCACTCAGACTACATGAA
CATGACTCCTCGGCGGCCGGGGCCGACTCGGAAGCACTACCAGCCTTACGCACC
CCCGAGAGATTTCGCGGCCTACCGCTCCCGGGTCAAGTTTTCCCGGTCTGCCGAC
GCTCCGGCGTACCAGCAGGGGCAGAACCAGCTCTACAATGAGCTGAATCTGGGT
CGGAGAGAAGAGTACGATGTGCTGGATAAGCGGAGAGGCAGAGATCCAGAAAT
GGGAGGAAAGCCTCGGAGAAAGAACCCACAGGAGGGACTGTATAATGAGCTGC
AGAAGGACAAAATGGCCGAAGCCTACAGCGAGATCGGCATGAAGGGAGAGCGG
CGCAGAGGGAAGGGACATGACGGCCTGTACCAGGGTCTGAGCACCGCGACTAAG
GACACCTACGATGCCCTTCATATGCAAGCACTCCCTCCGCGC

SEQ ID NO: 62 amino acid sequence of D0047:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGD
VEENPGPRAKRVDMALPVTALLLPLALLLHAARPEVQLQQSGAELVKPGASVKMSC
KASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSST
AYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSG
GGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNL
ASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGS
GGGGSGGGGSGGGGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWY
QQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLP
YTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVS
LPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSL
QTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSASATTTPAPRPPTPAPTIASQP
LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 65 nucleotide sequence of D0001:

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCT
GCTTATTCCCCAGGTACAGCTTCAACAGAGTGGGCCGGGACTGGTGAAACACTC
CCAAACACTTTCTCTGACGTGCGCTATATCAGGTGACTCTGTTTCATCTAATTCTG
CTGCGTGGAACTGGATTCGACAATCTCCCAGTCGCGGGTTGGAATGGCTGGGAC
GAACATATTATCGGTCTAAGTGGTATAACGATTATGCTGTATCTGTTAAATCTCG
AATTACGATTAATCCTGACACCTCCAAGAACCAGTTCTCCCTCCAGTTGAACTCA
GTCACACCGGAAGACACTGCGGTCTACTATTGCGCTCAAGAAGTCGAGCCACAT
GATGCATTCGACATCTGGGGCCAGGGAACGATGGTCACCGTCAGCAGTGGCGGC
GGCGGATCTGGGGGTGGCGGTTCTGGCGGTGGAGGATCAGACATACAAATGACG
CAGAGTCCCTCAAGTGTGTACGCGAGTGTGGGGGATAAGGTAACTATTACGTGC
AGAGCGTCACAGGATGTTAGTGGATGGCTTGCCTGGTATCAGCAGAAGCCAGGC
CTTGCTCCACAGCTCCTTATCAGTGGTGCTTCTACACTTCAGGGCGAGGTTCCGA
GTAGATTCTCTGGTTCTGGATCTGGTACTGACTTCACTCTTACAATTTCTTCTTTG
CAACCAGAAGACTTTGCGACTTATTACTGCCAACAGGCCAAATACTTCCCTTATA
CATTTGGCCAAGGTACCAAGTTGGAGATAAAGGCGGCCGCAACTACCACCCCTG
CCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCG
CCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGA
CTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTC
CTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTT
ACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACG
GATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCA
AGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTA

CAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGAC
GCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAA
GGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATC
GGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGG
ACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCA
CCCCGG

SEQ ID NO: 66 amino acid sequence of D0001:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKHSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPHDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSV
YASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLISGASTLQGEVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQAKYFPYTFGQGTKLEIKAAATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 67 nucleotide sequence of D0002:

ATGTTGCTGCTCGTGACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCT
GCTCATCCCTCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCC
CAGACTCTGAGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGG
CGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGC
GCACTTACTACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCG
GATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGC
GTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAG
GACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGA
GGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACC
CAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTA
GAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCT
TGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATC
ACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTC
CAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATA
CCTTCGGAAGAGGCACTAAGCTGGAAATCAAGGCGGCCGCAACTACCACCCCTG
CCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCG
CCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGA
CTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTC
CTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTT
ACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACG
GATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCA
AGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTA
CAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGAC
GCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAA
GGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATC
GGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGG
ACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCA
CCCCGG

SEQ ID NO: 68 amino acid sequence of D0002:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 69 nucleotide sequence of D0003:

ATGTTGCTGCTCGTGACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCT
GCTCATCCCTCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCC
CAGACTCTGAGCCTGACTTGCGCCATTAGCGGGAACTCAGTCTCGTCCAATTCGG
CGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGC
GCACTTACTACCGGTCCAAATGGTATAACGACTACGCCGTGTCCGTGAAGTCCCG
GATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGC
GTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAG
GACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGA
GGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACC
CAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTA
GAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCT
TGGCTCCTCAACTGCTGATCTTTGGCGCCAGCACTCTTCAGGGGGAGGTGCCATC
ACGCTTCTCCGGAGGTGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTC
CAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATA
CCTTCGGACAAGGCACTAAGCTGGAAATCAAGGCGGCCGCAACTACCACCCCTG
CCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCG
CCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGA
CTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTC
CTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTT
ACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACG
GATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCA
AGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTA
CAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGAC
GCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAA
GGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATC
GGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGG
ACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCA
CCCCGG

SEQ ID NO: 70 amino acid sequence of D0003:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGNSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGGGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGQGTKLEIKAAATTTPAPRPPTPAPTIASQ

PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 73 nucleotide sequence of LTG2273:

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGCACTCCG
ACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACTACCAGC
CGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCAGAGTGAAGTTCAGCCG
CTCAGCCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCT
CAACCTGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAG
ATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTAC
AACGAACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAA
GGGAGAACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAA
CTGCCACTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGG

SEQ ID NO: 74 amino acid sequence of LTG2273:

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRP
GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 75 nucleotide sequence of LTG2200:

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCT
GCTTATTCCCCAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAG
CCAGACGCTGTCCCTGACTTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGC
GCGGCGTGGAATTGGATTCGACAATCTCCTTCCCGAGGGTTGGAATGGCTTGGAC
GAACATATTACAGATCCAAATGGTATAACGACTATGCGGTATCAGTAAAGTCAA
GAATAACCATTAACCCCGACACAAGCAAGAACCAATTCTCTTTGCAGCTTAACTC
TGTCACGCCAGAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGA
CCTGGAAGACGCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTC
AGGGGGCGGTGGGAGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTC
AGATGACCCAGTCCCCTTCATCCTTGTCTGCCTCCGTCGGTGACAGGGTGACAAT
AACATGCAGAGCAAGCCAAACAATCTGGAGCTATCTCAACTGGTACCAGCAGCG
ACCAGGAAAAGCGCCAAACCTGCTGATTTACGCTGCTTCCTCCCTCCAATCAGGC
GTGCCTAGTAGATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTATAA
GCTCTCTTCAAGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTAT
ACCTCAGACTTTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCAACTAC
CACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTC
TCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGG
GGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCG
GCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAA
GCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAA
GAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACT
GCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAA
TCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGA
CAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACC
CTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACT
CAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTG
TACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAA
GCACTCCCACCCCGG

SEQ ID NO: 76 amino acid sequence of LTG2200:

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAREVTGDLEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
LSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGS
GTDFTLTISSLQAEDFATYYCQQSYSIPQTFGQGTKLEIKAAATTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 77 nucleotide sequence of GMCSF leader peptide

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCT
GCTTATTCCC

SEQ ID NO: 78 amino acid sequence of GMCSF leader peptide

MLLLVTSLLLCELPHPAFLLIP

SEQ ID NO: 79 nucleotide sequence of CD8a leader peptide

ATGGCCCTGCCCGTCACTGCGCTGCTTCTTCCACTTGCGCTTCTGCTGCACGCAGC
GCGCCCG

SEQ ID NO: 80 amino acid sequence of CD8a leader peptide

MALPVTALLLPLALLLHAARP

SEQ ID NO: 81 nucleotide sequence of CD8 hinge and transmembrane domain

GCGGCCGCTACCACAACCCCTGCGCCCCGGCCTCCTACCCCCGCACCCACGATTG
CTTCTCAACCTCTTTCACTCCGACCTGAGGCTTGTAGACCTGCAGCCGGGGGTGC
CGTCCACACACGGGGACTCGACTTCGCTTGTGATATATATATTTGGGCGCCCCTG
GCCGGCACTTGTGGAGTTCTTTTGCTCTCTCTTGTTATCACATTGTACTGC

SEQ ID NO: 82 amino acid sequence of CD8 hinge and transmembrane domain

AAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT
CGVLLLSLVITLYC

SEQ ID NO: 83 nucleotide sequence of 4-1BB/CD137 costimulatory domain

AAGCGAGGTAGGAAGAAATTGCTTTACATTTTTAAGCAGCCGTTCATGCGACCA
GTACAGACTACTCAAGAAGAAGATGGGTGCTCTTGTCGGTTCCCGGAAGAAGAA
GAGGGTGGTTGCGAGTTG

SEQ ID NO: 84 amino acid sequence of 4-1BB/CD137 costimulatory domain

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 85 nucleotide sequence of CD28 costimulatory domain nucleotide sequence

CGGTCGAAGCGCTCAAGACTGCTGCACTCAGACTACATGAACATGACTCCTCGG
CGGCCGGGGCCGACTCGGAAGCACTACCAGCCTTACGCACCCCCGAGAGATTTC
GCGGCCTACCGCTCC

SEQ ID NO: 86 amino acid sequence of CD28 costimulatory domain

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

SEQ ID NO: 87 nucleotide sequence of CD3 zeta

AGGGTGAAGTTCTCCCGCTCTGCCGACGCACCGGCATATCAGCAGGGACAAAAC
CAGCTCTACAACGAATTGAACCTGGGTCGGCGGGAAGAATATGACGTGCTCGAT
AAGCGGCGGGGTCGCGACCCAGAAATGGGAGGCAAACCGCGCAGGAAAAATCC
ACAGGAGGGACTTTATAACGAACTTCAAAAGGATAAGATGGCAGAGGCATACAG
CGAAATCGGGATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTT
ACCAGGGGCTTTCTACCGCGACGAAGGATACCTACGATGCTCTCCATATGCAAGC
ACTTCCTCCTAGA

SEQ ID NO: 88 amino acid sequence of CD3 zeta

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

SEQ ID NO: 89 nucleotide sequence of Furin P2A furin

CGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGAAGCAGGCCGGG
GATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAG

SEQ ID NO: 90 amino acid sequence Furin P2A furin

RAKRGSGATNFSLLKQAGDVEENPGPRAKR

SEQ ID NO: 95 nucleotide sequence of 16P17 CD22 scFv VH

CAGGTACAGCTTCAACAGAGTGGGCCGGGACTGGTGAAACACTCCCAAACACTT
TCTCTGACGTGCGCTATATCAGGTGACTCTGTTTCATCTAATTCTGCTGCGTGGAA
CTGGATTCGACAATCTCCCAGTCGCGGGTTGGAATGGCTGGGACGAACATATTAT
CGGTCTAAGTGGTATAACGATTATGCTGTATCTGTTAAATCTCGAATTACGATTA
ATCCTGACACCTCCAAGAACCAGTTCTCCCTCCAGTTGAACTCAGTCACACCGGA
AGACACTGCGGTCTACTATTGCGCTCAAGAAGTCGAGCCACATGATGCATTCGAC
ATCTGGGGCCAGGGAACGATGGTCACCGTCAGCAGT

SEQ ID NO: 96 amino acid sequence of 16P17 CD22 scFv VH

QVQLQQSGPGLVKHSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRS
KWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAQEVEPHDAFDIWG
QGTMVTVSS

SEQ ID NO: 97 nucleotide sequence of 16P17 CD22 scFv VL

GACATACAAATGACGCAGAGTCCCTCAAGTGTGTACGCGAGTGTGGGGGATAAG
GTAACTATTACGTGCAGAGCGTCACAGGATGTTAGTGGATGGCTTGCCTGGTATC
AGCAGAAGCCAGGCCTTGCTCCACAGCTCCTTATCAGTGGTGCTTCTACACTTCA
GGGCGAGGTTCCGAGTAGATTCTCTGGTTCTGGATCTGGTACTGACTTCACTCTT
ACAATTTCTTCTTTGCAACCAGAAGACTTTGCGACTTATTACTGCCAACAGGCCA
AATACTTCCCTTATACATTTGGCCAAGGTACCAAGTTGGAGATAAAG

SEQ ID NO: 98 amino acid sequence of 16P17 CD22 scFv VL

DIQMTQSPSSVYASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLISGASTLQGE
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKYFPYTFGQGTKLEIK

SEQ ID NO: 99 nucleotide sequence of 16P8 CD22 scFv VH

CAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTG
AGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGGCGGCCTGGA
ACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACT
ACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCGGATCACCA
TTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCC
CGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTT
CGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCC

SEQ ID NO: 100 amino acid sequence of 16P8 CD22 scFv VH

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRS
KWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPEDTAVYYCAQEVEPQDAFDIWG
QGTMVTVSS

SEQ ID NO: 101 nucleotide sequence of 16P8 CD22 scFv VL

GATATCCAGATGACCCAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAG
GTCACCATTACCTGTAGAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACC
AGCAGAAGCCAGGCTTGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCA
GGGGGAAGTGCCATCACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTG
ACCATCAGCAGCCTCCAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCA
AGTACTTCCCCTATACCTTCGGAAGAGGCACTAAGCTGGAAATCAAG

SEQ ID NO: 102 amino acid sequence of 16P8 CD22 scFv VL

DIQMTQSPSSVSASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGE
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIK

SEQ ID NO: 103 nucleotide sequence of 16P13 CD22 scFv VH

CAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTG
AGCCTGACTTGCGCCATTAGCGGGAACTCAGTCTCGTCCAATTCGGCGGCCTGGA
ACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACT
ACCGGTCCAAATGGTATAACGACTACGCCGTGTCCGTGAAGTCCCGGATCACCAT
TAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCCC
GAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTTC
GACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCC

SEQ ID NO: 104 amino acid sequence of 16P13 CD22 scFv VH

QVQLQQSGPGLVKPSQTLSLTCAISGNSVSSNSAAWNWIRQSPSRGLEWLGRTYYRS
KWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAQEVEPQDAFDIWG
QGTMVTVSS

SEQ ID NO: 105 nucleotide sequence of 16P13 CD22 scFv VL

GATATCCAGATGACCCAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAG
GTCACCATTACCTGTAGAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACC
AGCAGAAGCCAGGCTTGGCTCCTCAACTGCTGATCTTTGGCGCCAGCACTCTTCA
GGGGGAGGTGCCATCACGCTTCTCCGGAGGTGGTTCCGGCACCGACTTCACCCTG
ACCATCAGCAGCCTCCAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCA
AGTACTTCCCCTATACCTTCGGACAAGGCACTAAGCTGGAAATCAAG

SEQ ID NO: 106 amino acid sequence of 16P13 CD22 scFv VL

DIQMTQSPSSVSASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGE
VPSRFSGGGSGTDFTLTISSLQPEDFATYYCQQAKYFPYTFGQGTKLEIK

SEQ ID NO: 107 amino acid sequence of Whitlow linker

GSTSGSGKPGSGEGSTKG

SEQ ID NO: 108 amino acid sequence of flexible interchain linker

GGGGSGGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 109 nucleotide sequence of LTG 2948 DuoCAR D93 CAR2019 ICOZz 2A CAR22z

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCAACGACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCTGGCTGACAAAAAAGAAGTATTCATCTAGTG
TACATGATCCGAACGGTGAATACATGTTCATGCGCGCGGTGAACACGGCCAAGA
AGAGCAGACTGACCGACGTAACCCTTAGAGTGAAGTTTAGCCGCTCAGCCGATG
CACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCTCAACCTGGGTC
GGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAGATCCGGAGATG
GGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTACAACGAACTGCA
GAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAAGGGAGAACGCC
GGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCACTAAGG
ACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGGCGCGCGAAACGCG
GCAGCGGCGCGACCAACTTTAGCCTGCTGAAACAGGCGGGCGATGTGGAAGAAA

ACCCGGGCCCGCGAGCAAAGAGGAATATTATGGCTCTGCCTGTTACGGCACTGC
TCCTTCCGCTTGCATTGTTGTTGCACGCAGCGCGGCCCCAAGTGCAGCTGCAGCA
GTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTGAGCCTGACTTGCGCAATT
AGCGGGGACTCAGTCTCGTCCAATTCGGCGGCCTGGAACTGGATCCGGCAGTCA
CCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACTACCGGTCCAAATGGTAT
ACCGACTACGCCGTGTCCGTGAAGAATCGGATCACCATTAACCCCGACACCTCG
AAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCCCGAGGATACCGCGGTG
TACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTTCGACATTTGGGGACAG
GGAACGATGGTCACAGTGTCGTCCGGTGGAGGAGGTTCCGGAGGCGGTGGATCT
GGAGGCGGAGGTTCGGATATCCAGATGACCCAGAGCCCCTCCTCGGTGTCCGCA
TCCGTGGGCGATAAGGTCACCATTACCTGTAGAGCGTCCCAGGACGTGTCCGGAT
GGCTGGCCTGGTACCAGCAGAAGCCAGGCTTGGCTCCTCAACTGCTGATCTTCGG
CGCCAGCACTCTTCAGGGGGAAGTGCCATCACGCTTCTCCGGATCCGGTTCCGGC
ACCGACTTCACCCTGACCATCAGCAGCCTCCAGCCTGAGGACTTCGCCACTTACT
ACTGCCAACAGGCCAAGTACTTCCCCTATACCTTCGGAAGAGGCACTAAGCTGG
AAATCAAGGCTAGCGCAACCACTACGCCTGCTCCGCGGCCTCCAACGCCCGCGC
CCACGATAGCTAGTCAGCCGTTGTCTCTCCGACCAGAGGCGTGTAGACCGGCCGC
TGGCGGAGCCGTACATACTCGCGGACTCGACTTCGCTTGCGACATCTACATTTGG
GCACCCTTGGCTGGGACCTGTGGGGTGCTGTTGCTGTCCTTGGTTATTACGTTGTA
CTGCAGAGTCAAATTTTCCAGGTCCGCAGATGCCCCCGCGTACCAGCAAGGCCA
GAACCAACTTTACAACGAACTGAACCTGGGTCGCCGGGAGGAATATGATGTGCT
GGATAAACGAAGGGGGAGGGACCCTGAGATGGGAGGGAAACCTCGCAGGAAAA
ACCCGCAGGAAGGTTTGTACAACGAGTTGCAGAAGGATAAGATGGCTGAGGCTT
ACTCTGAAATAGGGATGAAGGGAGAGAGACGGAGAGGAAAAGGCCATGATGGC
CTTTACCAGGGCTTAAGCACAGCAACAAAGGATACTTACGACGCTCTTCACATGC
AAGCTCTGCCACCACGG

SEQ ID NO: 110 amino acid sequence of LTG 2948 DuoCAR D93 CAR2019 ICOZz 2A CAR22z

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCWLTKKKYSSSVHDPNGEYM
FMRAVNTAKKSRLTDVTLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIMAL
PVTALLLPLALLLHAARPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWI
RQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPEDTAV
YYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVSASV
GDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKASATTTPAPRPPTPAPTIASQPLSL
RPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRVKFSRSADA

PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 111 nucleotide sequence of LTG 2949 DuoCAR D94 CAR2019 OX40z 2A CAR22z

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCAACGACCACTCCAGCACCGA
GACCGCCAACCCCCGCGCCTACCATCGCAAGTCAACCACTTTCTCTCAGGCCTGA
AGCGTGCCGACCTGCAGCTGGTGGGGCAGTACATACCAGGGGTTTGGACTTCGC
ATGTGACGTGGCGGCAATTCTCGGCCTGGGACTTGTCCTTGGTCTGCTTGGTCCG
CTCGCAATACTTCTGGCCTTGTACCTGCTCCGCAGAGACCAAAGACTTCCGCCCG
ACGCCCACAAGCCCCCAGGAGGAGGTTCCTTCAGAACGCCTATACAAGAAGAAC
AAGCAGATGCCCACTCTACCCTGGCTAAAATCAGGGTGAAGTTTAGCCGCTCAG
CCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCTCAACC
TGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAGATCCG
GAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTACAACGA
ACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAAGGGAG
AACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCA
CTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGGCGCGCGAA
ACGCGGCAGCGGCGCGACCAACTTTAGCCTGCTGAAACAGGCGGGCGATGTGGA
AGAAAACCCGGGCCCGCGAGCAAAGAGGAATATTATGGCTCTGCCTGTTACGGC

ACTGCTCCTTCCGCTTGCATTGTTGTTGCACGCAGCGCGGCCCCAAGTGCAGCTG
CAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTGAGCCTGACTTGCG
CAATTAGCGGGGACTCAGTCTCGTCCAATTCGGCGGCCTGGAACTGGATCCGGC
AGTCACCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACTACCGGTCCAAAT
GGTATACCGACTACGCCGTGTCCGTGAAGAATCGGATCACCATTAACCCCGACA
CCTCGAAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCCCGAGGATACCG
CGGTGTACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTTCGACATTTGGG
GACAGGGAACGATGGTCACAGTGTCGTCCGGTGGAGGAGGTTCCGGAGGCGGTG
GATCTGGAGGCGGAGGTTCGGATATCCAGATGACCCAGAGCCCCTCCTCGGTGT
CCGCATCCGTGGGCGATAAGGTCACCATTACCTGTAGAGCGTCCCAGGACGTGTC
CGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCTTGGCTCCTCAACTGCTGATC
TTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATCACGCTTCTCCGGATCCGGTT
CCGGCACCGACTTCACCCTGACCATCAGCAGCCTCCAGCCTGAGGACTTCGCCAC
TTACTACTGCCAACAGGCCAAGTACTTCCCCTATACCTTCGGAAGAGGCACTAAG
CTGGAAATCAAGGCTAGCGCAACCACTACGCCTGCTCCGCGGCCTCCAACGCCC
GCGCCCACGATAGCTAGTCAGCCGTTGTCTCTCCGACCAGAGGCGTGTAGACCG
GCCGCTGGCGGAGCCGTACATACTCGCGGACTCGACTTCGCTTGCGACATCTACA
TTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTGTTGCTGTCCTTGGTTATTACG
TTGTACTGCAGAGTCAAATTTTCCAGGTCCGCAGATGCCCCCGCGTACCAGCAAG
GCCAGAACCAACTTTACAACGAACTGAACCTGGGTCGCCGGGAGGAATATGATG
TGCTGGATAAACGAAGGGGGAGGGACCCTGAGATGGGAGGGAAACCTCGCAGG
AAAAACCCGCAGGAAGGTTTGTACAACGAGTTGCAGAAGGATAAGATGGCTGAG
GCTTACTCTGAAATAGGGATGAAGGGAGAGAGACGGAGAGGAAAAGGCCATGA
TGGCCTTTACCAGGGCTTAAGCACAGCAACAAAGGATACTTACGACGCTCTTCAC
ATGCAAGCTCTGCCACCACGG

SEQ ID NO: 112 amino acid sequence of LTG 2949 DuoCAR D94 CAR2019 OX40z 2A CAR22z

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDVAAILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHKPPGGGS
FRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIMALP
VTALLLPLALLLHAARPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIR
QSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPEDTAVY
YCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVG
DKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKASATTTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRVKFSRSADAP

AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 113 nucleotide sequence of LTG 2950 DuoCAR D95 CAR2019 OX40z 2A CAR22 ICOSz

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCAACGACCACTCCAGCACCGA
GACCGCCAACCCCCGCGCCTACCATCGCAAGTCAACCACTTTCTCTCAGGCCTGA
AGCGTGCCGACCTGCAGCTGGTGGGGCAGTACATACCAGGGGTTTGGACTTCGC
ATGTGACGTGGCGGCAATTCTCGGCCTGGGACTTGTCCTTGGTCTGCTTGGTCCG
CTCGCAATACTTCTGGCCTTGTACCTGCTCCGCAGAGACCAAAGACTTCCGCCCG
ACGCCCACAAGCCCCCAGGAGGAGGTTCCTTCAGAACGCCTATACAAGAAGAAC
AAGCAGATGCCCACTCTACCCTGGCTAAAATCAGGGTGAAGTTTAGCCGCTCAG
CCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCTCAACC
TGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAGATCCG
GAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTACAACGA
ACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAAGGGAG
AACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCA
CTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGGCGCGCGAA
ACGCGGCAGCGGCGCGACCAACTTTAGCCTGCTGAAACAGGCGGGCGATGTGGA
AGAAAACCCGGGCCCGCGAGCAAAGAGGAATATTATGGCTCTGCCTGTTACGGC

ACTGCTCCTTCCGCTTGCATTGTTGTTGCACGCAGCGCGGCCCCAAGTGCAGCTG
CAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCCCAGACTCTGAGCCTGACTTGCG
CAATTAGCGGGGACTCAGTCTCGTCCAATTCGGCGGCCTGGAACTGGATCCGGC
AGTCACCATCAAGGGGCCTGGAATGGCTCGGGCGCACTTACTACCGGTCCAAAT
GGTATACCGACTACGCCGTGTCCGTGAAGAATCGGATCACCATTAACCCCGACA
CCTCGAAGAACCAGTTCTCACTCCAACTGAACAGCGTGACCCCCGAGGATACCG
CGGTGTACTACTGCGCACAAGAAGTGGAACCGCAGGACGCCTTCGACATTTGGG
GACAGGGAACGATGGTCACAGTGTCGTCCGGTGGAGGAGGTTCCGGAGGCGGTG
GATCTGGAGGCGGAGGTTCGGATATCCAGATGACCCAGAGCCCCTCCTCGGTGT
CCGCATCCGTGGGCGATAAGGTCACCATTACCTGTAGAGCGTCCCAGGACGTGTC
CGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCTTGGCTCCTCAACTGCTGATC
TTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATCACGCTTCTCCGGATCCGGTT
CCGGCACCGACTTCACCCTGACCATCAGCAGCCTCCAGCCTGAGGACTTCGCCAC
TTACTACTGCCAACAGGCCAAGTACTTCCCCTATACCTTCGGAAGAGGCACTAAG
CTGGAAATCAAGGCTAGCGCAACCACTACGCCTGCTCCGCGGCCTCCAACGCCC
GCGCCCACGATAGCTAGTCAGCCGTTGTCTCTCCGACCAGAGGCGTGTAGACCG
GCCGCTGGCGGAGCCGTACATACTCGCGGACTCGACTTCGCTTGCGACATCTACA
TTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTGTTGCTGTCCTTGGTTATTACG
TTGTACTGCTGGCTGACAAAAAAGAAGTATTCATCTAGTGTACATGATCCGAACG
GTGAATACATGTTCATGCGCGCGGTGAACACGGCCAAGAAGAGCAGACTGACCG
ACGTAACCCTTAGAGTCAAATTTTCCAGGTCCGCAGATGCCCCCGCGTACCAGCA
AGGCCAGAACCAACTTTACAACGAACTGAACCTGGGTCGCCGGGAGGAATATGA
TGTGCTGGATAAACGAAGGGGGAGGGACCCTGAGATGGGAGGGAAACCTCGCA
GGAAAAACCCGCAGGAAGGTTTGTACAACGAGTTGCAGAAGGATAAGATGGCTG
AGGCTTACTCTGAAATAGGGATGAAGGGAGAGAGACGGAGAGGAAAAGGCCAT
GATGGCCTTTACCAGGGCTTGAGCACAGCAACAAAGGATACTTACGACGCTCTTC
ACATGCAAGCTCTGCCACCACGG

SEQ ID NO: 114 amino acid sequence of LTG 2950 DuoCAR D95 CAR2019 OX40z 2A CAR22 ICOSz

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDVAAILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHKPPGGGS
FRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIMALP
VTALLLPLALLLHAARPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIR
QSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPEDTAVY
YCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVG
DKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKASATTTPAPRPPTPAPTIASQPLSLR

PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCWLTKKKYSSSV
HDPNGEYMFMRAVNTAKKSRLTDVTLRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 115 nucleotide sequence of LTG 2951 DuoCAR D96 CAR2019 27z 2A CAR22 ICOSz

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCAACGGCGCAAATACCGCTCCAATAAAGGCG
AAAGTCCGGTAGAACCCGCAGAACCTTGCCACTACAGTTGTCCCAGAGAAGAAG
AGGGTTCTACAATACCTATTCAAGAGGACTATAGGAAACCAGAGCCCGCATGTA
GTCCCAGAGTGAAGTTCAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGAC
AGAACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGTGC
TGGACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGAGGAAG
AACCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATGGCGGAAGC
CTACTCCGAGATCGGCATGAAGGGAGAACGCCGGAGAGGGAAGGGTCATGACG
GACTGTACCAGGGCCTGTCAACTGCCACTAAGGACACTTACGATGCGCTCCATAT

GCAAGCTTTGCCCCCGCGGCGCGCGAAACGCGGCAGCGGCGCGACCAACTTTAG
CCTGCTGAAACAGGCGGGCGATGTGGAAGAAAACCCGGGCCCGCGAGCAAAGA
GGAATATTATGGCTCTGCCTGTTACGGCACTGCTCCTTCCGCTTGCATTGTTGTTG
CACGCAGCGCGGCCCCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAG
CCGTCCCAGACTCTGAGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCA
ATTCGGCGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGC
TCGGGCGCACTTACTACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAA
GAATCGGATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTG
AACAGCGTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAA
CCGCAGGACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCC
GGTGGAGGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAG
ATGACCCAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTA
CCTGTAGAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGC
CAGGCTTGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGT
GCCATCACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTGACCATCAGC
AGCCTCCAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCC
CCTATACCTTCGGAAGAGGCACTAAGCTGGAAATCAAGGCTAGCGCAACCACTA
CGCCTGCTCCGCGGCCTCCAACGCCCGCGCCCACGATAGCTAGTCAGCCGTTGTC
TCTCCGACCAGAGGCGTGTAGACCGGCCGCTGGCGGAGCCGTACATACTCGCGG
ACTCGACTTCGCTTGCGACATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGG
GTGCTGTTGCTGTCCTTGGTTATTACGTTGTACTGCTGGCTGACAAAAAAGAAGT
ATTCATCTAGTGTACATGATCCGAACGGTGAATACATGTTCATGCGCGCGGTGAA
CACGGCCAAGAAGAGCAGACTGACCGACGTAACCCTTAGAGTCAAATTTTCCAG
GTCCGCAGATGCCCCCGCGTACCAGCAAGGCCAGAACCAACTTTACAACGAACT
GAACCTGGGTCGCCGGGAGGAATATGATGTGCTGGATAAACGAAGGGGGAGGG
ACCCTGAGATGGGAGGGAAACCTCGCAGGAAAAACCCGCAGGAAGGTTTGTACA
ACGAGTTGCAGAAGGATAAGATGGCTGAGGCTTACTCTGAAATAGGGATGAAGG
GAGAGAGACGGAGAGGAAAAGGCCATGATGGCCTTTACCAGGGCTTGAGCACA
GCAACAAAGGATACTTACGACGCTCTTCACATGCAAGCTCTGCCACCACGG

SEQ ID NO: 116 amino acid sequence of LTG2951 DuoCAR D96 CAR2019 27z 2A CAR22 ICOSz

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCQRRKYRSNKGESPVEPAEPC
HYSCPREEEGSTIPIQEDYRKPEPACSPRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQAGDVEENPGPR
AKRNIMALPVTALLLPLALLLHAARPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSN
SAAWNWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNS
VTPEDTAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQ

SPSSVSASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFS
GSGSGTDFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKASATTTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCW
LTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTLRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 117 nucleotide sequence of D088 CAR2019 ICOSz

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCTGGCTGACAAAAAAGAAGTATTCATCTAGTG
TACATGATCCGAACGGTGAATACATGTTCATGCGCGCGGTGAACACGGCCAAGA
AGAGCAGACTGACCGACGTAACCCTTAGAGTGAAGTTCAGCCGCTCAGCCGATG
CACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCTCAACCTGGGTC
GGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAGATCCGGAGATG
GGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTACAACGAACTGCA
GAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAAGGGAGAACGCC

GGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCACTAAGG
ACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGG

SEQ ID NO: 118 amino acid sequence of D088 CAR2019 ICOSz

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCWLTKKKYSSSVHDPNGEYM
FMRAVNTAKKSRLTDVTLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 119 nucleotide sequence of D089 CAR22 ICOSz

ATGTTGCTGCTCGTGACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCT
GCTCATCCCTCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCC
CAGACTCTGAGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGG
CGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGC
GCACTTACTACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCG
GATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGC
GTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAG
GACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGA
GGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACC
CAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTA
GAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCT
TGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATC
ACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTC
CAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATA
CCTTCGGAAGAGGCACTAAGCTGGAAATCAAGGCGGCCGCGACTACCACTCCTG
CACCACGGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCG
CCCCGAAGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGA
CTTCGCCTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTG
CTCTTGTCTCTGGTCATTACCCTGTACTGCTGGCTGACAAAAAAGAAGTATTCAT
CTAGTGTACATGATCCGAACGGTGAATACATGTTCATGCGCGCGGTGAACACGG
CCAAGAAGAGCAGACTGACCGACGTAACCCTTAGAGTGAAGTTCAGCCGCTCAG
CCGATGCACCGGCCTACCAGCAGGGACAGAACCAGCTCTACAACGAGCTCAACC
TGGGTCGGCGGGAAGAATATGACGTGCTGGACAAACGGCGCGGCAGAGATCCG
GAGATGGGGGGAAAGCCGAGGAGGAAGAACCCTCAAGAGGGCCTGTACAACGA
ACTGCAGAAGGACAAGATGGCGGAAGCCTACTCCGAGATCGGCATGAAGGGAG
AACGCCGGAGAGGGAAGGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCA
CTAAGGACACTTACGATGCGCTCCATATGCAAGCTTTGCCCCCGCGG

SEQ ID NO: 120 amino acid sequence of D089 CAR22 ICOSz

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCWLTKKK
YSSSVHDPNGEYMFMRAVNTAKKSRLTDVTLRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 121 nucleotide sequence of D090 CAR2019 OX40z

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA
CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCAACGACCACTCCAGCACCGA
GACCGCCAACCCCCGCGCCTACCATCGCAAGTCAACCACTTTCTCTCAGGCCTGA
AGCGTGCCGACCTGCAGCTGGTGGGGCAGTACATACCAGGGGTTTGGACTTCGC
ATGTGACGTGGCGGCAATTCTCGGCCTGGGACTTGTCCTTGGTCTGCTTGGTCCG
CTCGCAATACTTCTGGCCTTGTACCTGCTCCGCAGAGACCAAAGACTTCCGCCCG
ACGCCCACAAGCCCCCAGGAGGAGGTTCCTTCAGAACGCCTATACAAGAAGAAC
AAGCAGATGCCCACTCTACCCTGGCTAAAATCAGGGTGAAGTTTAGCCGGTCAG

CTGATGCACCTGCATATCAGCAGGGACAGAACCAGCTGTACAATGAGCTGAACC
TCGGACGAAGAGAGGAGTACGACGTGTTGGACAAAAGACGAGGTAGAGACCCC
GAGATGGGCGGCAAGCCGAGAAGAAAAAACCCACAAGAAGGGCTTTATAATGA
GCTTCAGAAAGATAAGATGGCAGAGGCCTACAGTGAGATTGGCATGAAGGGCGA
AAGAAGGAGGGGCAAAGGACACGACGGTCTCTACCAAGGCCTCAGCACGGCTA
CCAAAGATACGTATGACGCATTGCATATGCAGGCATTGCCGCCCCGC

SEQ ID NO: 122 amino acid sequence of D090 CAR2019 OX40z

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDVAAILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHKPPGGGS
FRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPR

SEQ ID NO: 123 nucleotide sequence of D091 CAR2019 CD27z

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCT
GCTGATTCCCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGG
AGCCAGCGTGAAGATGAGCTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAAC
ATGCACTGGGTGAAACAGACCCCGGGACAAGGGCTCGAATGGATTGGCGCCATC
TACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTCAAGGGAAAGGCCACC
CTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCCTGACCT
CCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTA
CTGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGC
GGAGGATCCGGTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACT
CAGTCCCCGGCAATCCTGTCGGCCTCACCGGGCGAAAAGGTCACGATGACTTGT
AGAGCGTCGTCCAGCGTGAACTACATGGATTGGTACCAAAAGAAGCCTGGATCG
TCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCCGGCGTGCCAGCGC
GGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCGTGGA
GGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACT
TTTGGAGGCGGTACTAAGCTGGAGATCAAAGGAGGCGGCGGCAGCGGCGGGGG
AGGGTCCGGAGGGGGTGGTTCTGGTGGAGGAGGATCGGGAGGCGGTGGCAGCG
ACATTCAGATGACTCAGACCACCTCCTCCCTGTCCGCCTCCCTGGGCGACCGCGT
GACCATCTCATGCCGCGCCAGCCAGGACATCTCGAAGTACCTCAACTGGTACCA
GCAGAAGCCCGACGGAACCGTGAAGCTCCTGATCTACCACACCTCCCGGCTGCA
CAGCGGAGTGCCGTCTAGATTCTCGGGTTCGGGGTCGGGAACTGACTACTCCCTT
ACTATTTCCAACCTGGAGCAGGAGGATATTGCCACCTACTTCTGCCAACAAGGAA
ACACCCTGCCGTACACTTTTGGCGGGGGAACCAAGCTGGAAATCACTGGCAGCA
CATCCGGTTCCGGGAAGCCCGGCTCCGGAGAGGGCAGCACCAAGGGGGAAGTCA
AGCTGCAGGAATCAGGACCTGGCCTGGTGGCCCCGAGCCAGTCACTGTCCGTGA

CTTGTACTGTGTCCGGAGTGTCGCTCCCGGATTACGGAGTGTCCTGGATCAGGCA
GCCACCTCGGAAAGGATTGGAATGGCTCGGAGTCATCTGGGGTTCCGAAACCAC
CTATTACAACTCGGCACTGAAATCCAGGCTCACCATTATCAAGGATAACTCCAAG
TCACAAGTGTTCCTGAAGATGAATAGCCTGCAGACTGACGACACGGCGATCTAC
TATTGCGCCAAGCACTACTACTACGGCGGATCCTACGCTATGGACTACTGGGGCC
AGGGGACCAGCGTGACCGTGTCATCCGCGGCCGCGACTACCACTCCTGCACCAC
GGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGA
AGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGACTTCGC
CTGTGACATCTACATCTGGGCCCCATTGGCTGGAACTTGCGGCGTGCTGCTCTTG
TCTCTGGTCATTACCCTGTACTGCCAACGGCGCAAATACCGCTCCAATAAAGGCG
AAAGTCCGGTAGAACCCGCAGAACCTTGCCACTACAGTTGTCCCAGAGAAGAAG
AGGGTTCTACAATACCTATTCAAGAGGACTATAGGAAACCAGAGCCCGCATGTA
GTCCCAGAGTGAAGTTCAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGAC
AGAACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGTGC
TGGACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGAGGAAG
AACCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATGGCGGAAGC
CTACTCCGAGATCGGCATGAAGGGAGAACGCCGGAGAGGGAAGGGTCATGACG
GACTGTACCAGGGCCTGTCAACTGCCACTAAGGACACTTACGATGCGCTCCATAT
GCAAGCTTTGCCCCCGCGG

SEQ ID NO: 124 amino acid sequence D091 CAR2019 CD27z

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMH
WVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS
ADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAI
LSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGSGS
GTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSGGGGSGGGGSGG
GGSGGGGSDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY
HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG
STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP
RKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH
YYYGGSYAMDYWGQGTSVTVSSAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCQRRKYRSNKGESPVEPAEPC
HYSCPREEEGSTIPIQEDYRKPEPACSPRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 125 nucleotide sequence of D92 CAR22z

ATGTTGCTGCTCGTGACCTCGCTCCTTCTGTGCGAGCTGCCCCATCCGGCTTTTCT
GCTCATCCCTCAAGTGCAGCTGCAGCAGTCCGGTCCTGGACTGGTCAAGCCGTCC
CAGACTCTGAGCCTGACTTGCGCAATTAGCGGGGACTCAGTCTCGTCCAATTCGG
CGGCCTGGAACTGGATCCGGCAGTCACCATCAAGGGGCCTGGAATGGCTCGGGC
GCACTTACTACCGGTCCAAATGGTATACCGACTACGCCGTGTCCGTGAAGAATCG
GATCACCATTAACCCCGACACCTCGAAGAACCAGTTCTCACTCCAACTGAACAGC
GTGACCCCCGAGGATACCGCGGTGTACTACTGCGCACAAGAAGTGGAACCGCAG
GACGCCTTCGACATTTGGGGACAGGGAACGATGGTCACAGTGTCGTCCGGTGGA
GGAGGTTCCGGAGGCGGTGGATCTGGAGGCGGAGGTTCGGATATCCAGATGACC
CAGAGCCCCTCCTCGGTGTCCGCATCCGTGGGCGATAAGGTCACCATTACCTGTA
GAGCGTCCCAGGACGTGTCCGGATGGCTGGCCTGGTACCAGCAGAAGCCAGGCT

TGGCTCCTCAACTGCTGATCTTCGGCGCCAGCACTCTTCAGGGGGAAGTGCCATC
ACGCTTCTCCGGATCCGGTTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTC
CAGCCTGAGGACTTCGCCACTTACTACTGCCAACAGGCCAAGTACTTCCCCTATA
CCTTCGGAAGAGGCACTAAGCTGGAAATCAAGGCGGCCGCAACCACTACACCAG
CTCCGCGGCCACCCACCCCAGCACCAACAATAGCCAGTCAGCCTTTGTCTCTGAG
ACCTGAGGCTTGTCGACCCGCTGCAGGTGGGGCAGTTCATACTCGGGGTCTTGAT
TTCGCCTGCGATATATATATTTGGGCCCCCTGGCGGGCACGTGTGGGGTGCTCC
TTCTTTCACTCGTAATTACTCTTTACTGTAGGGTTAAGTTCTCACGATCCGCCGAT
GCGCCAGCATACCAACAGGGACAGAACCAACTTTATAATGAGCTGAATCTTGGT
CGCAGGGAAGAATATGATGTACTTGATAAACGCAGAGGCCGGGATCCCGAGATG
GGAGGGAAACCTCGGAGAAAGAACCCCCAGGAGGGCCTGTATAATGAATTGCA
AAAAGATAAAATGGCTGAAGCTTATTCAGAGATTGGAATGAAAGGCGAGCGGAG
AAGAGGAAAAGGGCACGACGGGCTTTACCAAGGACTGTCCACCGCGACAAAGG
ACACGTACGACGCCCTTCATATGCAGGCGCTTCCTCCACGA

SEQ ID NO: 126 amino acid sequence of D92 CAR22z

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKNRITINPDTSKNQFSLQLNSVTPED
TAVYYCAQEVEPQDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDKVTITCRASQDVSGWLAWYQQKPGLAPQLLIFGASTLQGEVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQAKYFPYTFGRGTKLEIKAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 127 nucleotide sequence of HER2 scFv

GAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCTGGTGCAGCCGGGCGGCAGCCTGCGCCT
GAGCTGCGCGGCGAGCGGCTTTAACATTAAAGATACCTATATTCATTGGGTGCGCCAGGC
GCCGGGCAAAGGCCTGGAATGGGTGGCGCGCATTTATCCGACCAACGGCTATACCCGCT
ATGCGGATAGCGTGAAAGGCCGCTTTACCATTAGCGCGGATACCAGCAAAAACACCGCG
TATCTGCAGATGAACAGCCTGCGCGCGGAAGATACCGCGGTGTATTATTGCAGCCGCTG
GGGCGGCGATGGCTTTTATGCGATGGATTATTGGGGCCAGGGCACCCTGGTGACCGTGA
GCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGATATTCA
GATGACCCAGAGCCCGAGCAGCCTGAGCGCGAGCGTGGGCGATCGCGTGACCATTACCT
GCCGCGCGAGCCAGGATGTGAACACCGCGGTGGCGTGGTATCAGCAGAAACCGGGCAA
AGCGCCGAAACTGCTGATTTATAGCGCGAGCTTTCTGTATAGCGGCGTGCCGAGCCGCTT
TAGCGGCAGCCGCAGCGGCACCGATTTTACCCTGACCATTAGCAGCCTGCAGCCGGAAG
ATTTTGCGACCTATTATTGCCAGCAGCATTATACCACCCCGCCGACCTTTGGCCAGGGCA
CCAAAGTGGAAATTAAA

SEQ ID NO: 128 amino acid sequence of HER2 scFv

EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR IYPTNGYTRY
ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGTLVTVSS

GGGGSGGGGSGGGGS
DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS
RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ GTKVEIK

SEQ ID NO: 129 nucleotide sequence of Folate receptor alpha (FolR1) scFv

ATGGAAGTGCAGCTCGTGGAGTCCGGAGGCGGAGTCGTGCAGCCGGGCAGATCC
CTGCGCCTTTCCTGCTCGGCATCCGGGTTTACCTTCTCTGGCTACGGTCTGTCGTG
GGTCAGACAGGCTCCAGGGAAGGGCCTGGAATGGGTGGCCATGATCTCCTCGGG
GGGTTCGTACACCTACTACGCCGACTCAGTGAAGGGCCGGTTCGCCATCTCCCGC
GACAACGCCAAGAACACCCTGTTCCTGCAAATGGACTCGCTCCGGCCTGAGGAC
ACTGGGGTGTACTTCTGCGCGAGACACGGAGATGACCCAGCTTGGTTCGCCTACT
GGGGACAAGGCACCCCTGTGACCGTGTCCTCCGCGAGCACCAAGGGAGGCGGAG
GAGGTTCCGGTGGAGGGGGATCAGGGGGTGGAGGATCGGACATTCAGCTGACCC
AGAGCCCCTCAAGCCTGTCCGCGAGCGTTGGGGACCGCGTGACCATCACCTGTTC
GGTGTCCTCCTCCATCTCCTCCAACAATCTCCATTGGTACCAGCAGAAACCGGGG
AAAGCCCCCAAGCCGTGGATCTACGGAACCTCCAACCTGGCTAGCGGAGTGCCG
TCGAGGTTCTCGGGCTCCGGATCAGGGACTGACTACACTTTCACTATTTCCTCCCT
GCAACCGGAGGACATTGCCACCTACTACTGTCAGCAGTGGTCGTCCTACCCCTAC
ATGTATACCTTCGGTCAAGGAACCAAGGTCGAGATCAAG

SEQ ID NO: 130 amino acid sequence of FolR1 scFv
MEVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLSWVRQAPGKGLEWVAMISSGG
SYTYYADSVKGRFAISRDNAKNTLFLQMDSLRPEDTGVYFCARHGDDPAWFAYWG
QGTPVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVTITCSVSSSISSNNL
HWYQQKPGKAPKPWIYGTSNLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQ
WSSYPYMYTFGQGTKVEIK

SEQ ID NO: 131 nucleotide sequence of CA125/MUC16
GAAGTACAGCTCGTAGAGTCTGGGGGTGGTCTCGTTCAACCAGGTGGCTCTTTGA
GACTGTCATGCGCCGCGTCTGGGTACAGCATTACAAACGATTACGCATGGAATTG
GGTGAGACAGGCTCCCGGAAAGGGCCTCGAATGGGTAGGATACATCTCATATAG
CGGTTATACAACGTACAATCCTAGCTTGAAATCAAGGTTTACAATCTCCAGGGAC
ACGTCAAAAAATACGCTTTACCTTCAGATGAACTCCCTGCGAGCAGAAGACACG
GCTGTGTACTACTGTGCCCGATGGACAAGTGGCCTCGATTACTGGGGTCAAGGTA

CACTGGTGACAGTATCCTCTGGAGGTGGCGGATCAGGGGGCGGCGGCAGTGGTG
GAGGTGGTTCAGATATCCAGATGACTCAGTCCCCCTCTTCCCTCAGTGCCTCCGT
TGGTGACCGAGTTACTATCACGTGCAAAGCCAGTGACTTGATCCATAATTGGCTG
GCGTGGTATCAGCAAAAACCTGGCAAAGCACCCAAGCTTCTGATATATGGTGCA
ACATCCCTGGAAACGGGCGTTCCCAGTCGCTTTTCAGGGTCAGGGTCAGGAACTG
ATTTTACGCTCACCATTTCCAGCCTGCAGCCTGAAGATTTCGCTACATACTACTGT
CAGCAATATTGGACTACTCCATTTACCTTCGGGCAAGGCACGAAGGTTGAGATA
AAG

SEQ ID NO: 132 amino acid sequence of CA125/MUC16

EVQLVESGGG LVQPGGSLRL SCAASGYSIT NDYAWNWVRQ APGKGLEWVG YISYSGYTTY
NPSLKSRFTI SRDTSKNTLY LQMNSLRAED TAVYYCARWT SGLDYWGQGT LVTVSS
GGGGSGGGGSGGGGS
DIQMTQSPSS LSASVGDRVT ITCKASDLIH NWLAWYQQKP GKAPKLLIYG ATSLETGVPS
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YWTTPFTFGQ GTKVEIK

SEQ ID NO: 133 nucleotide sequence of CD276/B7-H3 scFv

GAAGTCCAGTTGGTTGAGTCAGGAGGGGGACTCGTGCAACCTGGTGGTAGCTTGCGCTT
GTCATGTGCTGCCTCCGGGTTTACATTCTCATCTTTCGGTATGCACTGGGTTAGACAAGCA
CCTGGGAAGGGCTTGGAATGGGTTGCTTACATTAGCAGTGATTCTAGCGCGATCTACTAC
GCTGACACCGTAAAGGGCAGATTTACCATCAGCAGAGATAACGCTAAGAACTCCCTCTA
CCTCCAGATGAACAGCCTCAGGGATGAAGACACTGCTGTTTATTACTGTGGGAGGGGCC
GCGAAAATATTTACTACGGGAGCCGATTGGATTATTGGGGTCAGGGGACAACAGTGACT
GTTTCAAGCGGTGGTGGGGGGTCCGGCGGTGGGGGAAGCGGCGGTGGGGGGTCAGATAT
ACAACTGACACAGAGCCCTAGCTTTTTGAGTGCGTCTGTCGGGGATAGAGTTACGATTAC
TTGTAAGGCGAGCCAGAACGTTGATACGAACGTGGCATGGTACCAGCAGAAGCCAGGGA
AAGCTCCGAAAGCCCTTATCTATTCTGCTAGTTACCGATACAGCGGCGTCCCCTCTCGGT
TCAGTGGGAGTGGAAGTGGAACGGACTTTACCCTTACGATCAGTTCCTTGCAACCGGAG
GATTTCGCCACCTACTACTGCCAGCAATACAATAACTATCCCTTTACTTTTGGCCAGGGC
ACAAAGCTTGAAATCAAA

SEQ ID NO: 134 amino acid sequence of CD276/B7-H3 scFv

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVAY ISSDSSAIYY
ADTVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCGRGR ENIYYGSRLD YWGQGTTVTV
SS
GGGGSGGGGSGGGGS

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS ASYRYSGVPS
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ GTKLEIK

SEQ ID NO: 135 nucleotide sequence of CD276/B7-H3 scFv

CAAGTTCAGTTGCAGCAGTCAGGCGCGGAGCTGGTGAAACCAGGTGCTTCAGTCAAGTT
GTCTTGTAAAGCAAGTGGCTATACATTCACAAATTATGATATCAACTGGGTGCGGCAGAG
GCCCGAACAGGGACTGGAATGGATCGGGTGGATCTTTCCTGGCGACGGTAGTACTCAAT
ACAACGAGAAATTCAAGGGAAAGGCTACTCTTACAACCGACACGTCATCATCTACAGCT
TATATGCAACTTAGTAGACTCACATCAGAAGACTCCGCTGTATACTTTTGTGCTCGACAG
ACGACGGCAACATGGTTCGCCTACTGGGGGCAAGGAACACTCGTAACCGTATCTGCAGG
CGGTGGTGGATCTGGAGGAGGTGGAAGCGGTGGTGGAGGGTCCGACATCGTTATGACGC
AAAGCCCCGCGACCCTCAGTGTGACCCCCGGTGACAGAGTTTCACTCAGTTGCAGAGCCT
CTCAGAGTATCTCAGATTACCTTCACTGGTATCAACAAAAAAGCCACGAAAGCCCCAGA
TTGCTCATAAAGTACGCGAGTCAATCAATCTCTGGTATTCCCTCTAGGTTCTCAGGCTCA
GGCAGCGGTAGCGATTTCACATTGTCTATAAATAGTGTGGAACCTGAGGATGTTGGCGTA
TATTACTGTCAGAACGGTCACTCCTTCCCGCTTACGTTTGGGGCGGGGACAAAATTGGAA
CTCAAG

SEQ ID NO: 136 amino acid sequence of CD276/B7-H3 scFv

QVQLQQSGAE LVKPGASVKL SCKASGYTFT NYDINWVRQR PEQGLEWIGW IFPGDGSTQY
NEKFKGKATL TTDTSSSTAY MQLSRLTSED SAVYFCARQT TATWFAYWGQ GTLVTVSA
GGGGSGGGGSGGGGS
DIVMTQSPAT LSVTPGDRVS LSCRASQSIS DYLHWYQQKS HESPRLLIKY ASQSISGIPS
RFSGSGSGSD FTLSINSVEP EDVGVYYCQN GHSFPLTFGA GTKLELK

SEQ ID NO: 137 nucleotide sequence of EGFR scFv

CAAGTTCAATTGAAACAGAGTGGTCCGGGTCTTGTTCAGCCAAGTCAGAGTTTGAGCATC
ACCTGTACTGTCTCCGGATTTAGTCTTACAAATTACGGCGTACATTGGGTCAGACAATCC
CCTGGGAAGGGTTTGGAATGGCTGGGGGTGATTTGGTCAGGTGGAAACACGGACTACAA
TACTCCCTTTACATCCCGATTGTCCATAAACAAAGATAATAGTAAATCTCAAGTATTTTTT
AAGATGAACAGTCTTCAATCTAACGATACAGCGATCTATTACTGCGCTCGCGCATTGACG
TACTATGACTATGAGTTTGCCTATTGGGGTCAAGGCACACTTGTCACAGTAAGCGCAGGG
GGAGGCGGGTCTGGAGGGGGCGGATCTGGCGGTGGCGGAAGCGATATCCTGTTGACTCA
GTCCCCAGTGATACTTTCAGTATCACCGGGCGAACGGGTGAGTTTCAGTTGCCGCGCCTC
TCAAAGTATCGGAACGAATATACACTGGTACCAGCAGCGGACAAACGGGAGCCCGCGCT
TGCTTATTAAGTACGCTTCCGAGTCTATATCAGGTATTCCATCCCGGTTTTCTGGTAGTGG

AAGTGGGACAGATTTCACACTGTCTATTAATTCAGTTGAGTCTGAAGACATCGCGGATTA
TTACTGCCAACAAAACAATAATTGGCCGACGACCTTCGGCGCTGGGACCAAGCTTGAGC
TTAAG

SEQ ID NO: 138 amino acid sequence of EGFR scFv

QVQLKQSGPG LVQPSQSLSI TCTVSGFSLT NYGVHWVRQS PGKGLEWLGV IWSGGNTDYN
TPFTSRLSIN KDNSKSQVFF KMNSLQSNDT AIYYCARALT YYDYEFAYWG QGTLVTVSA
GGGGSGGGGSGGGGS
DILLTQSPVI LSVSPGERVS FSCRASQSIG TNIHWYQQRT NGSPRLLIKY ASESISGIPS
RFSGSGSGTD FTLSINSVES EDIADYYCQQ NNNWPTTFGA GTKLELK

SEQ ID NO: 139 nucleotide sequence of GD2 scFv

GAGGTTCAGTTGCTCCAGTCTGGACCTGAGTTGGAGAAACCCGGTGCTAGTGTAATGATC
AGCTGCAAGGCATCAGGTTCCAGTTTCACCGGCTATAATATGAATTGGGTTCGGCAGAAC
ATAGGCAAAAGTCTCGAGTGGATAGGTGCGATTGACCCGTACTATGGCGGCACTTCATAT
AACCAAAAGTTCAAGGGTCGAGCTACACTCACTGTCGATAAAAGCAGCTCCACAGCCTA
TATGCACCTTAAGTCACTTACTAGCGAAGATTCTGCCGTATATTACTGCGTATCAGGTAT
GGAGTACTGGGGGCAGGGCACGTCCGTCACAGTATCATCCGGCGGCGGTGGTAGCGGGG
GAGGAGGTTCTGGTGGTGGGGGGAGTGAAATAGTCATGACTCAATCCCCTGCGACCCTG
TCCGTATCCCCGGGAGAACGCGCAACTTTGTCCTGTCGCAGCTCTCAGTCTTTGGTTCATC
GGAATGGTAATACATACCTGCACTGGTATTTGCAAAAACCCGGCCAGAGTCCGAAGCTG
CTCATCCATAAGGTCTCCAATCGCTTCTCTGGGGTACCTGATCGGTTTAGCGGGTCTGGA
TCAGGGACGGATTTTACACTGAAAATAAGTAGAGTTGAGGCAGAGGACCTTGGAGTCTA
CTTCTGCAGTCAGTCCACGCACGTACCTCCACTCACATTTGGGGCTGGGACCAAGTTGGA
ACTCAAA

SEQ ID NO: 140 amino acid sequence of GD2 scFv

EVQLLQSGPE LEKPGASVMI SCKASGSSFT GYNMNWVRQN IGKSLEWIGA IDPYYGGTSY
NQKFKGRATL TVDKSSSTAY MHLKSLTSED SAVYYCVSGM EYWGQGTSVT VSS
GGGGSGGGGSGGGGS
EIVMTQSPAT LSVSPGERAT LSCRSSQSLV HRNGNTYLHW YLQKPGQSPK LLIHKVSNRF
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP PLTFGAGTKL ELK

SEQ ID NO: 141 nucleotide sequence of NKGD2 scFv

CAAGTTCATTTGCAAGAGTCAGGCCCTGGCCTCGTTAAGCCCTCCGAGACGCTCTCTTTG
ACCTGCACAGTTTCAGATGATTCCATTTCATCATACTACTGGTCATGGATTCGGCAGCCG

CCAGGGAAGGGCCTCGAATGGATTGGACATATCAGCTACTCCGGAAGTGCTAACTATAA
CCCATCCTTGAAATCCAGAGTCACAATTTCCGTAGACACATCTAAGAACCAATTCAGCCT
GAAACTTAGTTCTGTTACTGCGGCGGATACTGCAGTGTATTATTGCGCTAATTGGGATGA
CGCCTTCAACATCTGGGGTCAAGGTACAATGGTGACCGTGAGTAGCGGGGGAGGAGGCT
CAGGCGGGGGTGGTTCAGGTGGTGGAGGCTCAGAAATCGTCTTGACGCAAAGTCCAGGA
ACTTTGAGTTTGTCTCCAGGAGAACGCGCGACGCTTTCTTGCCGAGCTTCACAATCCGTC
TCCAGCTCTTATTTGGCTTGGTATCAGCAGAAACCAGGTCAAGCTCCCAGGCTTCTGATC
TACGGTGCGTCTTCCCGAGCCACTGGGATTCCCGATCGGTTCAGCGGGTCCGGCAGCGGA
ACAGATTTCACTCTCACCATATCTAGACTTGAACCGGAGGACTTCGCAGTGTATTACTGT
CAGCAGTACGGCAGTTCACCCTGGACGTTTGGTCAGGGTACGAAAGTTGAGATCAAG

SEQ ID NO: 142 amino acid sequence of NKGD2 scFv

QVHLQESGPG LVKPSETLSL TCTVSDDSIS SYYWSWIRQP PGKGLEWIGH ISYSGSANYN
PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCANWDD AFNIWGQGTM VTVSS
GGGGSGGGGSGGGGS
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY GASSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIK

SEQ ID NO: 143 nucleotide sequence of ROR1 scFv4

CAAGTTCAGCTGCAAGAATCAGGACCTGGGCTTGTCAAACCATCTGAAACCCTC
AGCTTGACTTGTACCGTATCAGGAGGGTCAATTTCAAGCTCATCCTACTATTGGG
GATGGATCAGACAACCACCCGGGAAAGGGCTCGAGTGGATAGGGTCCATATATT
ACAGCGGATCTACATACTACAACCCGTCATTGAAGTCCAGGGTAACGATTCCGGT
GGACACTAGCAAGAATCAGTTTAGCCTCAAGTTGAGCAGTGTAACTGCTGCGGA
CACGGCGGTATATTATTGTGCTCGACACCTCGGTGGAGATGCTTTTGACATATGG
GGTCAAGGGACAACAGTCACCGTTAGCTCAGGTGGAGGGGGTAGCGGGGGGGG
CGGATCTGGGGGAGGCGGTTCATTGCCCGTACTTACACAGCCACCCTCTGTCAGC
GTCGCACCTGGACAAACCGCTCGCATCACCTGTGGCGGAAATAATATAGGTTCC
AAGTCTGTTCATTGGTATCAGCAGAAACCGGGACAGGCCCCCGTCCTTGTGGTGT
ATGATGATTCTGATAGGCCATCTGGTATCCCAGAACGGTTTTCAGGTAGCAATTC
AGGGAATACTGCCACTCTCACTATTAGCGGTACTCAAGCTATGGATGAGGCCGA
CTATTTTTGCCAGAGCTACGACTCTAGTAACCCAGTCGTGTTCGGGGGAGGGACC
CAGTTGACCGTGCTG

SEQ ID NO: 144 amino acid sequence of ROR1 scFv4

QVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSIYYSGS
TYYNPSLKSRVTIPVDTSKNQFSLKLSSVTAADTAVYYCARHLGGDAFDIWGQGTTV
TVSSGGGGSGGGGSGGGGSLPVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQ
KPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISGTQAMDEADYFCQSYDSSNP
VVFGGGTQLTVL

SEQ ID NO: 145 nucleotide sequence of ROR1 scFv9
CAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGAAGAAGCCTGG
GTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCT
ATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATC
AACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGGCAGGGTCACC
ATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGA
TCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTTTTGATATCT
GGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTGGTGGTG
GCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCACTCTGTGTC
GGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTGGCAGCAT
TGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCCCCACCAT
TGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGC
TCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGCAGAGTG
AGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGGTATTCG
GCGGAGGGACCAAGGTCACCGTCCTA

SEQ ID NO: 146 amino acid sequence of ROR1 scFv9
QAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGWIN
PNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCASYNDAFDIWGQ
GTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQ
WYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSSNSASLTISGLQSEDEADYYCQSY
EPGNGVFGGGTKVTVL

SEQ ID NO: 147 nucleotide sequence of ROR1 R12
CAAGAACAGCTTGTAGAGTCCGGCGGTAGATTGGTGACACCGGGGGGGAGCCTT
ACCCTGTCTTGTAAGGCATCTGGGTTCGATTTCAGTGCGTATTATATGAGCTGGG
TTCGGCAGGCGCCCGGGAAGGGGCTGGAATGGATAGCCACTATATACCCGTCAT
CCGGCAAGACTTACTACGCGACTTGGGTAAACGGGAGGTTTACGATAAGCTCAG

ATAACGCCCAAAACACGGTTGATCTCCAAATGAATAGCTTGACCGCCGCTGATA
GGGCGACCTATTTCTGTGCGCGGGACTCTTACGCTGATGACGGGGCCCTCTTCAA
TATATGGGGACCGGGAACGCTCGTAACCATATCATCTGGAGGAGGTGGGAGCGG
AGGCGGAGGGTCAGGTGGGGGCGGGAGCGAACTCGTACTTACACAATCTCCAAG
CGTAAGCGCAGCGTTGGGGAGTCCAGCAAAGATCACCTGCACTTTGTCAAGCGC
CCACAAAACGGATACGATAGATTGGTATCAGCAACTCCAAGGTGAAGCGCCACG
ATATCTCATGCAGGTACAGAGCGACGGGAGTTATACTAAGAGGCCCGGGGTCCC
AGACAGATTCAGTGGCAGCAGTTCAGGTGCCGACAGATACCTGATAATACCCTC
AGTTCAAGCCGATGATGAAGCCGATTACTACTGTGGGGCTGACTACATAGGTGG
GTATGTTTTCGGGGGCGGCACTCAATTGACAGTTACAGGG

SEQ ID NO: 148 amino acid sequence of ROR1 R12
QEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSWVRQAPGKGLEWIATIYPSSGK
TYYATWVNGRFTISSDNAQNTVDLQMNSLTAADRATYFCARDSYADDGALFNIWG
PGTLVTISSGGGGSGGGGSGGGGSELVLTQSPSVSAALGSPAKITCTLSSAHKTDTID
WYQQLQGEAPRYLMQVQSDGSYTKRPGVPDRFSGSSSGADRYLIIPSVQADDEADY
YCGADYIGGYVFGGGTQLTVTG

SEQ ID NO: 149 nucleotide sequence of MSLN M1-4S
GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGG
TCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATA
GTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAG
ACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACA
CGGCCTTGTATTACTGTGCAAAAGATTTATCGTCAGTGGCTGGACCCTTTAACTA
CTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTGGTGG
AGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGGACCCTGCTGTG
TCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAGA
AGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTC
ATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCA
GCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAGGATGAGG
CTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATCTGGTATTCGGCGG
AGGCACCCAGCTGACCGTCCTCGGT

SEQ ID NO: 150 amino acid sequence of MSLN M1-4S

EVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNS
GSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDLSSVAGPFNYWG
QGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSR
DSSGNHLVFGGGTQLTVLG

SEQ ID NO: 151 nucleotide sequence of CAR LTG2527 ROR1 IgG4 CD8 BBz

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAAGAACAGCTTGTAGAGTCCGGCGGTAGATTGGTGACACCGG
GGGGGAGCCTTACCCTGTCTTGTAAGGCATCTGGGTTCGATTTCAGTGCGTATTA
TATGAGCTGGGTTCGGCAGGCGCCCGGGAAGGGGCTGGAATGGATAGCCACTAT
ATACCCGTCATCCGGCAAGACTTACTACGCGACTTGGGTAAACGGGAGGTTTAC
GATAAGCTCAGATAACGCCCAAAACACGGTTGATCTCCAAATGAATAGCTTGAC
CGCCGCTGATAGGGCGACCTATTTCTGTGCGCGGGACTCTTACGCTGATGACGGG
GCCCTCTTCAATATATGGGGACCGGGAACGCTCGTAACCATATCATCTGGAGGA
GGTGGGAGCGGAGGCGGAGGGTCAGGTGGGGGCGGGAGCGAACTCGTACTTAC
ACAATCTCCAAGCGTAAGCGCAGCGTTGGGGAGTCCAGCAAAGATCACCTGCAC
TTTGTCAAGCGCCCACAAAACGGATACGATAGATTGGTATCAGCAACTCCAAGG
TGAAGCGCCACGATATCTCATGCAGGTACAGAGCGACGGGAGTTATACTAAGAG
GCCCGGGGTCCCAGACAGATTCAGTGGCAGCAGTTCAGGTGCCGACAGATACCT
GATAATACCCTCAGTTCAAGCCGATGATGAAGCCGATTACTACTGTGGGGCTGAC
TACATAGGTGGGTATGTTTTCGGGGGCGGCACTCAATTGACAGTTACAGGGGCG
GCCGCAGAGTCAAAATACGGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGG
CCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTA
CTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCG
GCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGA
GGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCC
CGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAG
AGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGG
GGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAA
GACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAG
GGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAAGGATAC
CTACGATGCCTTGCATATGCAAGCACTCCCACCCCGGTAG

SEQ ID NO: 152 amino acid sequence of CAR LTG2527 ROR1 IgG4 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSW
VRQAPGKGLEWIATIYPSSGKTYYATWVNGRFTISSDNAQNTVDLQMNSLTAADRA
TYFCARDSYADDGALFNIWGPGTLVTISSGGGGSGGGGSGGGGSELVLTQSPSVSAA
LGSPAKITCTLSSAHKTDTIDWYQQLQGEAPRYLMQVQSDGSYTKRPGVPDRFSGSS
SGADRYLIIPSVQADDEADYYCGADYIGGYVFGGGTQLTVTGAAAESKYGPPCPPCP
IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE
EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 153 nucleotide sequence of CAR LTG2528 ROR1scFv4 IgG4 CD8 BBz

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAAGTTCAGCTGCAAGAATCAGGACCTGGGCTTGTCAAACCATC
TGAAACCCTCAGCTTGACTTGTACCGTATCAGGAGGGTCAATTTCAAGCTCATCC
TACTATTGGGGATGGATCAGACAACCACCCGGGAAAGGGCTCGAGTGGATAGGG
TCCATATATTACAGCGGATCTACATACTACAACCCGTCATTGAAGTCCAGGGTAA
CGATTCCGGTGGACACTAGCAAGAATCAGTTTAGCCTCAAGTTGAGCAGTGTAA
CTGCTGCGGACACGGCGGTATATTATTGTGCTCGACACCTCGGTGGAGATGCTTT
TGACATATGGGGTCAAGGGACAACAGTCACCGTTAGCTCAGGTGGAGGGGGTAG
CGGGGGGGGCGGATCTGGGGGAGGCGGTTCATTGCCCGTACTTACACAGCCACC
CTCTGTCAGCGTCGCACCTGGACAAACCGCTCGCATCACCTGTGGCGGAAATAAT
ATAGGTTCCAAGTCTGTTCATTGGTATCAGCAGAAACCGGGACAGGCCCCCGTCC
TTGTGGTGTATGATGATTCTGATAGGCCATCTGGTATCCCAGAACGGTTTTCAGG
TAGCAATTCAGGGAATACTGCCACTCTCACTATTAGCGGTACTCAAGCTATGGAT
GAGGCCGACTATTTTTGCCAGAGCTACGACTCTAGTAACCCAGTCGTGTTCGGGG
GAGGGACCCAGTTGACCGTGCTGGCGGCCGCAGAGTCAAAATACGGTCCTCCGT
GCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCT
CCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTT
TACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGAC
GGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTC
AAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCT

ACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGA
CGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGA
AGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAAT
CGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGG
GACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCC
ACCCCGGTAG

SEQ ID NO: 154 amino acid sequence of CAR LTG2528 ROR1scFv4 IgG4 CD8 BBz
MLLLVTSLLLCELPHPAFLLIPQVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWG
WIRQPPGKGLEWIGSIYYSGSTYYNPSLKSRVTIPVDTSKNQFSLKLSSVTAADTAVY
YCARHLGGDAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSLPVLTQPPSVSVAPGQT
ARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTIS
GTQAMDEADYFCQSYDSSNPVVFGGGTQLTVLAAAESKYGPPCPPCPIYIWAPLAGT
CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 155 nucleotide sequence of CAR LTG2529 ROR1scFv9 IgG4 CD8 BBz
ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT

GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG
CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGTAG

SEQ ID NO: 156 amino acid sequence of CAR LTG2529 ROR1scFv9 IgG4 CD8 BBz
MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPR

SEQ ID NO: 157 nucleotide sequence of CAR D0181 MSLN M1-4S CD8 BBz
ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTG
GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGC
CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTAT
TAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCAC
CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG
AGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATTTATCGTCAGTGGCTGGA
CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
GGTCTGGTGGAGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGG
ACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAG
ACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCC

CTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
CTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCG
GAGGATGAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATCTG
GTATTCGGCGGAGGCACCCAGCTGACCGTCCTCGGTGCGGCCGCAACTACCACC
CCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCT
TGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGC
TGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGT
GCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTG
CTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAG
GACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGC
GTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAG
CTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAA
GCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTC
AGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCA
GAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTA
CCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGC
ACTCCCACCCCGGTAG

SEQ ID NO: 158 amino acid sequence of CAR D0181 MSLN M1-4S CD8 BBz
MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
WVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT
ALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSV
ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGAAATTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRG
RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 159 nucleotide sequence of CAR D0229 ROR1 scFv9 IgG4 CD8 BBz 2A mIL7
ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA

AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
TTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTC
TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
GCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCC
CCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTT
CTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGC
AGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGG
TATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCtGCCGCAGAGTCAAAATACGG
TCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTTGC
GGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGCGcGGccGcAAGAA
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GAAGATGGGTGCTCTTGTCGGTTCCCGGAAGAAGAAGAGGGTGGTTGCGAGTTG
AGGGTGAAGTTCTCCCGCTCTGCCGACGCACCGGCATATCAGCAGGGACAAAAC
CAGCTCTACAACGAATTGAACCTGGGTCGGCGGGAAGAATATGACGTGCTCGAT
AAGCGGCGGGGTCGCGACCCAGAAATGGGAGGCAAACCGCGCAGGAAAAATCC
ACAGGAGGGACTTTATAACGAACTTCAAAAGGATAAGATGGCAGAGGCATACAG
CGAAATCGGGATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTT
ACCAGGGGCTTTCTACCGCGACGAAGGATACCTACGATGCTCTCCATATGCAAGC
ACTTCCTCCTAGACGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTG
AAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAGTAATG
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AGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAATAC
TGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGGTGAAG
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GCACTCACTGACCTGTGCTTTTGAGGACCCAGATGTCAACATCACCAATCTGGAA
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CGTATGGCCCAGTCTCCCCGATCATAAGAAGACTCTGGAACATCTTTGTAAGAAA
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ATTCTCTCCTCTTCCAGGTCCCTAGACTGCAGGGAGAGTGGCAAGAATGGGCCTC
ATGTGTACCAGGACCTCCTTCTTAGCCTTGGGACTACAAACAGCACGCTGCCCCC
TCCATTTTCTCTCCAATCTGGAATCCTGACATTGAACCCAGTTGCTCAGGGTCAG
CCCATTCTTACTTCCCTGGGATCAAATCAAGAAGAAGCATATGTCACCATGTCCA
GCTTCTACCAAAACCAGCCCTAG

SEQ ID NO: 160 amino acid sequence of CAR D0229 ROR1 scFv9 IgG4 CD8 BBz 2A mIL7

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW

APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
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LIPMDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEG
MFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQP
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SGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNITNLEFEICGALV
EVKCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTTIVKPEAPFDLSVV
YREGANDFVVTFNTSHLQKKYVKVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQR
KLQPAAMYEIKVRSIPDHYFKGFWSEWSPSYYFRTPEINNSSGEMDPILLTISILSFFSV
ALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRV
DDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTC
LAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTL
NPVAQGQPILTSLGSNQEEAYVTMSSFYQNQP

SEQ ID NO: 161 nucleotide sequence of CAR D0228 ROR1 scFv9 IgG4 CD8 BBz 2A TGFbRIIdn

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
TTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTC
TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
GCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCC
CCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTT
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CGAAATCGGGATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTT
ACCAGGGGCTTTCTACCGCGACGAAGGATACCTACGATGCTCTCCATATGCAAGC
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TGTAAGATTTTCAACGTGCGATAACCAGAAAAGCTGTATGTCCAACTGCAGCATC
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GACGAAAATATCACACTGGAGACCGTTTGTCACGATCCTAAACTCCCTTATCATG
ACTTTATTCTGGAAGACGCAGCGTCACCGAAGTGTATAATGAAAGAGAAGAAGA
AGCCTGGAGAGACGTTTTTCATGTGCAGTTGCTCCTCAGATGAGTGTAATGACAA
CATCATTTTTTCCGAGGAGTACAATACGAGTAACCCAGACCTCCTGCTGGTTATT
TTCCAGGTAACCGGCATCAGTTTGTTGCCCCCACTGGGTGTTGCAATCAGTGTAA
TAATCATATTTTATTGTTACCGGGTGTGA

SEQ ID NO: 162 amino acid sequence of CAR D0228 ROR1 scFv9 IgG4 CD8 BBz 2A TGFbRIIdn

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRVDMGRGLLRGLWPLHIVL
WTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG
ETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQVTGISLLPPLGVAISVIIIFYCYRV

SEQ ID NO: 163 nucleotide sequence of CAR D0231 ROR1 scFv9 IgG4 CD8 BBz 2A tEGFR

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
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TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
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GCTAGGCCCAGGAAGGTTTGCAATGGAATCGGTATAGGGGAGTTTAAGGATTCA
CTTAGCATAAACGCTACTAAcATTAAACACTTCAAAAACTGTACGAGTATAAGTG
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ACATAGATGGACCTCACTGTGTGAAAACCTGCCCGGCCGGAGTTATGGGAGAAA
ACAACACTCTGGTGTGGAAATACGCTGATGCAGGCCACGTGTGCCACCTTTGTCA
CCCGAATTGcACATATGGGTGTACCGGTCCTGGACTTGAAGGTTGCCCTACCAAT
GGCCCTAAAATACCCAGTATCGCAACTGGCATGGTAGGCGCTCTTCTCTTGCTCT
TGGTAGTTGCTCTCGGCATAGGTCTTTTTATGTGA

SEQ ID NO: 164 amino acid sequence of CAR D0231 ROR1 scFv9 IgG4 CD8 BBz 2A tEGFR

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRASVYMALPVTALLLPLALL
LHAARPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPL

DPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNIT
SLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQ
VCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPE
CLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHV
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SEQ ID NO: 165 nucleotide sequence of CAR D0245 MSLN M1-4S CD8 BBz 2A mIL7
ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTG
GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGC
CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTAT
TAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCAC
CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG
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CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
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ACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAG
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TCCTAGACGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGAAGCA
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GTGTACCAGGACCTCCTTCTTAGCCTTGGGACTACAAACAGCACGCTGCCCCCTC

CATTTTCTCTCCAATCTGGAATCCTGACATTGAACCCAGTTGCTCAGGGTCAGCC
CATTCTTACTTCCCTGGGATCAAATCAAGAAGAAGCATATGTCACCATGTCCAGC
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SEQ ID NO: 166 amino acid sequence of CAR D0245 MSLN M1-4S CD8 BBz 2A mIL7
MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
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QLEVNGSQHSLTCAFEDPDVNITNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLIG
KSNICVKVGEKSLTCKKIDLTTIVKPEAPFDLSVVYREGANDFVVTFNTSHLQKKYV
KVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMYEIKVRSIPDHYFKGF
WSEWSPSYYFRTPEINNSSGEMDPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSL
PDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLE
ESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE
SGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVT
MSSFYQNQPR

SEQ ID NO: 167 nucleotide sequence of CAR D0284 MSLN M1-4S CD8 28z 2A mIL7
ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
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TAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCAC
CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG

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CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
GGTCTGGTGGAGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGG
ACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAG
ACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCC
CTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
CTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCG
GAGGATGAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATCTG
GTATTCGGCGGAGGCACCCAGCTGACCGTCCTCGGTGCGGCCGCAACCACTACG
CCTGCTCCGCGGCCTCCAACGCCCGCGCCCACGATAGCTAGTCAGCCGTTGTCTC
TCCGACCAGAGGCGTGTAGACCGGCCGCTGGCGGAGCCGTACATACTCGCGGAC
TCGACTTCGCTTGCGACATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGGGT
GCTGTTGCTGTCCTTGGTTATTACGTTGTACTGCCGGTCGAAGAGGTCCAGACTC
TTGCACTCCGACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAG
CACTACCAGCCGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCAGAGTCA
AATTTTCCAGGTCCGCAGATGCCCCCGCGTACCAGCAAGGCCAGAACCAACTTTA
CAACGAACTGAACCTGGGTCGCCGGGAGGAATATGATGTGCTGGATAAACGAAG
GGGGAGGGACCCTGAGATGGGAGGGAAACCTCGCAGGAAAAACCCGCAGGAAG
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GGATGAAGGGAGAGAGACGGAGAGGAAAAGGCCATGATGGCCTTTACCAGGGC
TTAAGCACAGCAACAAAGGATACTTACGACGCTCTTCACATGCAAGCTCTGCCAC
CACGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGAAGCAGG
CCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAGTAATGCTCTTGC
TCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTTTTGATACCT
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GGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAATACTGTTGA
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CAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACAGAAAAAACTGAATG
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TTTGATGGGCACTAAAGAACACTCCGGAGGTTCCGGTGGTGGCTCAGGTGGTGG
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TGTATGAGATTAAAGTTCGATCCATCCCTGATCACTATTTTAAAGGCTTCTGGAG
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GATTCATCCCTCACATGCCTGGCTGGGAATGTCAGTGCATGTGACGCCCCTATTC
TCTCCTCTTCCAGGTCCCTAGACTGCAGGGAGAGTGGCAAGAATGGGCCTCATGT
GTACCAGGACCTCCTTCTTAGCCTTGGGACTACAAACAGCACGCTGCCCCCTCCA
TTTTCTCTCCAATCTGGAATCCTGACATTGAACCCAGTTGCTCAGGGTCAGCCCA
TTCTTACTTCCCTGGGATCAAATCAAGAAGAAGCATATGTCACCATGTCCAGCTT
CTACCAAAACCAGCCCTAG

SEQ ID NO: 168 amino acid sequence of CAR D0284 MSLN M1-4S CD8 28z 2A mIL7
MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
WVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT
ALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSV
ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGAAATTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKR
SRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI

GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQA
GDVEENPGPRAKRVMLLLVTSLLLCELPHPAFLLIPMDCDIEGKDGKQYESVLMVSI
DQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFD
LHLLKVSEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKR
LLQEIKTCWNKILMGTKEHSGGSGGGSGGGSGESGYAQNGDLEDAELDDYSFSCYS
QLEVNGSQHSLTCAFEDPDVNITNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLIG
KSNICVKVGEKSLTCKKIDLTTIVKPEAPFDLSVVYREGANDFVVTFNTSHLQKKYV
KVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMYEIKVRSIPDHYFKGF
WSEWSPSYYFRTPEINNSSGEMDPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSL
PDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLE
ESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE
SGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVT
MSSFYQNQP

SEQ ID NO: 169 nucleotide sequence of CAR D0211 MSLN M1-4S CD8 BBz 2A TGFbRIIdn

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTG
GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGC
CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTAT
TAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCAC
CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG
AGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATTTATCGTCAGTGGCTGGA
CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
GGTCTGGTGGAGGCGGTAGCGGCGGTGGCGGATCCTCTTCTGAGCTGACTCAGG
ACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAG
ACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCC
CTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
CTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCG
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GTATTCGGCGGAGGCACCCAGCTGACCGTCCTCGGTGCGGCCGCTACCACAACC
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CCGACCTGAGGCTTGTAGACCTGCAGCCGGGGGTGCCGTCCACACACGGGGACT
CGACTTCGCTTGTGATATATATATTTGGGCGCCCCTGGCCGGCACTTGTGGAGTT

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TACAACGAATTGAACCTGGGTCGGCGGGAAGAATATGACGTGCTCGATAAGCGG
CGGGGTCGCGACCCAGAAATGGGAGGCAAACCGCGCAGGAAAAATCCACAGGA
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GGTAACCGGCATCAGTTTGTTGCCCCCACTGGGTGTTGCAATCAGTGTAATAATC
ATATTTTATTGTTACCGGGTGTGA

SEQ ID NO: 170 amino acid sequence of CAR D0211 MSLN M1-4S CD8 BBz 2A TGFbRIIdn

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
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ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGAAATTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRG
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QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQ

AGDVEENPGPRAKRVDMGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTD
NNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITL
ETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTS
NPDLLLVIFQVTGISLLPPLGVAISVIIIFYCYRV

SEQ ID NO: 171 nucleotide sequence of CAR D0246 MSLN M1-4S CD8 BBz 2A mIL7 2A tEGFR

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGGAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTACAGCCTG
GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGC
CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTAT
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CATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAG
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CCCTTTAACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCG
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CGGGATGAAAGGCGAGAGAAGAAGGGGGAAAGGGCACGATGGTCTTTACCAGG
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GGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCTAAGCGAGTAATGCTCTT
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GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAAGGT
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TGTTTGGCACTTCCGGCCAGAAAACCAAGATTATTTCAAATCGGGGTGAGAACA
GCTGCAAAGCCACCGGCCAGGTTTGTCATGCCTTGTGCTCTCCGGAAGGCTGTTG
GGGGCCAGAACCCAGGGACTGCGTCAGTTGCAGAAACGTCTCAAGAGGCCGCGA
ATGCGTTGACAAGTGTAACCTCCTTGAGGGTGAGCCACGAGAGTTTGTTGAGAA
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TGCACAGGCCGCGGGCCTGATAACTGCATCCAGTGTGCTCATTACATAGATGGAC
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TGTGGAAATACGCTGATGCAGGCCACGTGTGCCACCTTTGTCACCCGAATTGCAC
ATATGGGTGTACCGGTCCTGGACTTGAAGGTTGCCCTACCAATGGCCCTAAAATA
CCCAGTATCGCAACTGGCATGGTAGGCGCTCTTCTCTTGCTCTTGGTAGTTGCTCT
CGGCATAGGTCTTTTTATGTGA

SEQ ID NO: 172 amino acid sequence of CAR D0246 MSLN M1-4S CD8 BBz 2A mIL7 2A tEGFR

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
WVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT
ALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSV
ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGAAATTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRG
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QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATNFSLLKQ
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LLQEIKTCWNKILMGTKEHSGGSGGGSGGGSGESGYAQNGDLEDAELDDYSFSCYS
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KSNICVKVGEKSLTCKKIDLTTIVKPEAPFDLSVVYREGANDFVVTFNTSHLQKKYV
KVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMYEIKVRSIPDHYFKGF
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PDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLE
ESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE
SGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVT
MSSFYQNQPRRAKRGSGEGRGSLLTCGDVEENPGPMMALPVTALLLPLALLLHAAR
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DILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLR
SLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHAL
CSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQA
MNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCH
PNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

SEQ ID NO: 173 nucleotide sequence of CAR D0233 MSLN M1-4S ROR1 scFv9 IgG4 CD8 BBz

ATGCTGTTACTTGTGACAAGCTTGCTTCTATGTGAACTGCCGCATCCGGCGTTTCT
GCTGATTCCGGAAGTACAGCTGGTACAGTCTGGAGGGGGATTGGTTCAGCCGGG
CGGGTCTTTGCGCCTGTCCTGCGCAGCTAGTGGCTTCACTTTTGATGACTATGCTA
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GCTGGAATAGTGGATCCATCGGCTATGCCGATAGTGTAAAGGGCAGGTTCACGA
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CCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCC
TGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTA
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ATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAA
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CTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCAC
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SEQ ID NO: 174 amino acid sequence of CAR D0233 MSLN M1-4S ROR1 scFv9 IgG4 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
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ALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSV
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ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGGGGGSGGGGSGGGGSG
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YNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSESPGKTVTISCTR
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DEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIWAPLAGTCGVLLL
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DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
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SEQ ID NO: 175 nucleotide sequence of CAR D0279 MSLN M1-4S ROR1 scFv9 IgG4 CD8 BBz 2A mIL7

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CTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGGCAGGGTCACCATGA
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TTTTCTCTCCAATCTGGAATCCTGACATTGAACCCAGTTGCTCAGGGTCAGCCCA
TTCTTACTTCCCTGGGATCAAATCAAGAAGAAGCATATGTCACCATGTCCAGCTT
CTACCAAAACCAGCCCTAG

SEQ ID NO: 176 amino acid sequence of CAR D0279 MSLN M1-4S ROR1 scFv9 IgG4 CD8 BBz 2A mIL7

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
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SEQ ID NO: 177 nucleotide sequence of CAR D0280 MSLN M1-4S ROR1 scFv9 IgG4 CD28 28BBz 2A mIL7

ATGCTGTTACTTGTGACAAGCTTGCTTCTATGTGAACTGCCGCATCCGGCGTTTCT
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SEQ ID NO: 178 amino acid sequence of CAR D0280 MSLN M1-4S ROR1 scFv9 IgG4 CD28 28BBz 2A mIL7

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SEQ ID NO: 179 nucleotide sequence of CAR D0281 MSLN M1-4S ROR1 scFv9 IgG4 CD8 28BBz 2A mIL7

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GAAACTACAAGAGATATATTTCATCGAGACAAAGAAATTCTTACTGATTGGAAA
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SEQ ID NO: 180 amino acid sequence of CAR D0281 MSLN M1-4S ROR1 scFv9 IgG4 CD8 28BBz 2A mIL7

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SEQ ID NO: 181 nucleotide sequence of CAR D0282 ROR1 scFv9 IgG4 OX40 OX40BBz 2A MSLN M1-4S CD8 ICOSz2A mIL7

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SEQ ID NO: 182 amino acid sequence of CAR D0282 ROR1 scFv9 IgG4 OX40 OX40BBz 2A MSLN M1-4S CD8 ICOSz2A mIL7

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SEQ ID NO: 183 nucleotide sequence of CAR D0283 ROR1 scFv9 IgG4 CD8 BBz 2A MSLN M1-4S CD8 28z 2A mIL7

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SEQ ID NO: 184 amino acid sequence of CAR D0283 ROR1 scFv9 IgG4 CD8 BBz 2A MSLN M1-4S CD8 28z 2A mIL7

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SEQ ID NO: 185 nucleotide sequence of CAR D0344 MSLN M1-4S CD8 BBz 2A HPSE
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SEQ ID NO: 186 amino acid sequence of CAR D0344 MSLN M1-4S CD8 BBz 2A HPSE
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SEQ ID NO: 187 nucleotide sequence of CAR D0345 MSLN M1-4S CD8 BBz 2A MMP2
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SEQ ID NO: 188 amino acid sequence of CAR D0345 MSLN M1-4S CD8 BBz 2A MMP2
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SEQ ID NO: 189 nucleotide sequence of CAR D0346 MSLN M1-4S CD8 BBz 2A SP PH20 IgG1 Fc
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SEQ ID NO: 190 amino acid sequence of CAR D0346 MSLN M1-4S CD8 BBz 2A SP PH20 IgG1 Fc

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SEQ ID NO: 191 nucleotide sequence of CAR D0347 ROR1 scFv9 IgG4 CD8 BBz 2A HPSE

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SEQ ID NO: 192 amino acid sequence of CAR D0347 ROR1 scFv9 IgG4 CD8 BBz 2A HPSE

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SEQ ID NO: 193 nucleotide sequence of CAR D0348 ROR1 scFv9 IgG4 CD8 BBz 2A MMP2

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ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
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SEQ ID NO: 196 amino acid sequence of CAR D0349 ROR1 scFv9 IgG4 CD8 BBz 2A SP PH20 IgG1 Fc

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SEQ ID NO: 197 nucleotide sequence of CAR D0358 MSLN ROR1 scFv9 IgG4 CD28 TM CD28 BBz

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GCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCA
AACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGG
GCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCA
GCAGGGGCAAAACCAGCTGTACAACGAACTTAACCTCGGTCGCCGGGAAGAATA
TGACGTGCTGGACAAGCGGCGGGGAAGAGATCCCGAGATGGGTGGAAAGCCGC
GGCGGAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGCAAAAGGACAAAATG
GCCGAAGCCTACTCCGAGATTGGCATGAAGGGAGAGCGCAGACGCGGGAAGGG
ACACGATGGACTGTACCAGGGACTGTCAACCGCGACTAAGGACACTTACGACGC
CCTGCACATGCAGGCCCTGCCCCCGCGCTAA

SEQ ID NO: 198 amino acid sequence of CAR D0358 MSLN ROR1 scFv9 IgG4 CD28 TM CD28 BBz

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGGGLVQPGGSLRLSCAASGFTFDDYAMH
WVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT
ALYYCAKDLSSVAGPFNYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSV
ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGTQLTVLGGGGGSGGGGSGGGGSG
GGGSGGGGSQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ
GLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAS
YNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSESPGKTVTISCTR
SSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSSNSASLTISGLQSE
DEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPFWVLVVVGGVLACY
SLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRG
RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 199 nucleotide sequence of EF1a Promoter
CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCG
AGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGC
GGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG
GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGG
GTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTC
TTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGT
GATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGC
GCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGG
GCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAA
GTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAG
ATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGC
CGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGG
CCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCC
TGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGG
CTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCT
GCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGA
GTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGAC
TCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGA
GTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACAC
TGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTG
GAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGT
TCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTG

SEQ ID NO: 200 nucleotide sequence of MND Promoter
AGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGAAGCCTATAGAG
TACGAGCCATAGATAGAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGA
ATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGGATCAAGGTTAGGAACAGAG
AGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCG
GCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTG
TGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGC
GGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAA
GGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGC
TTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCA
CTCGGCG

SEQ ID NO: 201 nucleotide sequence of MSCV Promoter
TGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAA
GGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTTAGGAACA
GAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCC
CCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGT
TTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCT
GTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGTTTCT
GCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACT

SEQ ID NO: 202 nucleotide sequence of PGK Promoter
TCCACGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGAC
GCGGCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTC
GCACATTCTTCACGTCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTG
GGCCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGT
TCGCGGCGTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCG
CAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTG
GCCAATAGCGGCTGCTCAGCGGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGC
GGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGCGCG
GTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCCCTCGTT
GACCGAATCACCGACCTCTCTCCCCG

SEQ ID NO: 203 nucleotide sequence of NFAT Promoter
TGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAG
AAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTC
ATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAA
CTGTTTCATACAGAAGGCGTCTGCAGGAGACTCTAGAGGGTATATAATGGTTTAA
ACTTAAGCTTGGTACCGGGCCCCCGAAG

SEQ ID NO: 204 nucleotide sequence of AP1/NFKb Promoter
TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAGCTCGAG
GATCTCGCTAGCGGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCG
GGAATTTCC

SEQ ID NO 205: nucleotide sequence of NAP (Helicobacter pylori neutrophil-activating protein A)

ATGAAAACGTTTGAGATACTTAAACATCTCCAGGCCGACGCCATTGTCCTGTTCA
TGAAGGTTCATAATTTTCATTGGAACGTGAAAGGAACTGATTTCTTTAATGTCCA
CAAAGCCACCGAGGAAATTTATGAGGAGTTTGCGGATATGTTTGACGATTTGGCT
GAACGAATAGTGCAGTTGGGTCATCATCCGTTGGTAACTCTGTCCGAGGCAATCA
AGCTTACGAGGGTGAAAGAGGAGACAAAGACATCATTCCACTCTAAGGACATTT
TCAAAGAAATTTTGGAAGATTATAAATACCTGGAAAAGGAGTTCAAGGAGCTTT
CCAACACGGCCGAAAAGGAGGGAGACAAAGTTACAGTCACATATGCGGACGAT
CAACTGGCCAAGCTCCAGAAGAGTATCTGGATGCTCCAAGCCCATTTGGCC

SEQ ID NO 206: amino acid sequence of NAP

MKTFEILKHLQADAIVLFMKVHNFHWNVKGTDFFNVHKATEEIYEEFADMFDDLAE
RIVQLGHHPLVTLSEAIKLTRVKEETKTSFHSKDIFKEILEDYKYLEKEFKELSNTAEK
EGDKVTVTYADDQLAKLQKSIWMLQAHLA

SEQ ID NO 207: amino acid sequence of MMP-2

MEALMARGALTGPLRALCLLGCLLSHAAAAPSPIIKFPGDVAPKTDKELAVQYLNTF
YGCPKESCNLFVLKDTLKKMQKFFGLPQTGDLDQNTIETMRKPRCGNPDVANYNFF
PRKPKWDKNQITYRIIGYTPDLDPETVDDAFARAFQVWSDVTPLRFSRIHDGEADIMI
NFGRWEHGDGYPFDGKDGLLAHAFAPGTGVGGDSHFDDDELWTLGEGQVVRVKY
GNADGEYCKFPFLFNGKEYNSCTDTGRSDGFLWCSTTYNFEKDGKYGFCPHEALFT
MGGNAEGQPCKFPFRFQGTSYDSCTTEGRTDGYRWCGTTEDYDRDKKYGFCPETA
MSTVGGNSEGAPCVFPFTFLGNKYESCTSAGRSDGKMWCATTANYDDDRKWGFCP
DQGYSLFLVAAHEFGHAMGLEHSQDPGALMAPIYTYTKNFRLSQDDIKGIQELYGAS
PDIDLGTGPTPTLGPVTPEICKQDIVFDGIAQIRGEIFFFKDRFIWRTVTPRDKPMGPLL
VATFWPELPEKIDAVYEAPQEEKAVFFAGNEYWIYSASTLERGYPKPLTSLGLPPDV
QRVDAAFNWSKNKKTYIFAGDKFWRYNEVKKKMDPGFPKLIADAWNAIPDNLDAV
VDLQGGGHSYFFKGAYYLKLENQSLKSVKFGSIKSDWLGC

SEQ ID NO 208: nucleotide sequence of MMP-2

ATGGAAGCTCTTATGGCTAGGGGAGCGCTCACAGGTCCATTGCGGGCACTGTGC
CTGTTGGGGTGCCTGTTGTCTCATGCGGCTGCGGCTCCGTCACCAATTATCAAAT
TTCCTGGCGACGTTGCCCCGAAGACAGACAAGGAACTTGCCGTGCAGTACCTCA

ATACGTTCTATGGATGTCCTAAAGAATCATGCAATCTGTTCGTTTTGAAAGATAC
CCTTAAGAAGATGCAAAAGTTCTTTGGCTTGCCACAGACTGGGGACCTTGACCAG
AATACaATTGAAACTATGAGAAAACCGAGATGCGGCAACCCCGATGTGGCTAAC
TACAACTTTTTTCCCAGAAAGCCTAAATGGGATAAGAACCAGATTACATACCGG
ATAATAGGATATACACCCGACCTGGACCCGGAGACAGTTGACGACGCATTTGCG
CGCGCCTTTCAGGTTTGGTCAGATGTAACTCCGCTTCGCTTTTCaCGAATaCATGA
CGGAGAAGCTGACATCATGATTAATTTCGGTCGGTGGGAGCATGGGGATGGTTA
TCCTTTCGACGGCAAAGACGGGCTGCTCGCCCATGCCTTTGCGCCTGGGACCGGC
GTCGGTGGTGATAGCCACTTCGATGACGATGAACTCTGGACCCTCGGAGAGGGA
CAAGTGGTGAGAGTAAAATACGGAAACGCCGACGGAGAATATTGCAAGTTCCCC
TTTCTaTTCAATGGTAAGGAATATAATAGCTGTACTGATACAGGTAGATCAGACG
GCTTCCTTTGGTGCTCAACCACCTACAATTTCGAAAAAGATGGTAAGTACGGCTT
CTGCCCTCATGAGGCCCTGTTCACTATGGGAGGCAATGCAGAGGGACAGCCGTG
CAAATTTCCATTTCGCTTTCAAGGTACGAGCTACGATTCTTGTACGACGGAGGGG
AGAACGGATGGGTATAGATGGTGTGGCACAACAGAGGATTACGATAGAGACAA
GAAATATGGGTTCTGTCCCGAGACCGCTATGAGTACAGTTGGGGGTAATTCCGA
GGGAGCTCCCTGCGTGTTCCCGTTCACATTCTTGGGTAACAAGTACGAGTCCTGT
ACCAGCGCTGGGCGGTCTGATGGTAAAATGTGGTGTGCAACGACGGCAAATTAC
GACGACGATCGGAAGTGGGGTTTTTGTCCTGACCAGGGTTACTCTCTGTTTCTCG
TTGCAGCGCATGAATTtGGACAcGCAATGGGTCTTGAGCACTCACAGGACCCCGG
CGCACTTATGGCGCCAATATACACTTACACCAAGAACTTTAGATTGAGTCAGGAC
GATATTAAGGGCATCCAGGAGCTTTATGGAGCCTCACCAGACATCGATCTGGGG
ACTGGTCCCACTCCCACTCTTGGTCCTGTCACACCAGAAATTTGTAAACAGGATA
TAGTCTTTGATGGTATAGCCCAGATTCGCGGAGAGATCTTTTTCTTTAAGGACAG
GTTCATCTGGAGGACAGTGACGCCAAGAGATAAACCCATGGGTCCTCTGTTGGT
AGCAACCTTCTGGCCCGAGCTCCCAGAGAAGATAGATGCAGTGTATGAGGCCCC
ACAAGAGGAGAAAGCGGTCTTTTTCGCGGGGAATGAGTATTGGATCTACTCAGC
CTCCACTCTGGAAAGAGGGTACCCAAAACCACTGACTTCTCTGGGTTTGCCCCCA
GATGTACAGCGAGTAGATGCTGCATTTAATTGGAGCAAGAATAAGAAGACCTAC
ATTTTCGCGGGGGATAAGTTCTGGAGGTACAATGAAGTCAAGAAGAAAATGGAT
CCCGGATTTCCAAAGCTCATAGCCGACGCATGGAATGCCATCCCGGACAACCTG
GATGCCGTCGTAGACTTGCAGGGTGGGGGACACTCCTATTTTTTCAAAGGAGCGT
ATTATTTGAAATTGGAGAATCAAAGTCTTAAGTCAGTTAAGTTTGGATCAATCAA
GAGCGACTGGCTCGGGTGT

SEQ ID NO 209: amino acid sequence of MMP-9

MSLWQPLVLVLLVLGCCFAAPRQRQSTLVLFPGDLRTNLTDRQLAEEYLYRYGYTR
VAEMRGESKSLGPALLLLQKQLSLPETGELDSATLKAMRTPRCGVPDLGRFQTFEGD
LKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPLTFTRVYSRDADIVIQF
GVAEHGDGYPFDGKDGLLAHAFPPGPGIQGDAHFDDDELWSLGKGVVVPTRFGNA
DGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDRFGFCPSERLYTQDGN
ADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADSTVM
GGNSAGELCVFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFDSDKKWGFCPDQGYS
LFLVAAHEFGHALGLDHSSVPEALMYPMYRFTEGPPLHKDDVNGIRHLYGPRPEPEP
RPPTTTTPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSP
VDDACNVNIFDAIAEIGNQLYLFKDGKYWRFSEGRGSRPQGPFLIADKWPALPRKLD
SVFEERLSKKLFFFSGRQVWVYTGASVLGPRRLDKLGLGADVAQVTGALRSGRGK
MLLFSGRRLWRFDVKAQMVDPRSASEVDRMFPGVPLDTHDVFQYREKAYFCQDRF
YWRVSSRSELNQVDQVGYVTYDILQCPED

SEQ ID NO 210: nucleotide sequence of MMP-9

ATGAGCCTCTGGCAGCCCCTGGTCCTGGTGCTCCTGGTGCTGGGCTGCTGCTTTG
CTGCCCCCAGACAGCGCCAGTCCACCCTTGTGCTCTTCCCTGGAGACCTGAGAAC
CAATCTCACCGACAGGCAGCTGGCAGAGGAATACCTGTACCGCTATGGTTACAC
TCGGGTGGCAGAGATGCGTGGAGAGTCGAAATCTCTGGGGCCTGCGCTGCTGCT
TCTCCAGAAGCAACTGTCCCTGCCCGAGACCGGTGAGCTGGATAGCGCCACGCT
GAAGGCCATGCGAACCCCACGGTGCGGGGTCCCAGACCTGGGCAGATTCCAAAC
CTTTGAGGGCGACCTCAAGTGGCACCACCACAACATCACCTATTGGATCCAAAA
CTACTCGGAAGACTTGCCGCGGGCGGTGATTGACGACGCCTTTGCCCGCGCCTTC
GCACTGTGGAGCGCGGTGACGCCGCTCACCTTCACTCGCGTGTACAGCCGGGAC
GCAGACATCGTCATCCAGTTTGGTGTCGCGGAGCACGGAGACGGGTATCCCTTCG
ACGGGAAGGACGGGCTCCTGGCACACGCCTTTCCTCCTGGCCCCGGCATTCAGG
GAGACGCCCATTTCGACGATGACGAGTTGTGGTCCCTGGGCAAGGGCGTCGTGG
TTCCAACTCGGTTTGGAAACGCAGATGGCGCGGCCTGCCACTTCCCCTTCATCTT
CGAGGGCCGCTCCTACTCTGCCTGCACCACCGATGGACGGTCCGACGGCTTGCCC
TGGTGCAGTACCACGGCCAACTACGACACCGACGACCGGTTTGGCTTCTGCCCCA
GCGAGAGACTCTACACCCAGGACGGCAATGCTGATGGGAAACCCTGCCAGTTTC
CATTCATCTTCCAAGGCCAATCCTACTCCGCCTGCACCACGGACGGTCGCTCCGA

CGGGTACCGCTGGTGCGCCACCACCGCCAACTACGACCGGGACAAGCTCTTCGG
CTTCTGCCCGACCCGAGCTGACTCGACGGTGATGGGGGGCAACTCGGCGGGGGA
GCTGTGCGTCTTCCCCTTCACTTTCCTGGGTAAGGAGTACTCGACCTGTACCAGC
GAGGGCCGCGGAGATGGGCGCCTCTGGTGCGCTACCACCTCGAACTTTGACAGC
GACAAGAAGTGGGGCTTCTGCCCGGACCAAGGATACAGTTTGTTCCTCGTGGCG
GCGCATGAGTTCGGCCACGCGCTGGGCTTAGATCATTCCTCAGTGCCGGAGGCGC
TCATGTACCCTATGTACCGCTTCACTGAGGGGCCCCCCTTGCATAAGGACGACGT
GAATGGCATCCGGCACCTCTATGGTCCTCGCCCTGAACCTGAGCCACGACCTCCA
ACAACCACCACACCGCAGCCCACGGCTCCACCGACGGTCTGCCCCACCGGACCC
CCCACTGTCCACCCCTCAGAGCGCCCCACTGCTGGCCCAACAGGACCTCCCTCAG
CTGGCCCCACAGGTCCCCCAACTGCTGGCCCTTCTACGGCCACTACTGTGCCTTT
GAGTCCGGTGGACGATGCCTGCAACGTGAACATCTTCGACGCCATCGCGGAGAT
TGGGAACCAGCTGTATTTGTTCAAGGATGGGAAGTACTGGCGATTCTCTGAGGGC
AGGGGGAGCCGGCCGCAGGGCCCCTTCCTTATCGCCGACAAGTGGCCCGCGCTG
CCCCGCAAGCTGGACTCGGTCTTTGAGGAGCGGCTCTCCAAGAAGCTTTTCTTCT
TCTCTGGTCGCCAGGTGTGGGTGTACACAGGTGCGTCGGTGCTGGGACCGAGGC
GTCTAGACAAGCTAGGCCTGGGAGCAGACGTGGCCCAGGTGACCGGGGCCCTCC
GGAGTGGCAGGGGGAAGATGCTGCTGTTCAGCGGGCGGCGCCTCTGGAGGTTCG
ACGTGAAGGCGCAGATGGTGGATCCCCGGAGCGCCAGCGAGGTGGACCGGATGT
TCCCCGGGGTGCCTTTGGACACGCACGACGTCTTCCAGTACCGAGAGAAAGCCT
ATTTCTGCCAGGACCGCTTCTACTGGCGCGTGAGTTCCCGGAGTGAGTTGAACCA
GGTGGACCAAGTGGGCTACGTGACCTATGACATCCTGCAGTGCCCTGAGGAC

SEQ ID NO 211: amino acid sequence of HPSE

MLLRSKPALPPPLMLLLLGPLGPLSPGALPRPAQAQDVVDLDFFTQEPLHLVSPSFLS
VTIDANLATDPRFLILLGSPKLRTLARGLSPAYLRFGGTKTDFLIFDPKKESTFEERSY
WQSQVNQDICKYGSIPPDVEEKLRLEWPYQEQLLLREHYQKKFKNSTYSRSSVDVL
YTFANCSGLDLIFGLNALLRTADLQWNSSNAQLLLDYCSSKGYNISWELGNEPNSFL
KKADIFINGSQLGEDFIQLHKLLRKSTFKNAKLYGPDVGQPRRKTAKMLKSFLKAGG
EVIDSVTWHHYYLNGRTATKEDFLNPDVLDIFISSVQKVFQVVESTRPGKKVWLGET
SSAYGGGAPLLSDTFAAGFMWLDKLGLSARMGIEVVMRQVFFGAGNYHLVDENFD
PLPDYWLSLLFKKLVGTKVLMASVQGSKRRKLRVYLHCTNTDNPRYKEGDLTLYAI
NLHNVTKYLRLPYPFSNKQVDKYLLRPLGPHGLLSKSVQLNGLTLKMVDDQTLPPL
MEKPLRPGSSLGLPAFSYSFFVIRNAKVAACI

SEQ ID NO 212: nucleotide sequence of HPSE

ATGCTTCTACGTTCTAAACCTGCACTTCCTCCACCACTTATGCTACTTCTTCTAGG
ACCTCTTGGTCCTCTATCACCTGGAGCTCTACCTCGACCTGCACAAGCACAGGAC
GTCGTGGACCTGGACTTCTTCACCCAGGAGCCGCTGCACCTGGTGAGCCCCTCGT
TCCTGTCCGTCACCATTGACGCCAACCTGGCCACGGACCCGCGGTTCCTCATCCT
CCTGGGTTCTCCAAAGCTTCGTACCTTGGCCAGAGGCTTGTCTCCTGCGTACCTG
AGGTTTGGTGGCACCAAGACAGACTTCCTAATTTTCGATCCCAAGAAGGAATCA
ACCTTTGAAGAGAGAAGTTACTGGCAATCTCAAGTCAACCAGGATATTTGCAAA
TATGGATCCATCCCTCCTGATGTGGAGGAGAAGTTACGGTTGGAATGGCCCTACC
AGGAGCAATTGCTACTCCGAGAACACTACCAGAAAAAGTTCAAGAACAGCACCT
ACTCAAGAAGCTCTGTAGATGTGCTATACACTTTTGCAAACTGCTCAGGACTGGA
CTTGATCTTTGGCCTAAATGCGTTATTAAGAACAGCAGATTTGCAGTGGAACAGT
TCTAATGCTCAGTTGCTCCTGGACTACTGCTCTTCCAAGGGGTATAACATTTCTTG
GGAACTAGGCAATGAACCTAACAGTTTCCTTAAGAAGGCTGATATTTTCATCAAT
GGGTCGCAGTTAGGAGAAGATTTTATTCAATTGCATAAACTTCTAAGAAAGTCCA
CCTTCAAAAATGCAAAACTCTATGGTCCTGATGTTGGTCAGCCTCGAAGAAAGAC
GGCTAAGATGCTGAAGAGCTTCCTGAAGGCTGGTGGAGAAGTGATTGATTCAGT
TACATGGCATCACTACTATTTGAATGGACGGACTGCTACCAAGGAAGATTTTCTA
AACCCTGATGTATTGGACATTTTTATTTCATCTGTGCAAAAAGTTTTCCAGGTGGT
TGAGAGCACCAGGCCTGGCAAGAAGGTCTGGTTAGGAGAAACAAGCTCTGCATA
TGGAGGCGGAGCGCCCTTGCTATCCGACACCTTTGCAGCTGGCTTTATGTGGCTG
GATAAATTGGGCCTGTCAGCCCGAATGGGAATAGAAGTGGTGATGAGGCAAGTA
TTCTTTGGAGCAGGAAACTACCATTTAGTGGATGAAAACTTCGATCCTTTACCTG
ATTATTGGCTATCTCTTCTGTTCAAGAAATTGGTGGGCACCAAGGTGTTAATGGC
AAGCGTGCAAGGTTCAAAGAGAAGGAAGCTTCGAGTATACCTTCATTGCACAAA
CACTGACAATCCAAGGTATAAAGAAGGAGATTTAACTCTGTATGCCATAAACCT
CCATAATGTCACCAAGTACTTGCGGTTACCCTATCCTTTTTCTAACAAGCAAGTG
GATAAATACCTTCTAAGACCTTTGGGACCTCATGGATTACTTTCCAAATCTGTCC
AACTCAATGGTCTAACTCTAAAGATGGTGGATGATCAAACCTTGCCACCTTTAAT
GGAAAAACCTCTCCGGCCAGGAAGTTCACTGGGCTTGCCAGCTTTCTCATATAGT
TTTTTTGTGATAAGAAATGCCAAAGTTGCTGCTTGCATC

SEQ ID NO 213: amino acid sequence of tPA-SP PH-20 GPI

MDAMKRGLCCVLLLCGAVFVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPL
DMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKA
KKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLT
EATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSC
FNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAK
SPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLL
LDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLE
KGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDA
FLKPPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVASL

SEQ ID NO 214: nucleotide sequence of tPA-SP PH-20 GPI

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCT
TCGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT
GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT
CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA
CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA

AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCT
CAACACTTAGTGCTACTATGTTTATAGTTTCTATTTTGTTCCTTATTATTTCAAGTG
TAGCTAGTCTT

SEQ ID NO 215: amino acid sequence of tPA-SP PH-20 7 A.A. of GPI
MDAMKRGLCCVLLLCGAVFVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPL
DMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKA
KKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLT
EATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSC
FNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAK
SPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLL
LDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLE
KGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDA
FLKPPMETEEPQIFYNASPSTLS

SEQ ID NO 216: nucleotide sequence of tPA-SP PH-20 7 A.A. of GPI
ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCT
TCGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT
GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT

CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA
CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA
AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCT
CAACACTTAGT

SEQ ID NO 217: amino acid sequence of tPA-SP PH-20
MDAMKRGLCCVLLLCGAVFVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPL
DMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKA
KKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLT
EATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSC
FNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAK
SPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLL
LDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLE
KGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDA
FLKPPMETEEPQIFYN

SEQ ID NO 218: nucleotide sequence of tPA-SP PH-20
ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCT
TCGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT

GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT
CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA
CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA
AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAAC

SEQ ID NO 219: amino acid sequence of NSP PH-20 GPI
MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEF
CLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKIS
LQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQ
QQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHY
KKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAI
RVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIM
RSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNP
DNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCI
ADGVCIDAFLKPPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVASL

SEQ ID NO 220: nucleotide sequence of NSP PH-20 GPI
ATGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTG
GAGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAAC
TTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGC
CTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGT

TTTATTGGGTCACCAAGGATTAACGCGACTGGACAAGGAGTGACGATATTTTATG
TCGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAA
TGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAA
AGACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGG
GAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAA
AAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGTGCAATTGTCCTTGACTGAG
GCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTT
GAGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACC
TCTTCCCTGACTGCTACAATCACCATTACAAGAAACCGGGCTACAATGGCTCTTG
TTTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATC
CACCGCACTCTACCCCAGCATCTATCTGAACACCCAGCAGAGTCCTGTAGCAGCA
ACGCTGTACGTCCGGAACCGGGTACGAGAGGCAATCAGAGTATCTAAGATCCCG
GATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACC
AGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCTATACGTTTGGAGAGACAGT
AGCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCA
ATGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATA
TCATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC
CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA
GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCTCAACACTTAGTGCTACTAT
GTTTATAGTTTCTATTTTGTTCCTTATTATTTCAAGTGTAGCTAGTCTT

SEQ ID NO 221: amino acid sequence of NSP PH-20 7 A.A. of GPI
MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEF
CLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKIS
LQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQ
QQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHY
KKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAI
RVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIM
RSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNP

DNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCI
ADGVCIDAFLKPPMETEEPQIFYNASPSTLS

SEQ ID NO 222: nucleotide sequence of NSP PH-20 7 A.A. of GPI

ATGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTG
GAGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAAC
TTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGC
CTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGT
TTTATTGGGTCACCAAGGATTAACGCGACTGGACAAGGAGTGACGATATTTTATG
TCGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAA
TGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAA
AGACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGG
GAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAA
AAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGTGCAATTGTCCTTGACTGAG
GCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTT
GAGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACC
TCTTCCCTGACTGCTACAATCACCATTACAAGAAACCGGGCTACAATGGCTCTTG
TTTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATC
CACCGCACTCTACCCCAGCATCTATCTGAACACCCAGCAGAGTCCTGTAGCAGCA
ACGCTGTACGTCCGGAACCGGGTACGAGAGGCAATCAGAGTATCTAAGATCCCG
GATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACC
AGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCTATACGTTTGGAGAGACAGT
AGCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCA
ATGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATA
TCATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC
CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA
GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCTCAACACTTAGT

SEQ ID NO 223: amino acid sequence of NSP PH-20

MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEF
CLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKIS
LQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQ
QQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHY
KKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAI
RVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIM
RSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNP
DNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCI
ADGVCIDAFLKPPMETEEPQIFYN

SEQ ID NO 224: nucleotide sequence of NSP PH-20

ATGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTG
GAGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAAC
TTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGC
CTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGT
TTTATTGGGTCACCAAGGATTAACGCGACTGGACAAGGAGTGACGATATTTTATG
TCGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAA
TGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAA
AGACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGG
GAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAA
AAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGTGCAATTGTCCTTGACTGAG
GCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTT
GAGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACC
TCTTCCCTGACTGCTACAATCACCATTACAAGAAACCGGGCTACAATGGCTCTTG
TTTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATC
CACCGCACTCTACCCCAGCATCTATCTGAACACCCAGCAGAGTCCTGTAGCAGCA
ACGCTGTACGTCCGGAACCGGGTACGAGAGGCAATCAGAGTATCTAAGATCCCG
GATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACC
AGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCTATACGTTTGGAGAGACAGT
AGCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCA
ATGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATA
TCATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC

CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA
GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAAC

SEQ ID NO: 225 amino acid sequence of CAR CAR D0351 Farle CD8 BBz

MLLLVTSLLLCELPHPAFLLIPMEVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLS
WVRQAPGKGLEWVAMISSGGSYTYYADSVKGRFAISRDNAKNTLFLQMDSLRPEDT
GVYFCARHGDDPAWFAYWGQGTPVTVSSASTKGGGGGSGGGGSGGGGSDIQLTQS
PSSLSASVGDRVTITCSVSSSISSNNLHWYQQKPGKAPKPWIYGTSNLASGVPSRFSGS
GSGTDYTFTISSLQPEDIATYYCQQWSSYPYMYTFGQGTKVEIKRTAAATTTPAPRPP
TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 226 nucleotide sequence of CAR D0351 Farle CD8 BBz

ATGTTGCTGCTCGTGACCAGCCTCCTTCTGTGCGAACTTCCCCACCCCGCATTCCT
GCTGATTCCTATGGAAGTGCAGCTCGTGGAGTCCGGAGGCGGAGTCGTGCAGCC
GGGCAGATCCCTGCGCCTTTCCTGCTCGGCATCCGGGTTTACCTTCTCTGGCTACG
GTCTGTCGTGGGTCAGACAGGCTCCAGGGAAGGGCCTGGAATGGGTGGCCATGA
TCTCCTCGGGGGGTTCGTACACCTACTACGCCGACTCAGTGAAGGGCCGGTTCGC
CATCTCCCGCGACAACGCCAAGAACACCCTGTTCCTGCAAATGGACTCGCTCCGG
CCTGAGGACACTGGGGTGTACTTCTGCGCGAGACACGGAGATGACCCAGCTTGG
TTCGCCTACTGGGGACAAGGCACCCCTGTGACCGTGTCCTCCGCGAGCACCAAG
GGAGGCGGAGGAGGTTCCGGTGGAGGGGGATCAGGGGGTGGAGGATCGGACAT
TCAGCTGACCCAGAGCCCCTCAAGCCTGTCCGCGAGCGTTGGGGACCGCGTGAC
CATCACCTGTTCGGTGTCCTCCTCCATCTCCTCCAACAATCTCCATTGGTACCAGC
AGAAACCGGGGAAAGCCCCCAAGCCGTGGATCTACGGAACCTCCAACCTGGCTA
GCGGAGTGCCGTCGAGGTTCTCGGGCTCCGGATCAGGGACTGACTACACTTTCAC
TATTTCCTCCCTGCAACCGGAGGACATTGCCACCTACTACTGTCAGCAGTGGTCG
TCCTACCCCTACATGTATACCTTCGGTCAAGGAACCAAGGTCGAGATCAAGAGG
ACAGCGGCCGCAACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACC
ATTGCCAGCCAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGC
GGAGCCGTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCAC
CACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTG
CAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCT
GTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAA
GAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGACGCTCCGGCG
TACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAACCTCGGTCGCCGGGAA
GAATATGACGTGCTGGACAAGCGGCGGGGAAGAGATCCCGAGATGGGTGGAAA
GCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGCAAAAGGACA
AAATGGCCGAAGCCTACTCCGAGATTGGCATGAAGGGAGAGCGCAGACGCGGG
AAGGGACACGATGGACTGTACCAGGGACTGTCAACCGCGACTAAGGACACTTAC
GACGCCCTGCACATGCAGGCCCTGCCCCCGCGC

SEQ ID NO: 227 amino acid sequence of CAR D0373 MMP-9 2A ROR1 ScFv9 IgG4H CD8TM BBz

MSLWQPLVLVLLVLGCCFAAPRQRQSTLVLFPGDLRTNLTDRQLAEEYLYRYGYTR
VAEMRGESKSLGPALLLLQKQLSLPETGELDSATLKAMRTPRCGVPDLGRFQTFEGD
LKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPLTFTRVYSRDADIVIQF
GVAEHGDGYPFDGKDGLLAHAFPPGPGIQGDAHFDDDELWSLGKGVVVPTRFGNA
DGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDRFGFCPSERLYTQDGN
ADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADSTVM
GGNSAGELCVFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFDSDKKWGFCPDQGYS
LFLVAAHEFGHALGLDHSSVPEALMYPMYRFTEGPPLHKDDVNGIRHLYGPRPEPEP
RPPTTTTPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSP
VDDACNVNIFDAIAEIGNQLYLFKDGKYWRFSEGRGSRPQGPFLIADKWPALPRKLD
SVFEERLSKKLFFFSGRQVWVYTGASVLGPRRLDKLGLGADVAQVTGALRSGRGK
MLLFSGRRLWRFDVKAQMVDPRSASEVDRMFPGVPLDTHDVFQYREKAYFCQDRF
YWRVSSRSELNQVDQVGYVTYDILQCPEDRAKRGSGATNFSLLKQAGDVEENPGPR
AKRMLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTF
SSYAISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSR
LRSDDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHS
VSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSID
TSSNSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPI
YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE
EEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 228 nucleotide sequence of CAR D0373 MMP-9 2A ROR1 ScFv9 IgG4H CD8TM BBz

ATGAGCCTCTGGCAGCCCCTGGTCCTGGTGCTCCTGGTGCTGGGCTGCTGCTTTG
CTGCCCCCAGACAGCGCCAGTCCACCCTTGTGCTCTTCCCTGGAGACCTGAGAAC
CAATCTCACCGACAGGCAGCTGGCAGAGGAATACCTGTACCGCTATGGTTACAC
TCGGGTGGCAGAGATGCGTGGAGAGTCGAAATCTCTGGGGCCTGCGCTGCTGCT
TCTCCAGAAGCAACTGTCCCTGCCCGAGACCGGTGAGCTGGATAGCGCCACGCT
GAAGGCCATGCGAACCCCACGGTGCGGGGTCCCAGACCTGGGCAGATTCCAAAC

CTTTGAGGGCGACCTCAAGTGGCACCACCACAACATCACCTATTGGATCCAAAA
CTACTCGGAAGACTTGCCGCGGGCGGTGATTGACGACGCCTTTGCCCGCGCCTTC
GCACTGTGGAGCGCGGTGACGCCGCTCACCTTCACTCGCGTGTACAGCCGGGAC
GCAGACATCGTCATCCAGTTTGGTGTCGCGGAGCACGGAGACGGGTATCCCTTCG
ACGGGAAGGACGGGCTCCTGGCACACGCCTTTCCTCCTGGCCCCGGCATTCAGG
GAGACGCCCATTTCGACGATGACGAGTTGTGGTCCCTGGGCAAGGGCGTCGTGG
TTCCAACTCGGTTTGGAAACGCAGATGGCGCGGCCTGCCACTTCCCCTTCATCTT
CGAGGGCCGCTCCTACTCTGCCTGCACCACCGATGGACGGTCCGACGGCTTGCCC
TGGTGCAGTACCACGGCCAACTACGACACCGACGACCGGTTTGGCTTCTGCCCCA
GCGAGAGACTCTACACCCAGGACGGCAATGCTGATGGGAAACCCTGCCAGTTTC
CATTCATCTTCCAAGGCCAATCCTACTCCGCCTGCACCACGGACGGTCGCTCCGA
CGGGTACCGCTGGTGCGCCACCACCGCCAACTACGACCGGGACAAGCTCTTCGG
CTTCTGCCCGACCCGAGCTGACTCGACGGTGATGGGGGGCAACTCGGCGGGGGA
GCTGTGCGTCTTCCCCTTCACTTTCCTGGGTAAGGAGTACTCGACCTGTACCAGC
GAGGGCCGCGGAGATGGGCGCCTCTGGTGCGCTACCACCTCGAACTTTGACAGC
GACAAGAAGTGGGGCTTCTGCCCGGACCAAGGATACAGTTTGTTCCTCGTGGCG
GCGCATGAGTTCGGCCACGCGCTGGGCTTAGATCATTCCTCAGTGCCGGAGGCGC
TCATGTACCCTATGTACCGCTTCACTGAGGGGCCCCCCTTGCATAAGGACGACGT
GAATGGCATCCGGCACCTCTATGGTCCTCGCCCTGAACCTGAGCCACGACCTCCA
ACAACCACCACACCGCAGCCCACGGCTCCACCGACGGTCTGCCCCACCGGACCC
CCCACTGTCCACCCCTCAGAGCGCCCCACTGCTGGCCCAACAGGACCTCCCTCAG
CTGGCCCCACAGGTCCCCCAACTGCTGGCCCTTCTACGGCCACTACTGTGCCTTT
GAGTCCGGTGGACGATGCCTGCAACGTGAACATCTTCGACGCCATCGCGGAGAT
TGGGAACCAGCTGTATTTGTTCAAGGATGGGAAGTACTGGCGATTCTCTGAGGGC
AGGGGGAGCCGGCCGCAGGGCCCCTTCCTTATCGCCGACAAGTGGCCCGCGCTG
CCCCGCAAGCTGGACTCGGTCTTTGAGGAGCGGCTCTCCAAGAAGCTTTTCTTCT
TCTCTGGTCGCCAGGTGTGGGTGTACACAGGTGCGTCGGTGCTGGGACCGAGGC
GTCTAGACAAGCTAGGCCTGGGAGCAGACGTGGCCCAGGTGACCGGGGCCCTCC
GGAGTGGCAGGGGGAAGATGCTGCTGTTCAGCGGGCGGCGCCTCTGGAGGTTCG
ACGTGAAGGCGCAGATGGTGGATCCCCGGAGCGCCAGCGAGGTGGACCGGATGT
TCCCCGGGGTGCCTTTGGACACGCACGACGTCTTCCAGTACCGAGAGAAAGCCT
ATTTCTGCCAGGACCGCTTCTACTGGCGCGTGAGTTCCCGGAGTGAGTTGAACCA
GGTGGACCAAGTGGGCTACGTGACCTATGACATCCTGCAGTGCCCTGAGGACCG
GGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCACTGTTGAAGCAGGCCGGGGA

TGTGGAGGAGAATCCTGGTCCTAGAGCAAAGCGAATGCTGCTGCTGGTGACCAG
CCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTCTGCTGATTCCGCAGGCGGCC
CAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG
AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG
TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACA
GTGGTGGCACAAACTATGCACAGAGGTTTCAGGGCAGGGTCACCATGACCAGGG
ACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACA
CGGCCGTGTATTACTGTGCGAGTTATAATGATGCTTTTGATATCTGGGGCCAAGG
CACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTGGTGGTGGCGGTAGCGGT
GGTGGCGGATCCAATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGG
GGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTGGCAGCATTGCCAGCAACT
ATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCCCCACCATTGTGATCTATGA
GGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACACC
TCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGCAGAGTGAGGACGAGGCTG
ACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGGTATTCGGCGGAGGGACCA
AGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATACGGTCCTCCGTGCCCTCCGTG
TCCGATCTACATTTGGGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCG
CTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCA
AGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGT
GCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCAC
GGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGC
TGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGC
GACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTA
CAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGA
AGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTGAGC
ACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 229 amino acid sequence of CAR D0368, D0369 Farle CD8 BBz 2A HPSE
MLLLVTSLLLCELPHPAFLLIPMEVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLS
WVRQAPGKGLEWVAMISSGGSYTYYADSVKGRFAISRDNAKNTLFLQMDSLRPEDT
GVYFCARHGDDPAWFAYWGQGTPVTVSSASTKGGGGGSGGGGSGGGGSDIQLTQS
PSSLSASVGDRVTITCSVSSSISSNNLHWYQQKPGKAPKPWIYGTSNLASGVPSRFSGS
GSGTDYTFTISSLQPEDIATYYCQQWSSYPYMYTFGQGTKVEIKRTAAATTTPAPRPP
TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL

YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATN
FSLLKQAGDVEENPGPRAKRMLLRSKPALPPPLMLLLLGPLGPLSPGALPRPAQAQD
VVDLDFFTQEPLHLVSPSFLSVTIDANLATDPRFLILLGSPKLRTLARGLSPAYLRFGG
TKTDFLIFDPKKESTFEERSYWQSQVNQDICKYGSIPPDVEEKLRLEWPYQEQLLLRE
HYQKKFKNSTYSRSSVDVLYTFANCSGLDLIFGLNALLRTADLQWNSSNAQLLLDY
CSSKGYNISWELGNEPNSFLKKADIFINGSQLGEDFIQLHKLLRKSTFKNAKLYGPDV
GQPRRKTAKMLKSFLKAGGEVIDSVTWHHYYLNGRTATKEDFLNPDVLDIFISSVQK
VFQVVESTRPGKKVWLGETSSAYGGGAPLLSDTFAAGFMWLDKLGLSARMGIEVV
MRQVFFGAGNYHLVDENFDPLPDYWLSLLFKKLVGTKVLMASVQGSKRRKLRVYL
HCTNTDNPRYKEGDLTLYAINLHNVTKYLRLPYPFSNKQVDKYLLRPLGPHGLLSKS
VQLNGLTLKMVDDQTLPPLMEKPLRPGSSLGLPAFSYSFFVIRNAKVAACI

SEQ ID NO: 230 nucleotide sequence of CAR D0368, D0369 Farle CD8 BBz 2A HPSE
ATGTTGCTGCTCGTGACCAGCCTCCTTCTGTGCGAACTTCCCCACCCCGCATTCCT
GCTGATTCCTATGGAAGTGCAGCTCGTGGAGTCCGGAGGCGGAGTCGTGCAGCC
GGGCAGATCCCTGCGCCTTTCCTGCTCGGCATCCGGGTTTACCTTCTCTGGCTACG
GTCTGTCGTGGGTCAGACAGGCTCCAGGGAAGGGCCTGGAATGGGTGGCCATGA
TCTCCTCGGGGGGTTCGTACACCTACTACGCCGACTCAGTGAAGGGCCGGTTCGC
CATCTCCCGCGACAACGCCAAGAACACCCTGTTCCTGCAAATGGACTCGCTCCGG
CCTGAGGACACTGGGGTGTACTTCTGCGCGAGACACGGAGATGACCCAGCTTGG
TTCGCCTACTGGGGACAAGGCACCCCTGTGACCGTGTCCTCCGCGAGCACCAAG
GGAGGCGGAGGAGGTTCCGGTGGAGGGGGATCAGGGGGTGGAGGATCGGACAT
TCAGCTGACCCAGAGCCCCTCAAGCCTGTCCGCGAGCGTTGGGGACCGCGTGAC
CATCACCTGTTCGGTGTCCTCCTCCATCTCCTCCAACAATCTCCATTGGTACCAGC
AGAAACCGGGGAAAGCCCCCAAGCCGTGGATCTACGGAACCTCCAACCTGGCTA
GCGGAGTGCCGTCGAGGTTCTCGGGCTCCGGATCAGGGACTGACTACACTTTCAC
TATTTCCTCCCTGCAACCGGAGGACATTGCCACCTACTACTGTCAGCAGTGGTCG
TCCTACCCCTACATGTATACCTTCGGTCAAGGAACCAAGGTCGAGATCAAGAGG
ACAGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACC
ATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTG
GAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCC
GCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGC

AAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCC
GTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAA
GAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCA
TATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAG
GAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAA
ACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACA
AGATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGA
AAGGGTCACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTAC
GATGCCTTGCATATGCAAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGG
GCGACTAACTTTTCACTGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGT
CCTAGAGCaAAGCGAATGCTtCTaCGtTCtAAaCCTGCaCTtCCtCCaCCaCTtATGCTaC
TtCTtCTaGGaCCtCTtGGTCCtCTaTCaCCTGGaGCtCTaCCtCGACCTGCaCAAGCACAG
GACGTCGTGGACCTGGACTTCTTCACCCAGGAGCCGCTGCACCTGGTGAGCCCCT
CGTTCCTGTCCGTCACCATTGACGCCAACCTGGCCACGGACCCGCGGTTCCTCAT
CCTCCTGGGTTCTCCAAAGCTTCGTACCTTGGCCAGAGGCTTGTCTCCTGCGTACC
TGAGGTTTGGTGGCACCAAGACAGACTTCCTAATTTTCGATCCCAAGAAGGAATC
AACCTTTGAAGAGAGAAGTTACTGGCAATCTCAAGTCAACCAGGATATTTGCAA
ATATGGATCCATCCCTCCTGATGTGGAGGAGAAGTTACGGTTGGAATGGCCCTAC
CAGGAGCAATTGCTACTCCGAGAACACTACCAGAAAAAGTTCAAGAACAGCACC
TACTCAAGAAGCTCTGTAGATGTGCTATACACTTTTGCAAACTGCTCAGGACTGG
ACTTGATCTTTGGCCTAAATGCGTTATTAAGAACAGCAGATTTGCAGTGGAACAG
TTCTAATGCTCAGTTGCTCCTGGACTACTGCTCTTCCAAGGGGTATAACATTTCTT
GGGAACTAGGCAATGAACCTAACAGTTTCCTTAAGAAGGCTGATATTTTCATCAA
TGGGTCGCAGTTAGGAGAAGATTTTATTCAATTGCATAAACTTCTAAGAAAGTCC
ACCTTCAAAAATGCAAAACTCTATGGTCCTGATGTTGGTCAGCCTCGAAGAAAG
ACGGCTAAGATGCTGAAGAGCTTCCTGAAGGCTGGTGGAGAAGTGATTGATTCA
GTTACATGGCATCACTACTATTTGAATGGACGGACTGCTACCAAGGAAGATTTTC
TAAACCCTGATGTATTGGACATTTTTATTTCATCTGTGCAAAAAGTTTTCCAGGTG
GTTGAGAGCACCAGGCCTGGCAAGAAGGTCTGGTTAGGAGAAACAAGCTCTGCA
TATGGAGGCGGAGCGCCCTTGCTATCCGACACCTTTGCAGCTGGCTTTATGTGGC
TGGATAAATTGGGCCTGTCAGCCCGAATGGGAATAGAAGTGGTGATGAGGCAAG
TATTCTTTGGAGCAGGAAACTACCATTTAGTGGATGAAAACTTCGATCCTTTACC
TGATTATTGGCTATCTCTTCTGTTCAAGAAATTGGTGGGCACCAAGGTGTTAATG
GCAAGCGTGCAAGGTTCAAAGAGAAGGAAGCTTCGAGTATACCTTCATTGCACA

AACACTGACAATCCAAGGTATAAAGAAGGAGATTTAACTCTGTATGCCATAAAC
CTCCATAATGTCACCAAGTACTTGCGGTTACCCTATCCTTTTTCTAACAAGCAAGT
GGATAAATACCTTCTAAGACCTTTGGGACCTCATGGATTACTTTCCAAATCTGTC
CAACTCAATGGTCTAACTCTAAAGATGGTGGATGATCAAACCTTGCCACCTTTAA
TGGAAAAACCTCTCCGGCCAGGAAGTTCACTGGGCTTGCCAGCTTTCTCATATAG
TTTTTTTGTGATAAGAAATGCCAAAGTTGCTGCTTGCATC

SEQ ID NO: 231 amino acid sequence of CAR D0423, D0424 Farle CD8 BBz 2A tPA-SP PH-20 GPI

MLLLVTSLLLCELPHPAFLLIPMEVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLS
WVRQAPGKGLEWVAMISSGGSYTYYADSVKGRFAISRDNAKNTLFLQMDSLRPEDT
GVYFCARHGDDPAWFAYWGQGTPVTVSSASTKGGGGGSGGGGSGGGGSDIQLTQS
PSSLSASVGDRVTITCSVSSSISSNNLHWYQQKPGKAPKPWIYGTSNLASGVPSRFSGS
GSGTDYTFTISSLQPEDIATYYCQQWSSYPYMYTFGQGTKVEIKRTAAATTTPAPRPP
TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAKRGSGATN
FSLLKQAGDVEENPGPRAKRMDAMKRGLCCVLLLCGAVFVSPSLNFRAPPVIPNVPF
LWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITG
VTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKD
VYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGY
YLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAA
TLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALG
ASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRK
NWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKA
DVKDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVA
SL

SEQ ID NO: 232 nucleotide sequence of CAR D0423, D0424 Farle CD8 BBz 2A tPA-SP PH-20 GPI

ATGTTGCTGCTCGTGACCAGCCTCCTTCTGTGCGAACTTCCCCACCCCGCATTCCT
GCTGATTCCTATGGAAGTGCAGCTCGTGGAGTCCGGAGGCGGAGTCGTGCAGCC
GGGCAGATCCCTGCGCCTTTCCTGCTCGGCATCCGGGTTTACCTTCTCTGGCTACG

GTCTGTCGTGGGTCAGACAGGCTCCAGGGAAGGGCCTGGAATGGGTGGCCATGA
TCTCCTCGGGGGGTTCGTACACCTACTACGCCGACTCAGTGAAGGGCCGGTTCGC
CATCTCCCGCGACAACGCCAAGAACACCCTGTTCCTGCAAATGGACTCGCTCCGG
CCTGAGGACACTGGGGTGTACTTCTGCGCGAGACACGGAGATGACCCAGCTTGG
TTCGCCTACTGGGGACAAGGCACCCCTGTGACCGTGTCCTCCGCGAGCACCAAG
GGAGGCGGAGGAGGTTCCGGTGGAGGGGGATCAGGGGGTGGAGGATCGGACAT
TCAGCTGACCCAGAGCCCCTCAAGCCTGTCCGCGAGCGTTGGGGACCGCGTGAC
CATCACCTGTTCGGTGTCCTCCTCCATCTCCTCCAACAATCTCCATTGGTACCAGC
AGAAACCGGGGAAAGCCCCCAAGCCGTGGATCTACGGAACCTCCAACCTGGCTA
GCGGAGTGCCGTCGAGGTTCTCGGGCTCCGGATCAGGGACTGACTACACTTTCAC
TATTTCCTCCCTGCAACCGGAGGACATTGCCACCTACTACTGTCAGCAGTGGTCG
TCCTACCCCTACATGTATACCTTCGGTCAAGGAACCAAGGTCGAGATCAAGAGG
ACAGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACC
ATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTG
GAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCC
GCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGC
AAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCC
GTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAA
GAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCA
TATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAG
GAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAA
ACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACA
AGATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGA
AAGGGTCACGACGGGCTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTAC
GATGCCTTGCATATGCAAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGG
GCGACTAACTTTTCACTGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGT
CCTAGAGCaAAGCGAATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTG
CTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGA
TCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGA
AAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGAT
TAACGCGACTGGACAAGGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTAC
CCCTACATAGATTCCATTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGA
TCTCTCTTCAAGACCACTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCC
GGTGGATAACCTGGGGATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTG

GGCTAGAAACTGGAAGCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGT
TCAGCAGCAGAACGTGCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCA
AGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAA
ACTGCTGCGACCTAATCATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATC
ACCATTACAAGAAACCGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACG
AAACGACGACCTGAGCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCAT
CTATCTGAACACCCAGCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCG
GGTACGAGAGGCAATCAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCC
GGTATTTGCGTACACCCGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCC
AGGACGAACTTGTCTATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCA
TTGTTATATGGGGAACCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTT
GATAACTATATGGAGACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAG
CAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAA
ATTGGAACAGTTCCGACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCT
TGAAAAGGGCGGAAAATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGA
GCAATTCTCTGAAAAGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAA
AAGGCGGACGTCAAGGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTT
TGCATCGACGCATTTCTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCT
ATAACGCTTCTCCCTCAACACTTAGTGCTACTATGTTTATAGTTTCTATTTTGTTCC
TTATTATTTCAAGTGTAGCTAGTCTT

SEQ ID NO: 233 amino acid sequence of CAR D0422 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20 GPI

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMDAMKRGLCCVLLLCGAV
FVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVT
IFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVID

WEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLV
ETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNEST
ALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFL
SQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAK
MCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE
KFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTL
SATMFIVSILFLIISSVASL

SEQ ID NO: 234 nucleotide sequence of CAR D0422 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20 GPI

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT
GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG

CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA
TGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTT
CGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT
GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT
CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA
CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA
AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCT
CAACACTTAGTGCTACTATGTTTATAGTTTCTATTTTGTTCCTTATTATTTCAAGTG
TAGCTAGTCTT

SEQ ID NO: 235 amino acid sequence of CAR D0460 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20 7 A.A. of GPI

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMDAMKRGLCCVLLLCGAV
FVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVT
IFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVID
WEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLV
ETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNEST
ALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFL
SQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAK
MCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE
KFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTL
S

SEQ ID NO: 236 nucleotide sequence of CAR D0460 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20 7 A.A. of GPI

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC

CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT
GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG
CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA
TGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTT
CGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT
GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT
CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA

CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA
AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCT
CAACACTTAGT

SEQ ID NO: 237 amino acid sequence of CAR D0459 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMDAMKRGLCCVLLLCGAV
FVSPSLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVT
IFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVID
WEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLV
ETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNEST
ALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFL
SQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAK
MCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE
KFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYN

SEQ ID NO: 238 nucleotide sequence of CAR D0459 ROR1 ScFv9 IgG4H CD8TM BBz 2A tPA-SP PH20

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA

AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT
GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG
CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA
TGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTT
CGTTTCGCCCAGCCTGAACTTTCGCGCCCCACCAGTGATCCCTAATGTGCCATTC
CTTTGGGCTTGGAATGCGCCTTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCT
GGATATGTCTCTTTTCAGTTTTATTGGGTCACCAAGGATTAACGCGACTGGACAA
GGAGTGACGATATTTTATGTCGATAGGCTCGGCTACTACCCCTACATAGATTCCA
TTACCGGCGTAACCGTGAATGGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCA
CTTGGACAAAGCAAAAAAAGACATTACATTCTACATGCCGGTGGATAACCTGGG
GATGGCCGTTATCGATTGGGAGGAGTGGAGACCCACGTGGGCTAGAAACTGGAA
GCCGAAGGACGTCTATAAAAACAGGTCTATCGAATTGGTTCAGCAGCAGAACGT

GCAATTGTCCTTGACTGAGGCGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGC
GGGAAAGGACTTTTTGGTTGAGACCATTAAGCTCGGTAAACTGCTGCGACCTAAT
CATCTGTGGGGTTACTACCTCTTCCCTGACTGCTACAATCACCATTACAAGAAAC
CGGGCTACAATGGCTCTTGTTTTAATGTCGAAATCAAACGAAACGACGACCTGA
GCTGGCTTTGGAACGAATCCACCGCACTCTACCCCAGCATCTATCTGAACACCCA
GCAGAGTCCTGTAGCAGCAACGCTGTACGTCCGGAACCGGGTACGAGAGGCAAT
CAGAGTATCTAAGATCCCGGATGCTAAATCCCCACTGCCGGTATTTGCGTACACC
CGAATCGTGTTCACTGACCAGGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCT
ATACGTTTGGAGAGACAGTAGCACTCGGCGCATCAGGCATTGTTATATGGGGAA
CCCTTAGCATCATGCGGTCAATGAAGTCCTGCTTGCTTCTTGATAACTATATGGA
GACAATCTTGAACCCCTATATCATCAATGTAACACTTGCAGCAAAAATGTGCTCC
CAAGTACTCTGTCAAGAGCAGGGAGTATGCATACGAAAAAATTGGAACAGTTCC
GACTACCTGCACCTTAACCCCGATAATTTTGCTATACAGCTTGAAAAGGGCGGAA
AATTTACAGTCCGAGGGAAGCCGACATTGGAGGATCTCGAGCAATTCTCTGAAA
AGTTTTATTGCTCATGCTACAGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAA
GGATACTGACGCCGTGGACGTCTGCATCGCCGACGGAGTTTGCATCGACGCATTT
CTTAAACCTCCCATGGAAACCGAAGAGCCACAAATCTTCTATAAC

SEQ ID NO: 239 amino acid sequence of CAR D0461 ROR1 ScFv9 IgG4H CD8TM BBz 2A NSP PH20 GPI

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMGVLKFKHIFFRSFVKSSG
VSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSP
RINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPV
DNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEF
EKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD
LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRI

VFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP
YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKP
TLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEP
QIFYNASPSTLSATMFIVSILFLIISSVASL

SEQ ID NO: 240 nucleotide sequence of CAR D0461 ROR1 ScFv9 IgG4H CD8TM BBz 2A NSP PH20 GPI

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT
GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG
CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA

TGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTGG
AGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAACT
TTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGCC
TTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGTT
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CGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAAT
GGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAAA
GACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGGG
AGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAAA
AACAGGTCTATCGAATTGGTTCAGCAGCAGAACGTGCAATTGTCCTTGACTGAGG
CGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTTG
AGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACCT
CTTCCCTGACTGCTACAATCACCATTACAAGAAACCGGGCTACAATGGCTCTTGT
TTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATCC
ACCGCACTCTACCCCAGCATCTATCTGAACACCCAGCAGAGTCCTGTAGCAGCAA
CGCTGTACGTCCGGAACCGGGTACGAGAGGCAATCAGAGTATCTAAGATCCCGG
ATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACCA
GGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCTATACGTTTGGAGAGACAGTA
GCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCAA
TGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATAT
CATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC
CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA
GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCTCAACACTTAGTGCTACTAT
GTTTATAGTTTCTATTTTGTTCCTTATTATTTCAAGTGTAGCTAGTCTT

SEQ ID NO: 241 amino acid sequence of CAR D0463 ROR1 ScFv9 IgG4H CD8TM BBz 2A NSP PH20 7 A.A. of GPI

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE

SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMGVLKFKHIFFRSFVKSSG
VSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSP
RINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPV
DNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEF
EKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD
LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRI
VFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP
YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKP
TLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEP
QIFYNASPSTLS

SEQ ID NO: 242 nucleotide sequence of CAR D0463 ROR1 ScFv9 IgG4H CD8TM BBz 2A NSP PH20 7 A.A. of GPI

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG
CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT

GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
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GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
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ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
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AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA
TGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTGG
AGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAACT
TTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGCC
TTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGTT
TTATTGGGTCACCAAGGATTAACGCGACTGGACAAGGAGTGACGATATTTTATGT
CGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAAT
GGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAAA
GACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGGG
AGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAAA
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CGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTTG
AGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACCT
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TTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATCC
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ATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACCA
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GCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCAA
TGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATAT
CATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC
CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA

GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAACGCTTCTCCCTCAACACTTAGT

SEQ ID NO: 243 amino acid sequence of CAR D0462 ROR1 ScFv9 IgG4H CD8TM BBz 2A NSP PH20

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRMGVLKFKHIFFRSFVKSSG
VSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSP
RINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPV
DNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEF
EKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD
LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRI
VFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP
YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKP
TLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEP
QIFYN

SEQ ID NO: 244 nucleotide sequence of CAR D0462 ROR1 ScFv9 IgG4H CD8TM BBz 2A PH20

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAG

CAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCT
TTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGT
CTGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCA
CTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGT
GGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCC
CCCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGT
TCTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTG
CAGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGG
GTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATAC
GGTCCTCCGTGCCCTCCGTGTCCGATCTACATTTGGGCCCCGCTGGCCGGCACTT
GCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAA
GAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAG
GAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGA
ACTGCGCGTCAAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCA
GAATCAGCTCTACAACGAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCT
GGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAA
ACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCT
ACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGG
CTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGC
AAGCACTCCCACCCCGGCGGGCAAAGCGGGGCTCAGGGGCGACTAACTTTTCAC
TGTTGAAGCAGGCCGGGGATGTGGAGGAGAATCCTGGTCCTAGAGCaAAGCGAA
TGGGAGTGCTAAAATTCAAGCACATCTTTTTCAGAAGCTTTGTTAAATCAAGTGG
AGTATCCCAGATAGTTTTCACCTTCCTTCTGATTCCATGTTGCTTGACTCTGAACT
TTCGCGCCCCACCAGTGATCCCTAATGTGCCATTCCTTTGGGCTTGGAATGCGCC
TTCTGAATTCTGCTTGGGAAAATTTGATGAGCCTCTGGATATGTCTCTTTTCAGTT
TTATTGGGTCACCAAGGATTAACGCGACTGGACAAGGAGTGACGATATTTTATGT
CGATAGGCTCGGCTACTACCCCTACATAGATTCCATTACCGGCGTAACCGTGAAT
GGTGGTATCCCTCAAAAGATCTCTCTTCAAGACCACTTGGACAAAGCAAAAAAA
GACATTACATTCTACATGCCGGTGGATAACCTGGGGATGGCCGTTATCGATTGGG
AGGAGTGGAGACCCACGTGGGCTAGAAACTGGAAGCCGAAGGACGTCTATAAA
AACAGGTCTATCGAATTGGTTCAGCAGCAGAACGTGCAATTGTCCTTGACTGAGG
CGACAGAGAAGGCCAAGCAAGAGTTTGAGAAGGCGGGAAAGGACTTTTTGGTTG
AGACCATTAAGCTCGGTAAACTGCTGCGACCTAATCATCTGTGGGGTTACTACCT
CTTCCCTGACTGCTACAATCACCATTACAAGAAACCGGGCTACAATGGCTCTTGT

TTTAATGTCGAAATCAAACGAAACGACGACCTGAGCTGGCTTTGGAACGAATCC
ACCGCACTCTACCCCAGCATCTATCTGAACACCCAGCAGAGTCCTGTAGCAGCAA
CGCTGTACGTCCGGAACCGGGTACGAGAGGCAATCAGAGTATCTAAGATCCCGG
ATGCTAAATCCCCACTGCCGGTATTTGCGTACACCCGAATCGTGTTCACTGACCA
GGTTCTGAAGTTTCTCTCCCAGGACGAACTTGTCTATACGTTTGGAGAGACAGTA
GCACTCGGCGCATCAGGCATTGTTATATGGGGAACCCTTAGCATCATGCGGTCAA
TGAAGTCCTGCTTGCTTCTTGATAACTATATGGAGACAATCTTGAACCCCTATAT
CATCAATGTAACACTTGCAGCAAAAATGTGCTCCCAAGTACTCTGTCAAGAGCA
GGGAGTATGCATACGAAAAAATTGGAACAGTTCCGACTACCTGCACCTTAACCC
CGATAATTTTGCTATACAGCTTGAAAAGGGCGGAAAATTTACAGTCCGAGGGAA
GCCGACATTGGAGGATCTCGAGCAATTCTCTGAAAAGTTTTATTGCTCATGCTAC
AGTACCCTTAGCTGTAAAGAAAAGGCGGACGTCAAGGATACTGACGCCGTGGAC
GTCTGCATCGCCGACGGAGTTTGCATCGACGCATTTCTTAAACCTCCCATGGAAA
CCGAAGAGCCACAAATCTTCTATAAC

SEQ ID NO: 245 nucleotide sequence of CAR D0426 CD276-22 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCT
TTTGATACCTCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGAAGAAGCCTGG
AAGCTCAGTGAAGGTCTCCTGCAAGGATTCTGGAGGCACCCTCAGCAGCCATGC
TATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGAAT
CATCCCTATCCTTGGTATAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCAC
GATTACAGCGGACGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAG
ATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGGGTCCAGGGAGTTACCA
TATGGACGTCTGGGGCAAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGG
GTCTGGTGGAGGCGGCAGCGGTGGTGGCGGATCCGAAATTGTGCTGACTCAGTC
TCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCC
AGTCAGAGTGTTGGCAGCTCCTTAGGCTGGTACCAACAGAAACCTGGCCAGGCT
CCCAGGCTCCTCATCTATGATGTATCCAACAGGGCCTCTGGCATCCCAGCCAGGT
TCAGTGGCAGTGGGTCTGGGATAGACTTCACTCTCACCATCAGCAGCCTAGAGCC
TGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCCCCCATGTAC
ACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAGCGGCCGCAACGACCACTCCT
GCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCCTGC
GGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGACTGG

ATTTCGCCTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTGCT
GCTGCTGTCCCTTGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTTG
TACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGAC
GGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTG
AAGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTG
TACAACGAACTTAACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGG
CGGGGAAGAGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGA
GGGCTTGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGAT
TGGCATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGG
GACTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGC
CCCCGCGCTAA

SEQ ID NO: 246 amino acid sequence of CAR D0426 CD276-22 CD8 BBz
MLLLVTSLLLCELPHPAFLLIPQVQLQQSGAEVKKPGSSVKVSCKDSGGTLSSHAISW
VRQAPGQGLEWMGGIIPILGIANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVY
YCARGGPGSYHMDVWGKGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPG
ERATLSCRASQSVGSSLGWYQQKPGQAPRLLIYDVSNRASGIPARFSGSGSGIDFTLTI
SSLEPEDFAVYYCQQRSNWPPMYTFGQGTKLEIKAAATTTPAPRPPTPAPTIASQPLS
LRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLY
IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 247 nucleotide sequence of CAR D0427 CD276-30 CD8 BBz
ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCT
TTTGATACCTCAGCTGCAGCTGCAGGAGTCCGGCCCAGGACTGGTGAAGCCTTCG
GAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCGTCAGCAGTAGTAACT
GGTGGAGTTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAA
TCTATCATAGTGGGAGCACCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCAT
ATCAGTAGACAAGTCCAAGAATCAATTCTCCCTGCACCTGAACTCTGTGACTCCC
GAGGACACGGCTGTGTACTACTGTGCGAGAGAGGTGGCTGGTTCTGCGGCTTTTG
ACATCTGGGGCCAAGGGACAATGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTG
GTGGAGGCGGCAGCGGTGGTGGCGGATCCCAGTCTGTCGTGACGCAGCCGCCCT
CAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATCTCCTGCTCTGGAAGCAGCTC

CAACATTGGGAATAATTATGTATCCTGGTACCAGCAGCTCCCAGGAACAGCCCCC
AAACTCCTCATCTATGGAAATAATAAGCGACCCTCAGGGATTCCTGACCGATTCT
CTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGG
GGACGAGGCCGATTATTACTGCGGAACATGGGATAGCAGCCTGAGTGCGGTATT
CGGCGGAGGCACCCAGCTGACCGTCCTCGCGGCCGCAACGACCACTCCTGCACC
CCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCCTGCGGCCG
GAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGACTGGATTTC
GCCTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTGCTGCTGC
TGTCCCTTGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTTGTACAT
CTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTG
CTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTT
TTCCCGGTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAA
CGAACTTAACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGG
AAGAGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCT
TGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCA
TGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACTG
TCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCG
CGCTAA

SEQ ID NO: 248 amino acid sequence of CAR D0427 CD276-30 CD8 BBz
MLLLVTSLLLCELPHPAFLLIPQLQLQESGPGLVKPSETLSLTCAVSGGSVSSSNWWS
WVRQPPGKGLEWIGEIYHSGSTNYNPSLKSRVTISVDKSKNQFSLHLNSVTPEDTAV
YYCAREVAGSAAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSQSVVTQPPSVSAAP
GQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYGNNKRPSGIPDRFSGSKSGTSA
TLGITGLQTGDEADYYCGTWDSSLSAVFGGGTQLTVLAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 249 nucleotide sequence of CAR D0480 CD276 376.96 CD8 BBz
ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCT
TTTGATACCTGACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAATT
GGAGCCAGGGTCAGCATCACCTGCAAGGCCAGTCAGGATGTGAGAACTGCTGTA

GCCTGGTATCAACAGAAACCAGGCCAGTCTCCTAAACTTCTTATTTACTCAGCAT
CCTACCGGTACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGA
TTTCACTTTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGT
CAGCAACATTATGGTACTCCTCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAA
ATCAAAGGCGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGCTCTGA
AGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCTGGAGGGTCCCTGAA
ACTCTCCTGTGAAGCCTCCAGATTCACTTTCAGTAGCTATGCCATGTCTTGGGTTC
GCCAGACTCCGGAGAAGAGGCTGGAGTGGGTCGCAGCCATTAGTGGAGGTGGTA
GGTACACCTACTATCCAGACAGTATGAAGGGTCGATTCACCATCTCCAGAGACA
ATGCCAAGAATTTCCTGTACCTGCAAATGAGCAGTCTGAGGTCTGAGGACACGG
CCATGTATTACTGTGCAAGACACTATGATGGTTATCTTGACTACTGGGGCCAAGG
CACCACTCTCACAGTCTCCTCAGCGGCCGCAACGACCACTCCTGCACCCCGCCCT
CCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCCTGCGGCCGGAAGCCT
GCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGACTGGATTTCGCCTGCG
ATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCT
TGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAG
CAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGC
CGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGG
TCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTT
AACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGA
TCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACAA
CGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCATGAAGGG
AGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACTGTCAACCG
CGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCGCGCTAA

SEQ ID NO: 250 amino acid sequence of CAR D0480 CD276 376.96 CD8 BBz
MLLLVTSLLLCELPHPAFLLIPDIVMTQSHKFMSTSIGARVSITCKASQDVRTAVAWY
QQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYG
TPPWTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLKLSCEASRF
TFSSYAMSWVRQTPEKRLEWVAAISGGGRYTYYPDSMKGRFTISRDNAKNFLYLQM
SSLRSEDTAMYYCARHYDGYLDYWGQGTTLTVSSAAATTTPAPRPPTPAPTIASQPL
SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNE

LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 251 nucleotide sequence of CAR D0432 ROR1scFv9 IgG4 CD8 BBz 2A CD276-22 CD8 CD28 CCR

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
TTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTC
TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
GCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCC
CCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTT
CTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGC
AGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGG
TATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATACG
GTCCTCCGTGCCCTCCGTGTCCGATCTACATCTGGGCCCCATTGGCTGGAACTTG
CGGCGTGCTGCTCTTGTCTCTGGTCATTACCCTGTACTGCAAGCGCGGACGGAAG
AAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAG
AAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAA
CTGAGAGTGAAGTTTAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGACAG
AACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGTGCTG
GACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAA
CCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATGGCGGAAGCCTA
CTCCGAGATCGGCATGAAGGGAGAACGCCGGAGAGGGAAGGGTCATGACGGAC
TGTACCAGGGCCTGTCAACTGCCACTAAGGACACTTACGATGCGCTCCATATGCA
AGCTTTGCCCCCGCGGCGCGCGAAACGCGGCAGCGGCGCGACCAACTTTAGCCT
GCTGAAACAGGCGGGCGATGTGGAAGAAAACCCGGGCCCGCGAGCAAAGAGGA
ATATTATGGCTCTGCCTGTTACGGCACTGCTCCTTCCGCTTGCATTGTTGTTGCAC
GCAGCGCGGCCCCAAGTGCAACTGCAACAATCCGGTGCTGAAGTGAAGAAACCG

GGTAGCTCCGTCAAGGTGTCTTGTAAAGATTCAGGCGGAACTTTGTCTTCTCATG
CGATTTCATGGGTACGCCAAGCCCCAGGGCAGGGACTTGAATGGATGGGAGGAA
TCATCCCTATCCTTGGTATAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCAC
GATTACAGCGGACGAATCCACGAGCACTGCGTATATGGAGCTGAGTTCTCTGAG
GAGCGAAGATACTGCTGTCTACTACTGTGCGAGAGGGGGTCCAGGGAGTTACCA
TATGGACGTCTGGGGAAAGGGCACTTTGGTCACTGTTTCTAGCGGTGGTGGAGGC
AGTGGTGGCGGAGGATCAGGGGGCGGGGGGTCCGAAATTGTGCTGACTCAGTCT
CCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTGGCAGCTCCTTAGGCTGGTACCAACAGAAACCTGGCCAGGCTC
CCAGGCTCCTCATCTATGATGTATCCAACAGGGCCTCTGGCATCCCAGCCAGGTT
CAGTGGCAGTGGGTCTGGGATAGACTTCACTCTCACCATCAGCAGCCTAGAGCCT
GAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCCCCCATGTACA
CTTTTGGCCAGGGGACCAAGCTGGAGATCAAAGCTAGCGCAACTACCACTCCTG
CACCACGGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCG
CCCCGAAGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGA
CTTCGCCTGTGACATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTG
TTGCTGTCCTTGGTTATTACGTTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGC
ACTCCGACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACT
ACCAGCCGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCTGA

SEQ ID NO: 252 amino acid sequence of CAR D0432 ROR1scFv9 IgG4 CD8 BBz 2A CD276-22 CD8 CD28 CCR

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIMALPVTALLLPLALLLH
AARPQVQLQQSGAEVKKPGSSVKVSCKDSGGTLSSHAISWVRQAPGQGLEWMGGII
PILGIANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGGPGSYHMDV
WGKGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVGS

SLGWYQQKPGQAPRLLIYDVSNRASGIPARFSGSGSGIDFTLTISSLEPEDFAVYYCQQ
RSNWPPMYTFGQGTKLEIKASATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRS

SEQ ID NO: 253 nucleotide sequence of CAR D0433 ROR1scFv9 IgG4 CD8 BBz 2A CD276-30 CD8 CD28 CCR

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
TTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTC
TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
GCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCC
CCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTT
CTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGC
AGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGG
TATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATACG
GTCCTCCGTGCCCTCCGTGTCCGATCTACATCTGGGCCCCATTGGCTGGAACTTG
CGGCGTGCTGCTCTTGTCTCTGGTCATTACCCTGTACTGCAAGCGCGGACGGAAG
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CTGAGAGTGAAGTTTAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGACAG
AACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGTGCTG
GACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAA
CCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATGGCGGAAGCCTA
CTCCGAGATCGGCATGAAGGGAGAACGCCGGAGAGGGAAGGGTCATGACGGAC
TGTACCAGGGCCTGTCAACTGCCACTAAGGACACTTACGATGCGCTCCATATGCA
AGCTTTGCCCCCGCGGCGCGCGAAACGCGGCAGCGGCGCGACCAACTTTAGCCT
GCTGAAACAGGCGGGCGATGTGGAAGAAAACCCGGGCCCGCGAGCAAAGAGGA

ATATTATGGCTCTGCCTGTTACGGCACTGCTCCTTCCGCTTGCATTGTTGTTGCAC
GCAGCGCGGCCCCAGCTGCAGCTGCAGGAGTCCGGCCCAGGACTGGTGAAGCCT
TCGGAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCGTCAGCAGTAGTA
ACTGGTGGAGTTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGG
AAATCTATCATAGTGGGAGCACCAACTACAACCCGTCCCTCAAGAGTCGAGTCA
CCATATCAGTAGACAAGTCCAAGAATCAATTCTCCCTGCACCTGAACTCTGTGAC
TCCCGAGGACACGGCTGTGTACTACTGTGCGAGAGAGGTGGCTGGTTCTGCGGCT
TTCGACATCTGGGGTCAGGGAACGATGGTGACTGTCTCTTCTGGAGGCGGAGGG
TCTGGTGGCGGAGGCTCAGGTGGGGGCGGAAGCCAAAGTGTAGTGACGCAGCCG
CCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATCTCCTGCTCTGGAAGCA
GCTCCAACATTGGGAATAATTATGTATCCTGGTACCAGCAGCTCCCAGGAACAGC
CCCCAAACTCCTCATCTATGGAAATAATAAGCGACCCTCAGGGATTCCTGACCGA
TTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGA
CTGGGGACGAGGCCGATTATTACTGCGGAACATGGGATAGCAGCCTGAGTGCGG
TATTCGGCGGAGGCACCCAGCTGACCGTCCTCGCTAGCGCAACTACCACTCCTGC
ACCACGGCCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGC
CCCGAAGCGTGTAGACCAGCTGCTGGAGGAGCCGTGCATACCCGAGGGCTGGAC
TTCGCCTGTGACATCTACATTTGGGCACCCTTGGCTGGGACCTGTGGGGTGCTGT
TGCTGTCCTTGGTTATTACGTTGTACTGCCGGTCGAAGAGGTCCAGACTCTTGCA
CTCCGACTACATGAACATGACTCCTAGAAGGCCCGGACCCACTAGAAAGCACTA
CCAGCCGTACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCTGA

SEQ ID NO: 254 amino acid sequence of CAR D0433 ROR1scFv9 IgG4 CD8 BBz 2A CD276-30 CD8 CD28 CCR

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGWINPNSGGTNYAQRFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
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MQALPPRRAKRGSGATNFSLLKQAGDVEENPGPRAKRNIMALPVTALLLPLALLLH
AARPQLQLQESGPGLVKPSETLSLTCAVSGGSVSSSNWWSWVRQPPGKGLEWIGEIY

HSGSTNYNPSLKSRVTISVDKSKNQFSLHLNSVTPEDTAVYYCAREVAGSAAFDIWG
QGTMVTVSSGGGGSGGGGSGGGGSQSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNY
VSWYQQLPGTAPKLLIYGNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCG
TWDSSLSAVFGGGTQLTVLASATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRS

SEQ ID NO: 255 nucleotide sequence of CAR D0397 ROR1scFv9 IgG4 CD8 BBz 2A CD276-376.96 CD8 CD28 CCR

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTC
TGCTGATTCCGCAGGCGGCCCAGGTACAGCTGCAGCAGTCAGGGGCTGAGGTGA
AGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAGGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGtTGAGC
AGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGTTATAATGATGCTT
TTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTC
TGGTGGTGGCGGTAGCGGTGGTGGCGGATCCAATTTTATGCTGACTCAGCCCCAC
TCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTG
GCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCC
CCACCATTGTGATCTATGAGGATGATCAAAGACCCTCTGGGGTCCCTGATCGGTT
CTCTGGCTCCATCGACACCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGC
AGAGTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGAGCCCGGCAATGGGG
TATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCGGCCGCAGAGTCAAAATACG
GTCCTCCGTGCCCTCCGTGTCCGATCTACATCTGGGCCCCATTGGCTGGAACTTG
CGGCGTGCTGCTCTTGTCTCTGGTCATTACCCTGTACTGCAAGCGCGGACGGAAG
AAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAG
AAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAA
CTGAGAGTGAAGTTTAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGACAG
AACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGTGCTG
GACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGAGGAAGAA
CCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATGGCGGAAGCCTA
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TAGCCTGGTATCAACAGAAACCAGGCCAGTCTCCTAAACTTCTTATTTACTCAGC
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GTCAGCAACATTATGGTACTCCTCCGTGGACGTTCGGTGGAGGCACCAAGCTGG
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CGGCCATGTATTACTGTGCAAGACACTATGATGGTTATCTTGACTACTGGGGCCA
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CCACCTACCCCAGCCCCCACCATTGCAAGCCAGCCACTTTCACTGCGCCCCGAAG
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TACGCCCCTCCTCGGGATTTCGCCGCATACCGGTCCTGA

SEQ ID NO: 256 amino acid sequence of CAR D0397 ROR1scFv9 IgG4 CD8 BBz 2A CD276-376.96 CD8 CD28 CCR

MLLLVTSLLLCELPHPAFLLIPQAAQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSY
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DDTAVYYCASYNDAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQPHSVSE
SPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTIVIYEDDQRPSGVPDRFSGSIDTSS
NSASLTISGLQSEDEADYYCQSYEPGNGVFGGGTKVTVLAAAESKYGPPCPPCPIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
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RYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYGTPPWTFGGGTKLEIKGG

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TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRS

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor (CAR) comprising at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276/B7-H3 antigen binding domain operationally linked to one or more booster elements, at least one transmembrane domain, and at least one intracellular signaling domain, which boosted single, tandem, multi-targeting, or DuoCARs CAR is encoded by a nucleotide sequence comprising SEQ ID NO: 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 245, 247, 249, 251, 253, or 255, or any combination thereof.

2. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR further comprises one or more booster elements comprising: i) an armor element so as to overcome immunosuppression in a tumor microenvironment (TME); ii) a cytokine stimulated element to promote autonomous T cell stimulation with cytokines; iii) a digestive enzyme element to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration; iv) neutrophil-activating protein (NAP); or v) an on-switch element or off-switch element, to control the expression of the CAR; or any combination thereof, and wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR exhibits one or more properties in a patient-specific manner.

3. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR further comprises one or more booster elements comprising an armor element comprising TGFβRIIdn, truncated PD-1, PD-1dn, synthetic PD-1 activating receptor, truncated CTLA-4, truncated Tim-3, truncated TIGIT or any combination thereof, so as to overcome immunosuppression in a tumor microenvironment (TME) in a patient-specific manner.

4. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR further comprises one or more booster elements comprising a cytokine stimulated element comprising membrane bound IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21 TNFα, IFNγ, or any combination thereof, to promote autonomous T cell stimulation with cytokines in a patient-specific manner.

5. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR further comprises one or more booster elements comprising a digestive enzyme element comprising heparinase/HPSE, MMP-1, MMP-2, MMP-9, MMP-12, MMP-13 and hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, PH-20, and HYALP1, or any combination thereof, to overcome the physical barrier of tumor stroma/extracellular matrix (ECM) and enable CAR T tumor penetration in a patient-specific manner.

6. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, HPSE, MMP-2, MMP-9 and PH-20 or DuoCARs CAR further comprises one or more booster elements comprising an on-switch element or off-switch element, comprising truncated EGF receptor, truncated TGFβ receptor (TGFβRIIdn), truncated CD19, truncated CD20, CD20 mimotope, truncated CD34, truncated LNGF receptor, chimeric costimulatory receptor (CCR), or any combination thereof, to control the expression of the CAR in a patient-specific manner.

7. The isolated nucleic acid molecule of claim 1, wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR exhibits one or more properties in a patient-specific manner, wherein said properties further comprise i) multi-targeting to overcome antigen escape; ii) a high-degree of surface expression on transduced T cells; or iii) a high degree of cytolysis and transduced T cell *in vivo* expansion and persistence to promote *in vivo* expansion, persistence of patient-specific anti-tumor T-cells resulting in tumor stabilization, reduction, elimination, remission of cancer or autoimmune, alloimmune, or autoaggressive disease, or prevention or amelioration of relapse of cancer or autoimmune, alloimmune, or autoaggressive disease, or any combination thereof.

8. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one ROR1 and/or MSLN antigen binding domain, the at least one intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

9. The isolated nucleic acid molecule of claim 8, wherein the encoded linker or spacer domain is derived from the extracellular domain of IgG1, IgG2, IgG3 or IgG4, CD8, TNFRSF19, or CD28, and is linked to a transmembrane domain.

10. The isolated nucleic acid molecule of claim 1, wherein the encoded extracellular ROR1 and/or MSLN antigen binding domain is preceded by a leader nucleotide sequence encoding a leader peptide.

11. The isolated nucleic acid molecule of claim 10, wherein the leader nucleotide sequence comprises a nucleotide sequence comprising SEQ ID NO: 13 encoding the leader amino acid sequence of SEQ ID NO: 14, or SEQ ID NO: 39 encoding the leader amino acid sequence of SEQ ID NO: 40, or SEQ ID NO: 41 encoding the leader amino acid sequence of SEQ ID NO: 42, or SEQ ID NO: 43 encoding the leader amino acid sequence of SEQ ID NO: 44.

12. The isolated nucleic acid molecule of claim 1, wherein the transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD83, CD86, CD134, CD137, CD154, and TNFRSF19, or any combination thereof.

13. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid sequence encoding the extracellular antigen binding domain comprises a nucleic sequence comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 73, 75, or 77, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

14. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one intracellular signaling domain further comprises a CD3 zeta intracellular domain.

15. The isolated nucleic acid molecule of claim 14, wherein the encoded at least one intracellular signaling domain is arranged on a C-terminal side relative to the CD3 zeta intracellular domain.

16. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, chimeric costimulatory receptor (CCR), or any combination thereof.

17. The isolated nucleic acid molecule of claim 16, wherein the encoded at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, 4-1BB (CD137), PD-1, GITR, and CTLA-4, or any combination thereof.

18. A boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor (CAR) encoded by the isolated nucleic acid molecule of claim 1.

19. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 18, comprising at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276 antigen binding domain, wherein the boosted single, tandem, multi-targeting, or DuoCARs comprise the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof, at least one transmembrane domain, and at least one intracellular signaling domain.

20. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 19, wherein the transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, and TNFRSF19 or any combination thereof.

21. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 20, wherein the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 28.

22. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 19, comprising at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276 antigen binding domain, the at least one intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain wherein the boosted single, tandem, multi-targeting, or DuoCARs comprise the amino acid sequence of SEQ ID

NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 98, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, or any combination thereof, at least one transmembrane domain, and at least one intracellular signaling domain.

23. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 22, wherein the linker or spacer domain is derived from the extracellular domain of CD8, TNFRSF19, IgG1, IgG2, IgG3, IgG4, or CD28, and is linked to a transmembrane domain.

24. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 19, wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

25. The boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 24, wherein the at least one intracellular signaling domain comprises a costimulatory domain comprising a functional signaling domain of a protein selected from the group consisting of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, 4-1BB (CD137), PD-1, GITR, and CTLA-4 or a combination thereof.

26. A vector comprising a nucleic acid molecule of claim 1.

27. The vector of claim 26, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid vector, a cosmid vector, a herpes virus vector, a measles virus vector, a lentivirus vector, adenoviral vector, or a retrovirus vector, or a combination thereof.

28. The vector of claim 26, further comprising a promoter.

29. The vector of claim 28, wherein the promoter is an inducible promoter, a constitutive promoter, a tissue specific promoter, a suicide promoter or any combination thereof.

30. A cell comprising the vector of claim 26.

31. The cell of claim 30, wherein the cell is a T cell, natural killer (NK) cell, natural killer T (NKT) cell, invariant natural killer T (iNKT) cell, T-regulatory cell (Treg), induced T-regulatory (iTreg) cell, B cell, dendritic cell (DCs), gamma delta T cell, macrophage, stem cell, or induced pluripotent stem (iPS) cell.

32. The cell of claim 30, wherein the T cell is a $CD8^+$ T cell, or a $CD4^+$ T cell.

33. The cell of claim 30, wherein the cell is a human cell.

34. A pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor (CAR), wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR comprises at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, at least one linker domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of a human having a cancer, autoimmune, alloimmune, or autoaggressive disease or any combination thereof.

35. The pharmaceutical composition of claim 34, wherein the at least one transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or any combination thereof.

36. The pharmaceutical composition of claim 34, wherein the T cells are T cells of a human having a hematological cancer.

37. The pharmaceutical composition of claim 36, wherein the hematological cancer is leukemia or lymphoma.

38. The pharmaceutical composition of claim 37, wherein the leukemia is acute myeloid leukemia (AML), blastic plasmacytoid dendritic cell neoplasm (BPDCN), chronic myelogenous

leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphoblastic T cell leukemia (T-ALL), or acute lymphoblastic B cell leukemia (B-ALL).

39. The pharmaceutical composition of claim 37, wherein the lymphoma is mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma.

40. The pharmaceutical composition of claim 36, wherein the hematological cancer is multiple myeloma.

41. The pharmaceutical composition of claim 36, wherein the autoimmune, alloimmune, or autoaggressive disease is rheumatoid arthritis, lupus, celiac disease, Sjögren's syndrome, multiple sclerosis, polymyalgia rheumatica, ankylosing spondylitis, type 1 diabetes, alopecia areata, vasculitis, temporal arteritis, post-streptococcal autoimmune disorder, antineuronal antibody-mediated neuropsychiatric disorder, immune-mediated extrapyramidal movement disorder, Sydenham chorea, alloimmune hemolytic disease, pulmonary fibrosis, systemic scleroderma, and fibrotic disease.

42. The pharmaceutical composition of claim 34, wherein the human cancer includes an adult carcinoma comprising oral and pharynx cancer (tongue, mouth, pharynx, head and neck), digestive system cancers (esophagus, stomach, small intestine, colon, rectum, anus, liver, intrahepatic bile duct, gallbladder, pancreas), respiratory system cancers (larynx, lung and bronchus), bones and joint cancers, soft tissue cancers, skin cancers (melanoma, basal and squamous cell carcinoma), pediatric tumors (neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma), tumors of the central nervous system (brain, astrocytoma, glioblastoma, glioma), and cancers of the breast, the genital system (uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, penis, endometrium), the urinary system (urinary bladder, kidney and renal pelvis, ureter), the eye and orbit, the endocrine system (thyroid), and the brain and other nervous system, or any combination thereof.

43. A method of making a cell comprising transducing a T cell with a vector of claim 26.

44. A method of generating a population of RNA-engineered cells comprising introducing an *in vitro* transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid molecule of claim 1.

45. A method of providing an anti-tumor immunity in a mammal comprising administering to the mammal an effective amount of a cell of claim 30.

46. A method of treating or preventing cancer in a mammal, comprising administering to the mammal the boosted single, tandem, multi-targeting, or DuoCARs CAR of claim 19, in an amount effective to treat or prevent cancer, autoimmune, alloimmune, autoaggressive disease, or any combination thereof in the mammal.

47. A method of treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor (CAR), wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR comprises at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having cancer.

48. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor (CAR), wherein the boosted single, tandem, multi-targeting, or DuoCARs CAR comprises at least one extracellular antigen binding domain comprising a ROR1, MSLN, FOLR1, and/or CD276 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 246, 248, 250, 252, 254, or 256, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having cancer.

49. The method of claim 47 or 48, wherein the at least one transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or any combination thereof.

50. A process for producing a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor-expressing cell, the process comprising introducing the isolated nucleic acid of claim 1 into a cell.

51. The process for producing a boosted single, tandem, multi-targeting, or DuoCARs chimeric antigen receptor-expressing cell according to claim 50, wherein the cell is a T cell, natural killer (NK) cell, natural killer T (NKT) cell, invariant natural killer T (iNKT) cell, dendritic cell (DCs), gamma delta T cell, macrophage, stem cell, or induced pluripotent stem (iPS) cell or a cell population containing a T cell, natural killer (NK) cell, natural killer T (NKT) cell, invariant natural killer T (iNKT) cell, dendritic cell (DCs), gamma delta T cell, macrophage, stem cell, or induced pluripotent stem (iPS) cell.

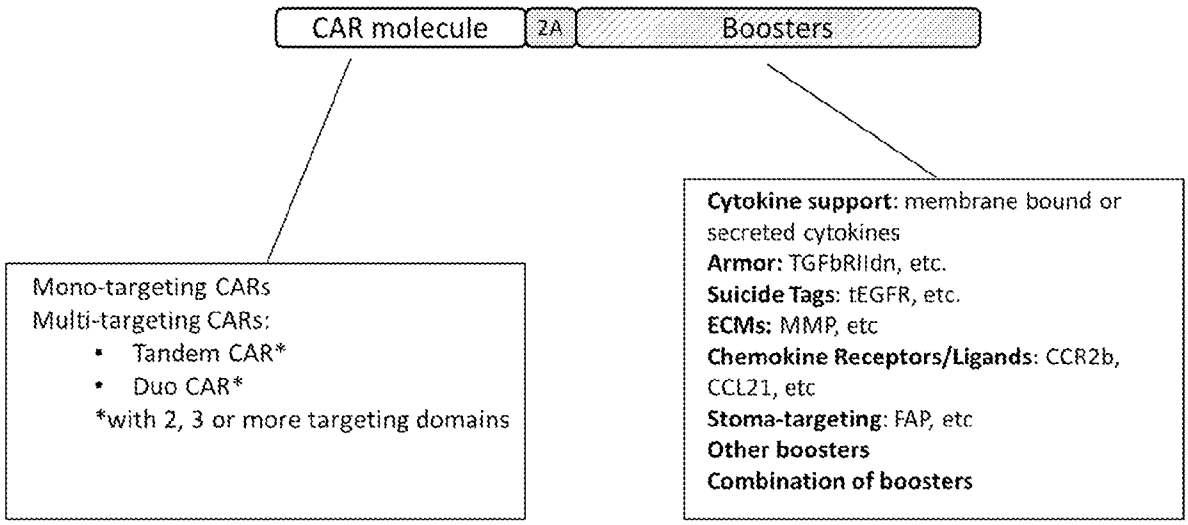
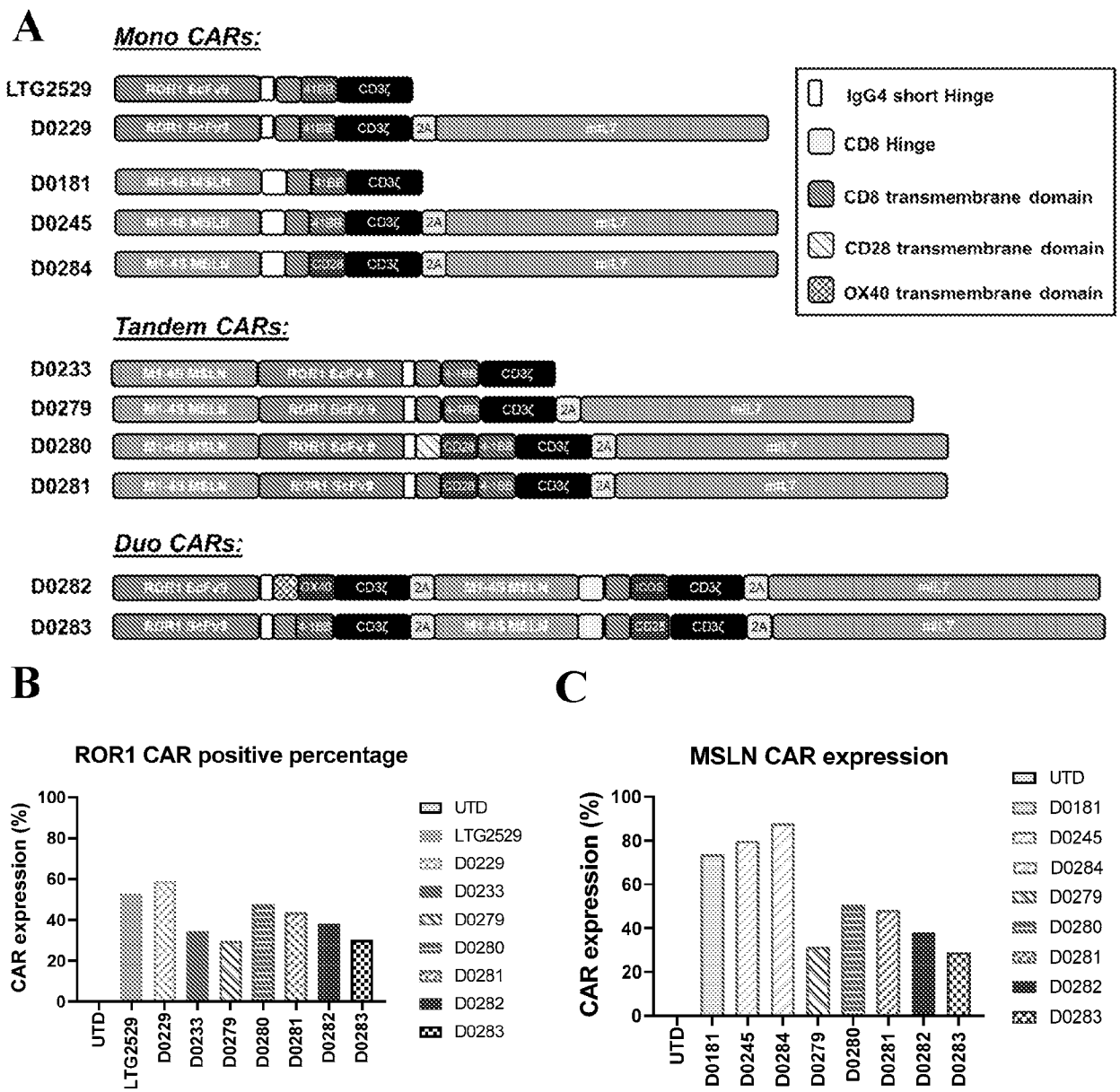
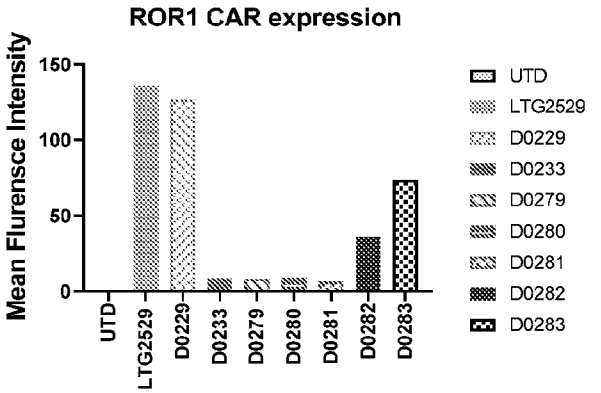

CAR molecule
2A
Boosters
Mono-targeting CARs
Multi-targeting CARs:
• Tandem CAR*
• Duo CAR*
*with 2, 3 or more targeting domains
Cytokine support: membrane bound or secreted cytokines
Armor: TGFbRIIdn, etc.
Suicide Tags: tEGFR, etc.
ECMs: MMP, etc
Chemokine Receptors/Ligands: CCR2b, CCL21, etc
Stoma-targeting: FAP, etc
Other boosters
Combination of boosters

FIGURE 1

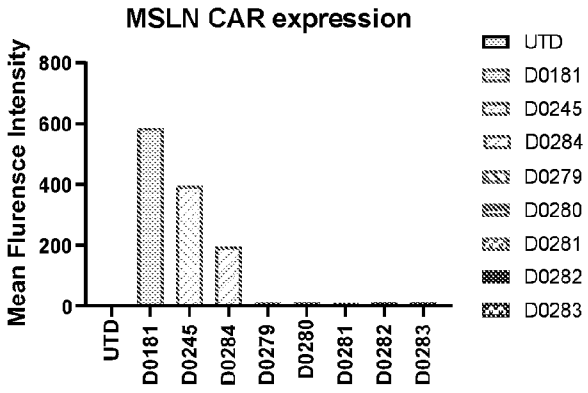


FIGURES 2A – 2C
(1/2)

D

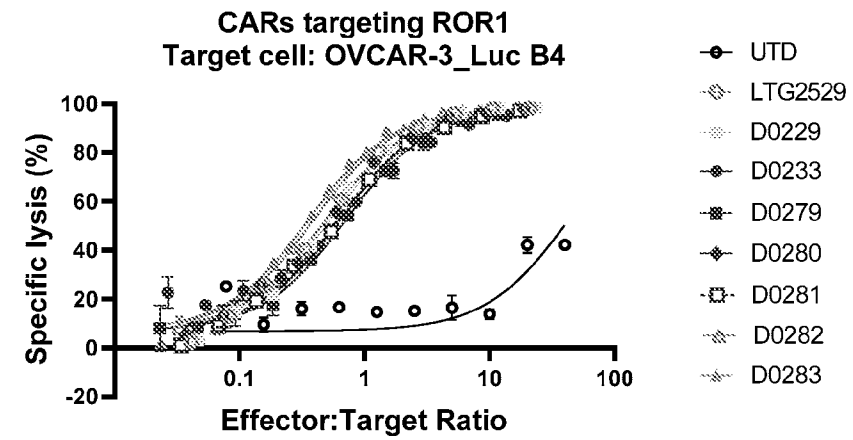


E

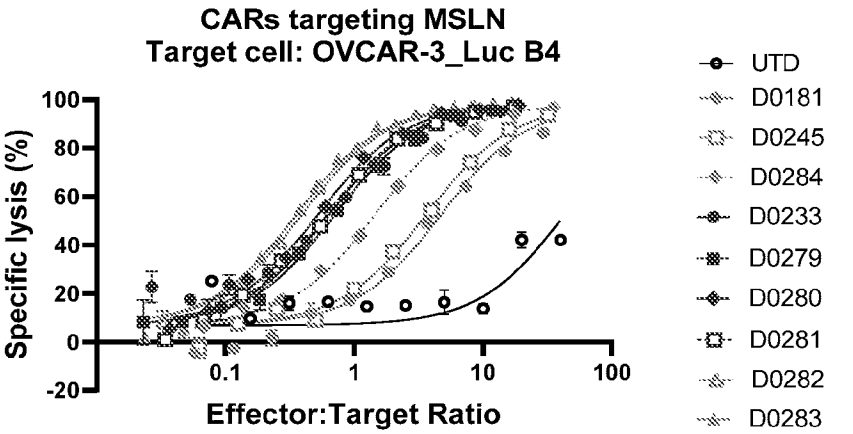


FIGURES 2D-2E
(2/2)

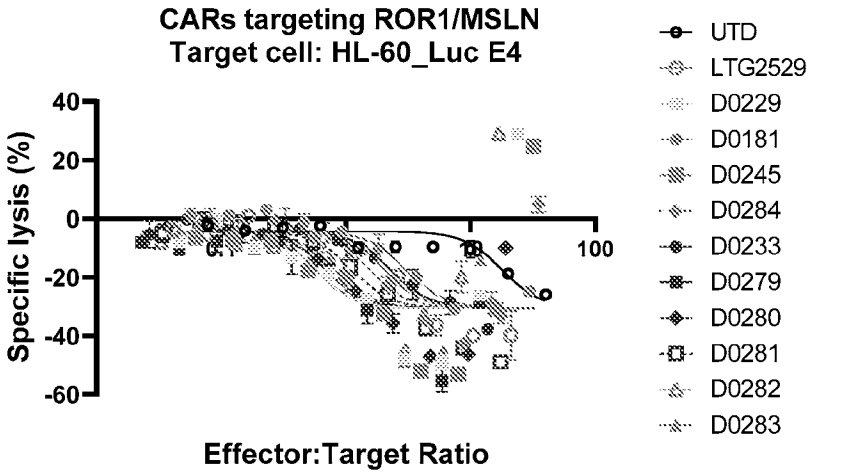
A



B

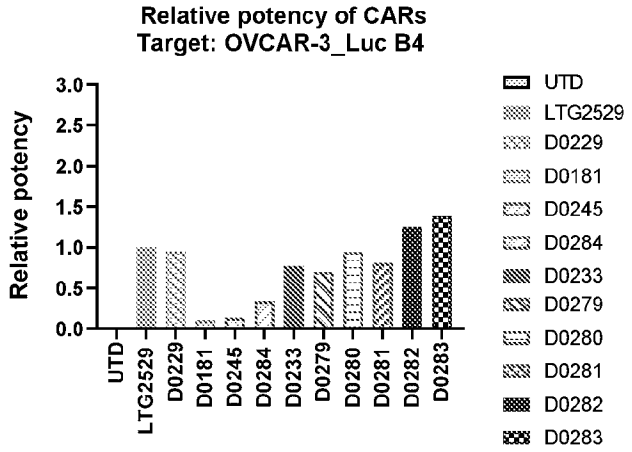


C

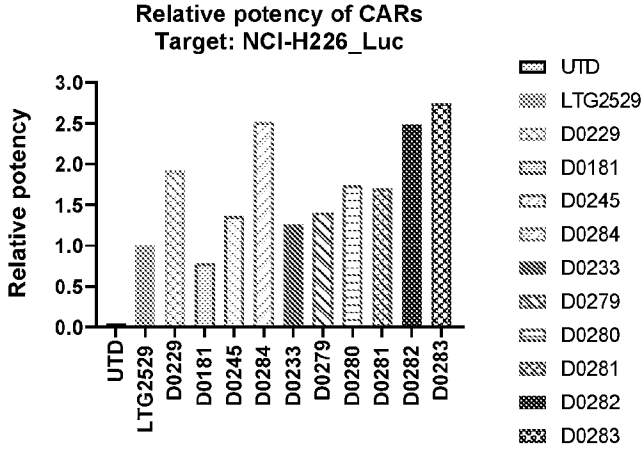


FIGURES 3A – 3C

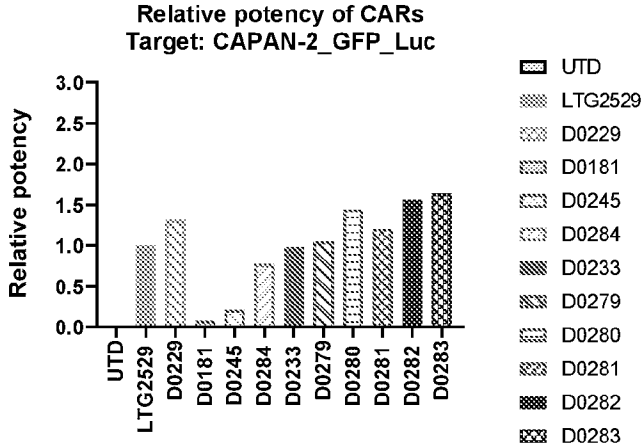
A



B

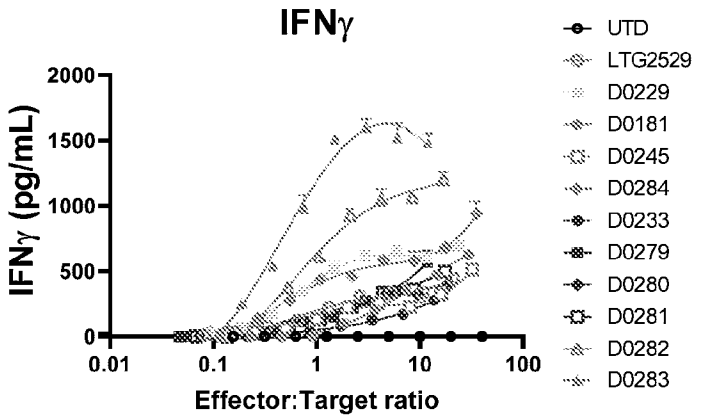


C

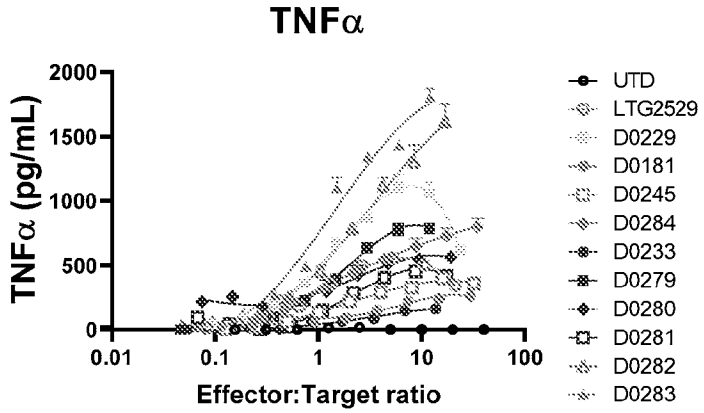


FIGURES 4A-4C

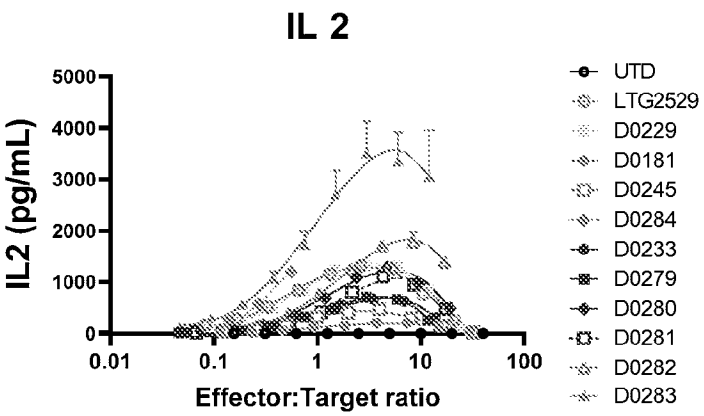
A



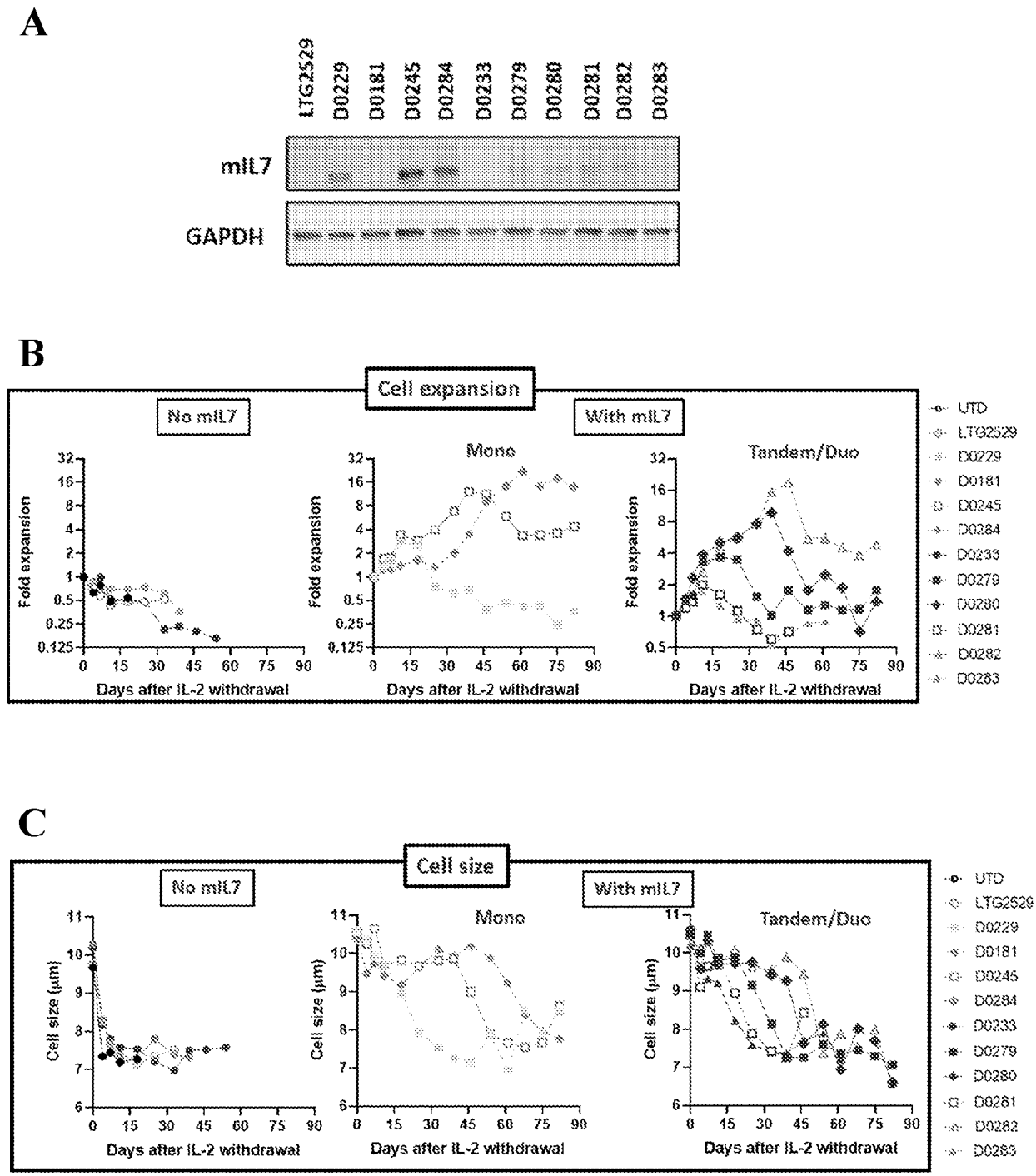
B



C

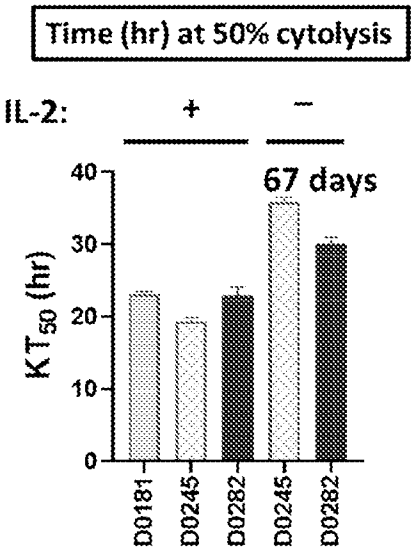


FIGURES 5A-5C

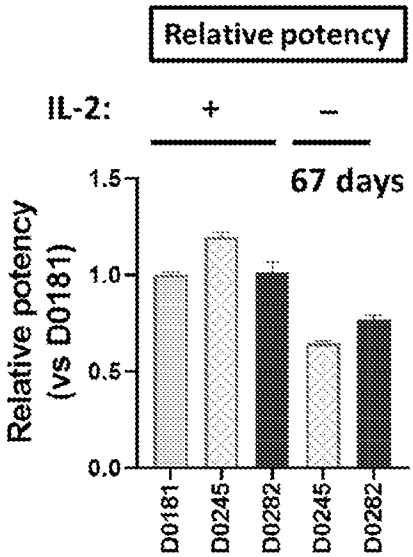


FIGURES 6A-6C

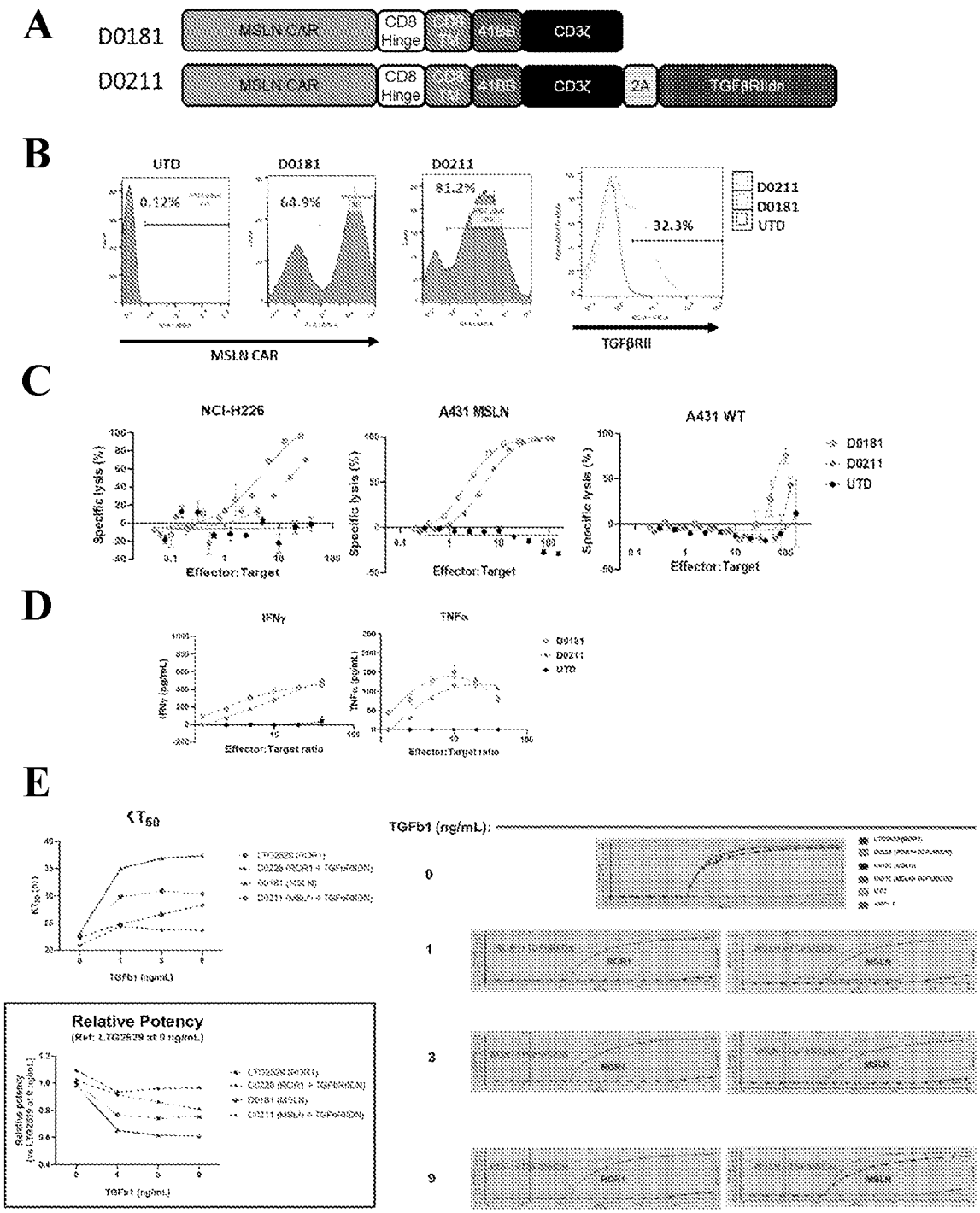
A



B

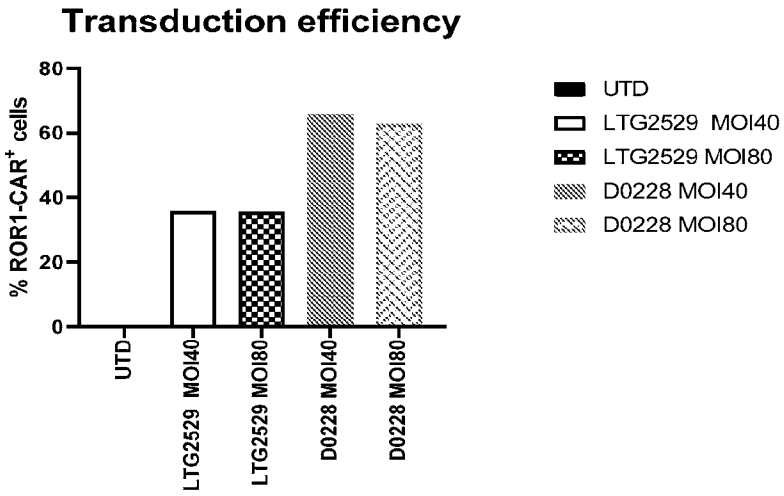


FIGURES 7A-7B

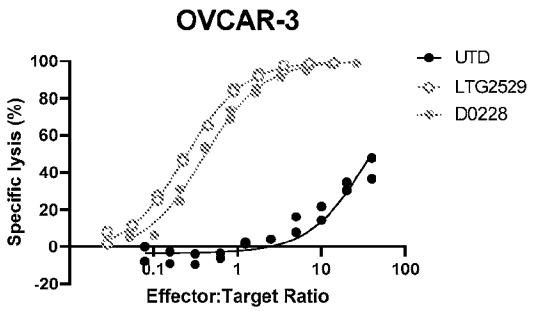


FIGURES 8A-8E

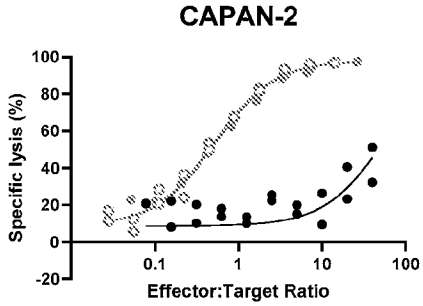
A



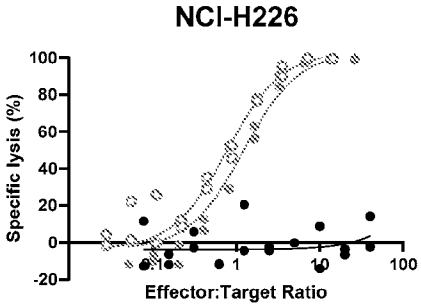
B



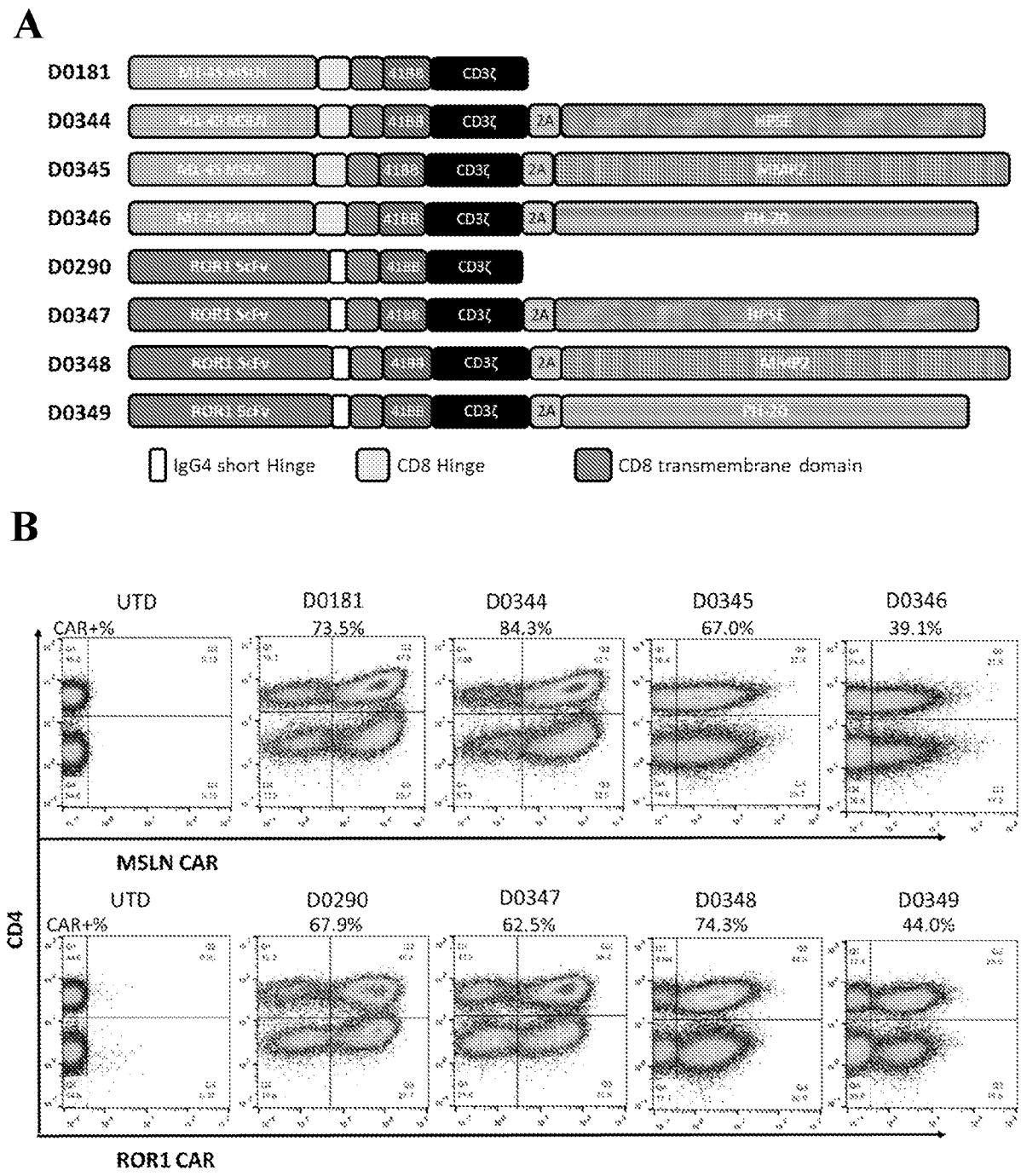
C



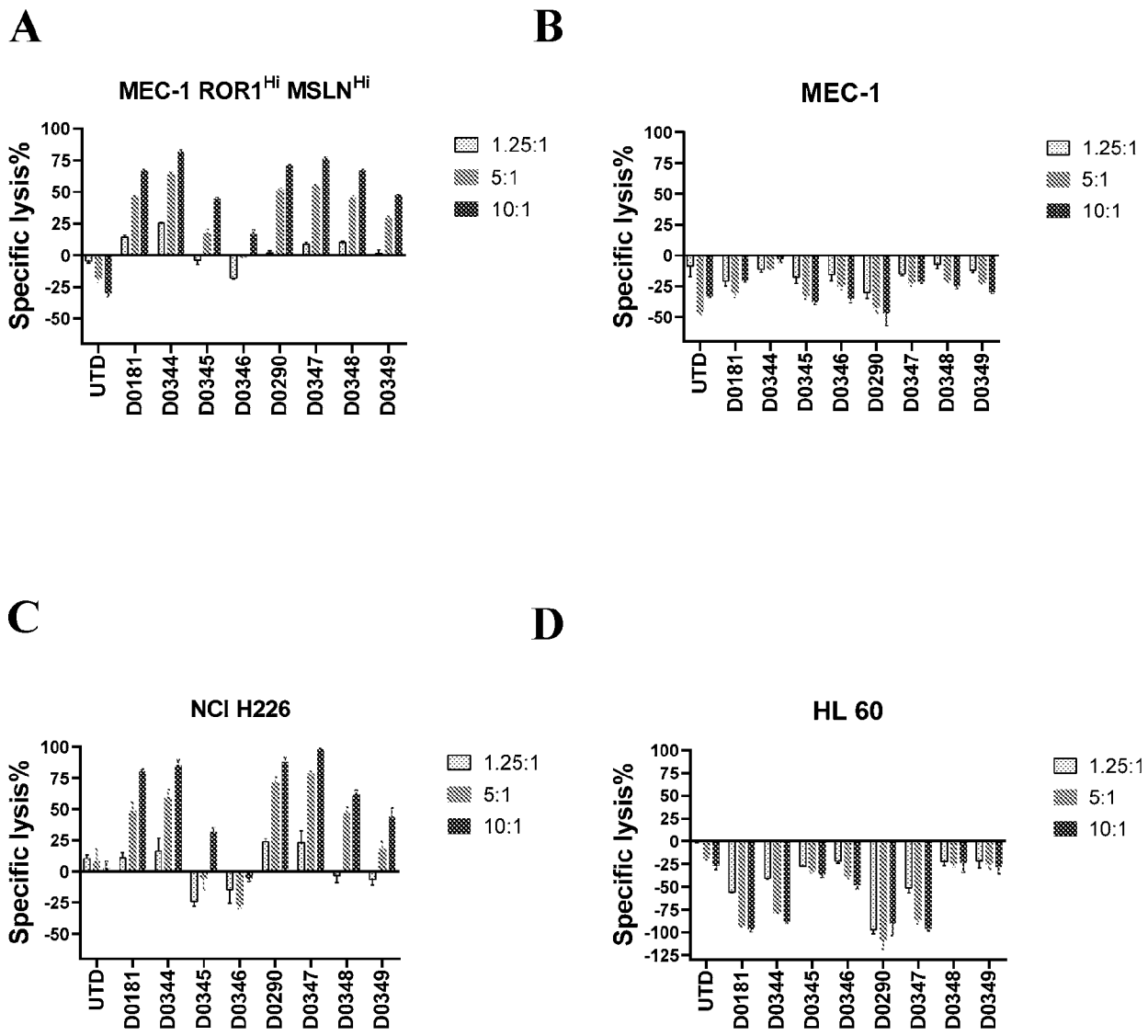
D



FIGURES 9A-9D

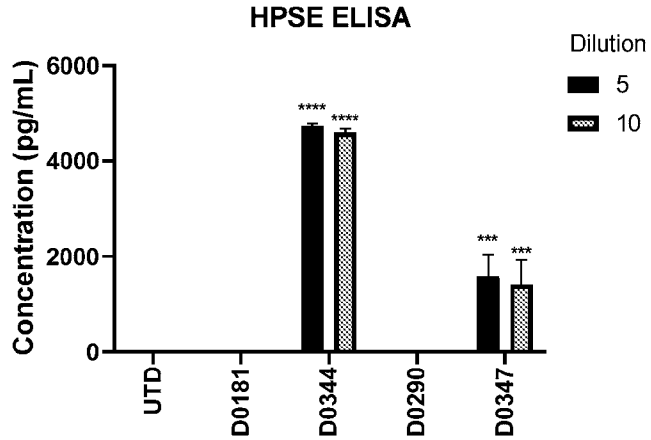


FIGURES 10A – 10B

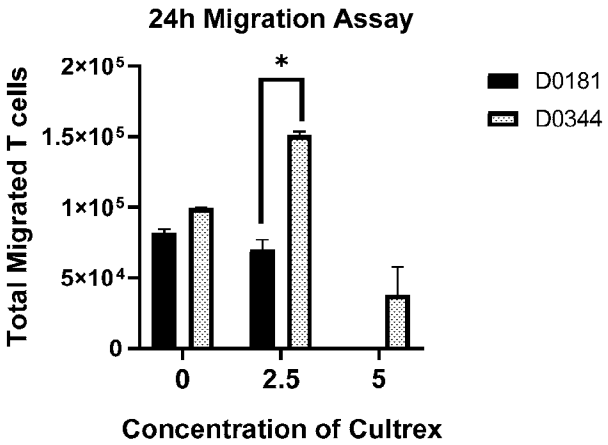


FIGURES 11A – 11D

A

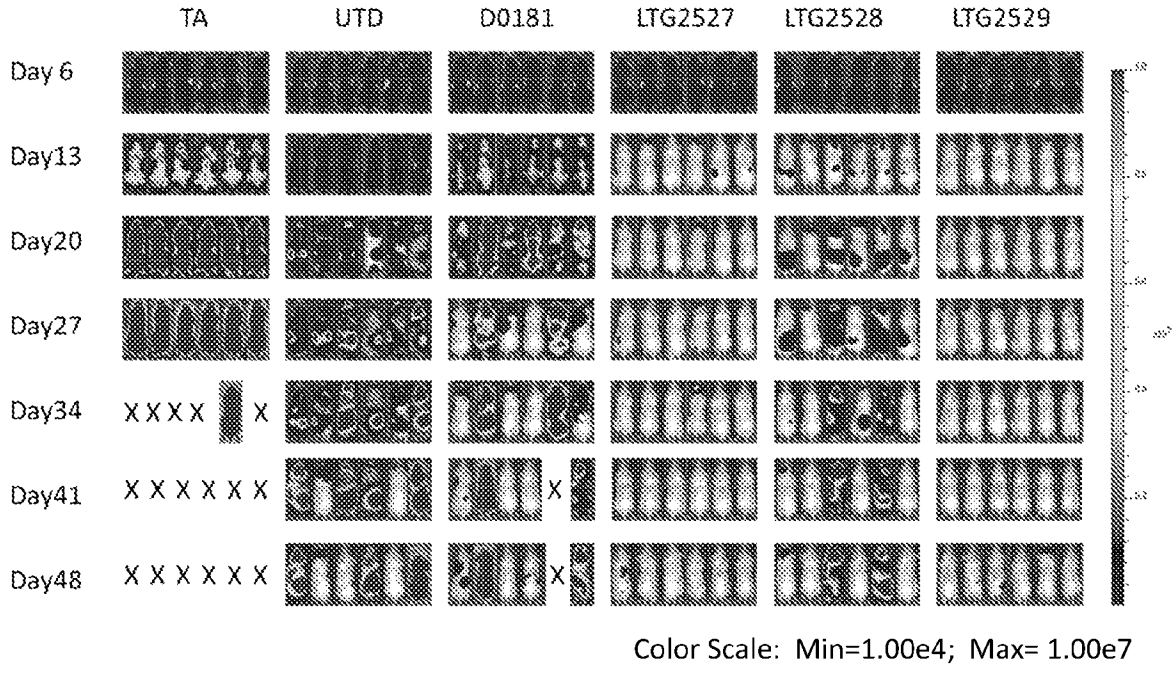


B

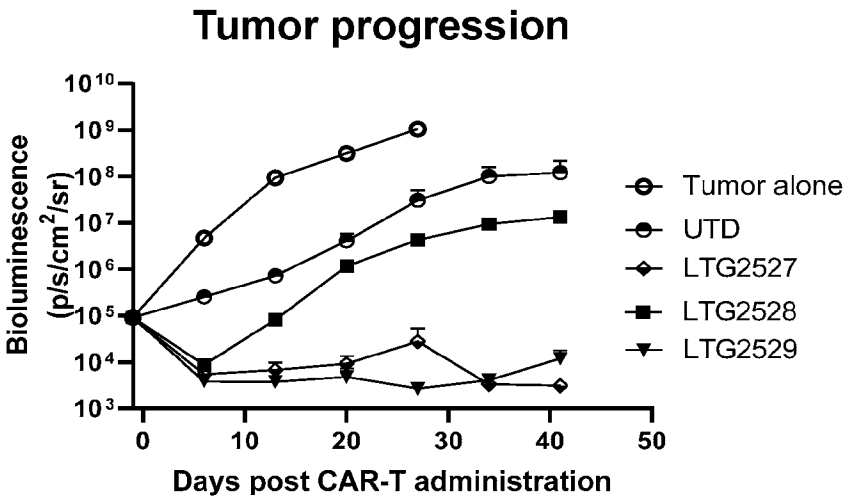


FIGURES 12A - 12B

A



B



FIGURES 13A-13B

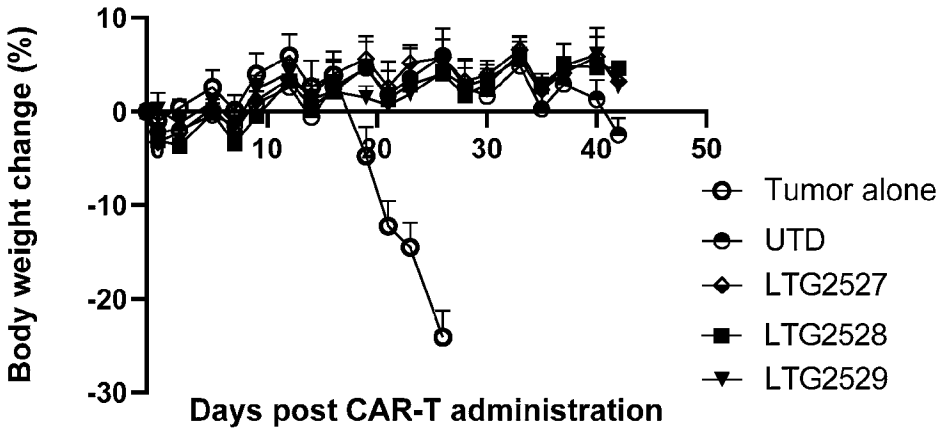
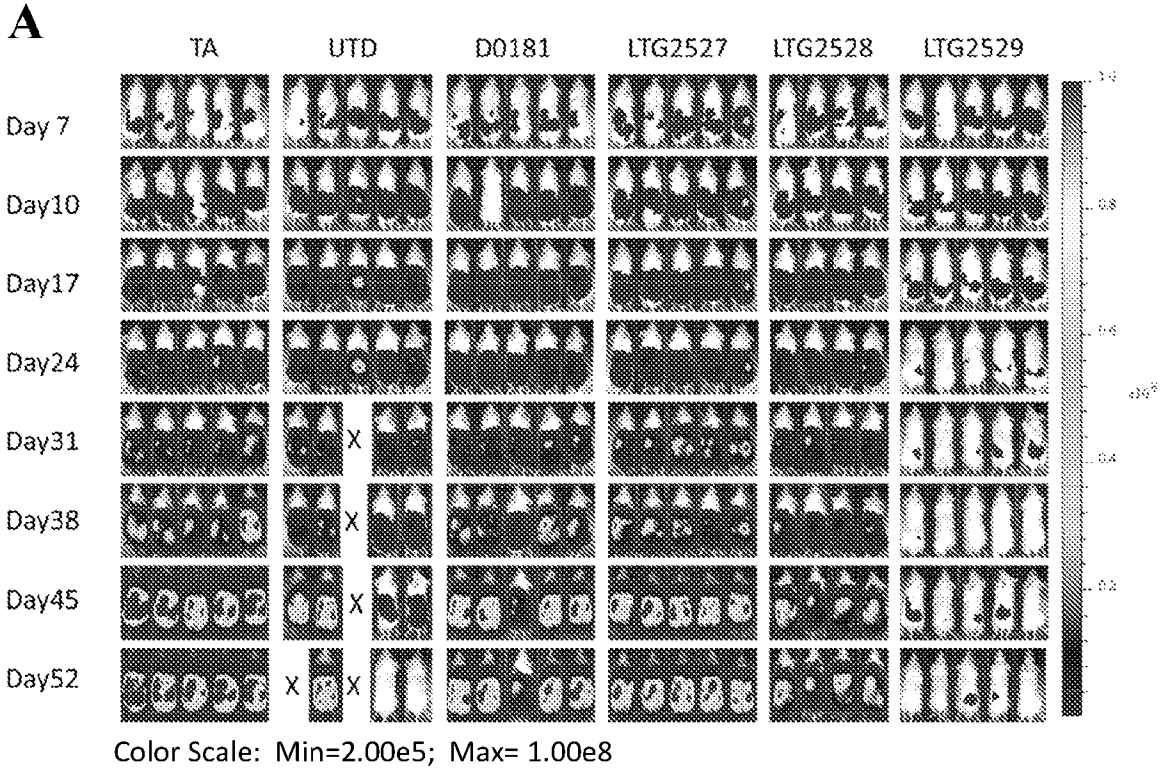
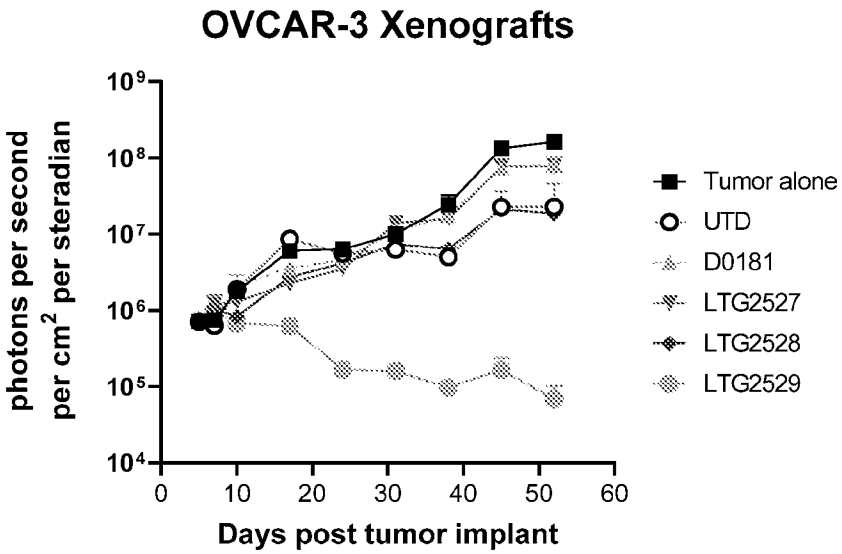

Body weight change (%)
10
0
-10
-20
-30
10
30
40
50
Days post CAR-T administration
Tumor alone
UTD
LTG2527
LTG2528
LTG2529

FIGURE 14

A



B



FIGURES 15A-15B

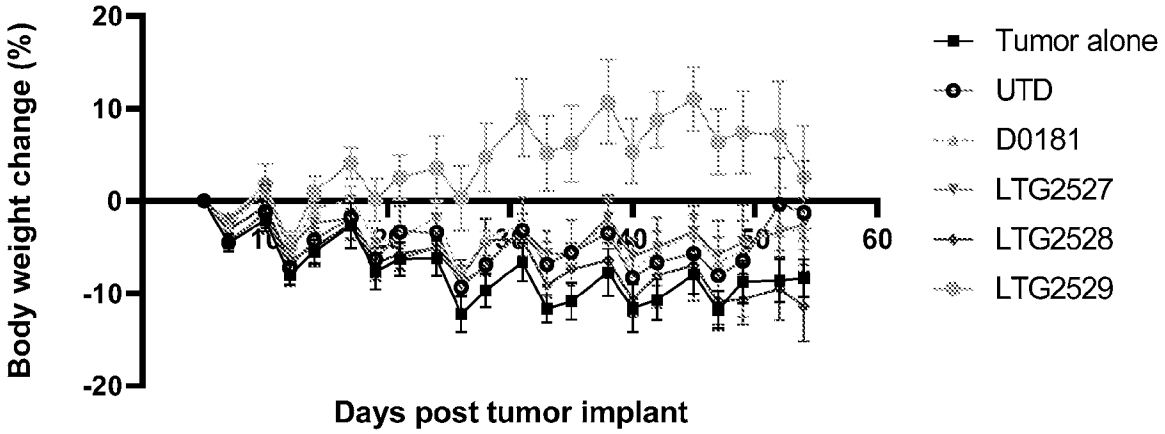

Body weight change (%)
20
10
0
-10
-20
10
20
30
40
50
60
Days post tumor implant
Tumor alone
UTD
D0181
LTG2527
LTG2528
LTG2529

FIGURE 16

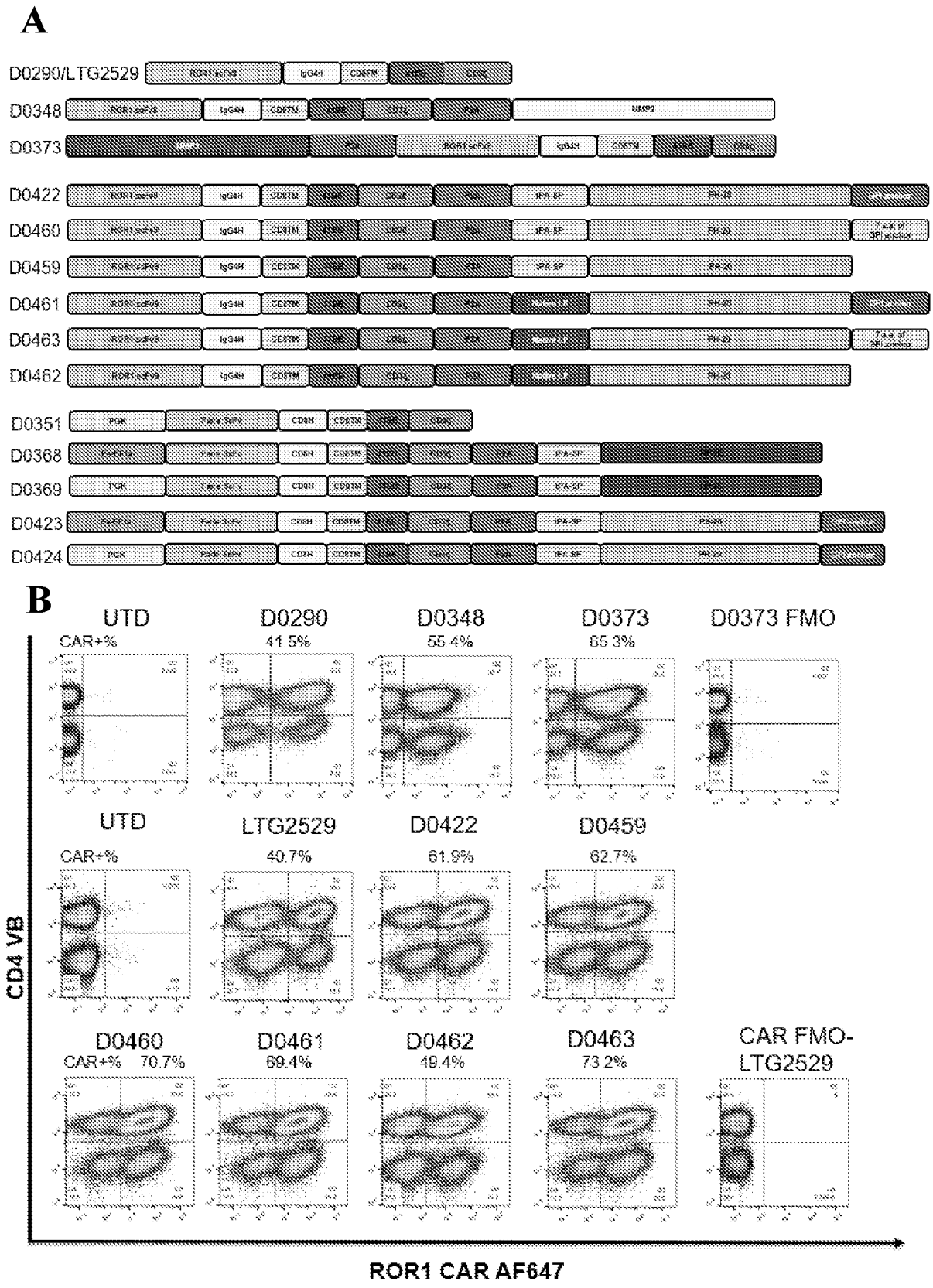

A
D0290/LTG2529
D0348
D0373
D0422
D0460
D0459
D0461
D0463
D0462
D0351
D0368
D0369
D0423
D0424
B
UTD
CAR+%
D0290
41.5%
D0348
55.4%
D0373
65.3%
D0373 FMO
UTD
CAR+%
LTG2529
40.7%
D0422
61.9%
D0459
62.7%
D0460
CAR+% 70.7%
D0461
69.4%
D0462
49.4%
D0463
73.2%
CAR FMO-
LTG2529
CD4 VB
ROR1 CAR AF647

FIGURE 17 (1/2)

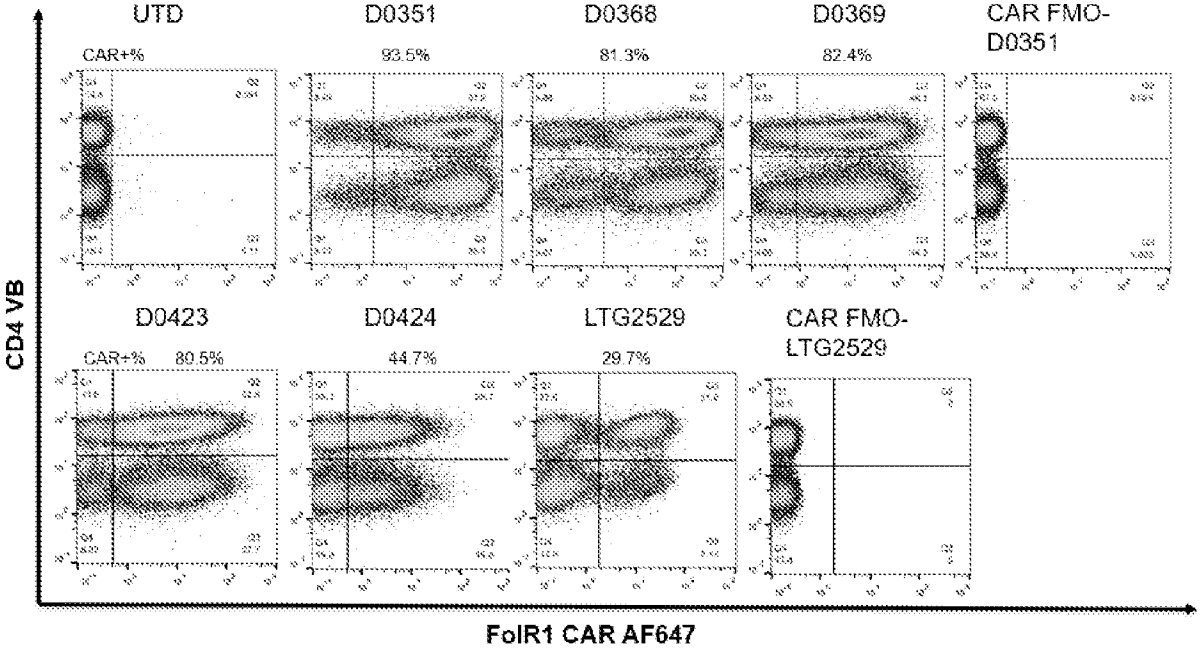
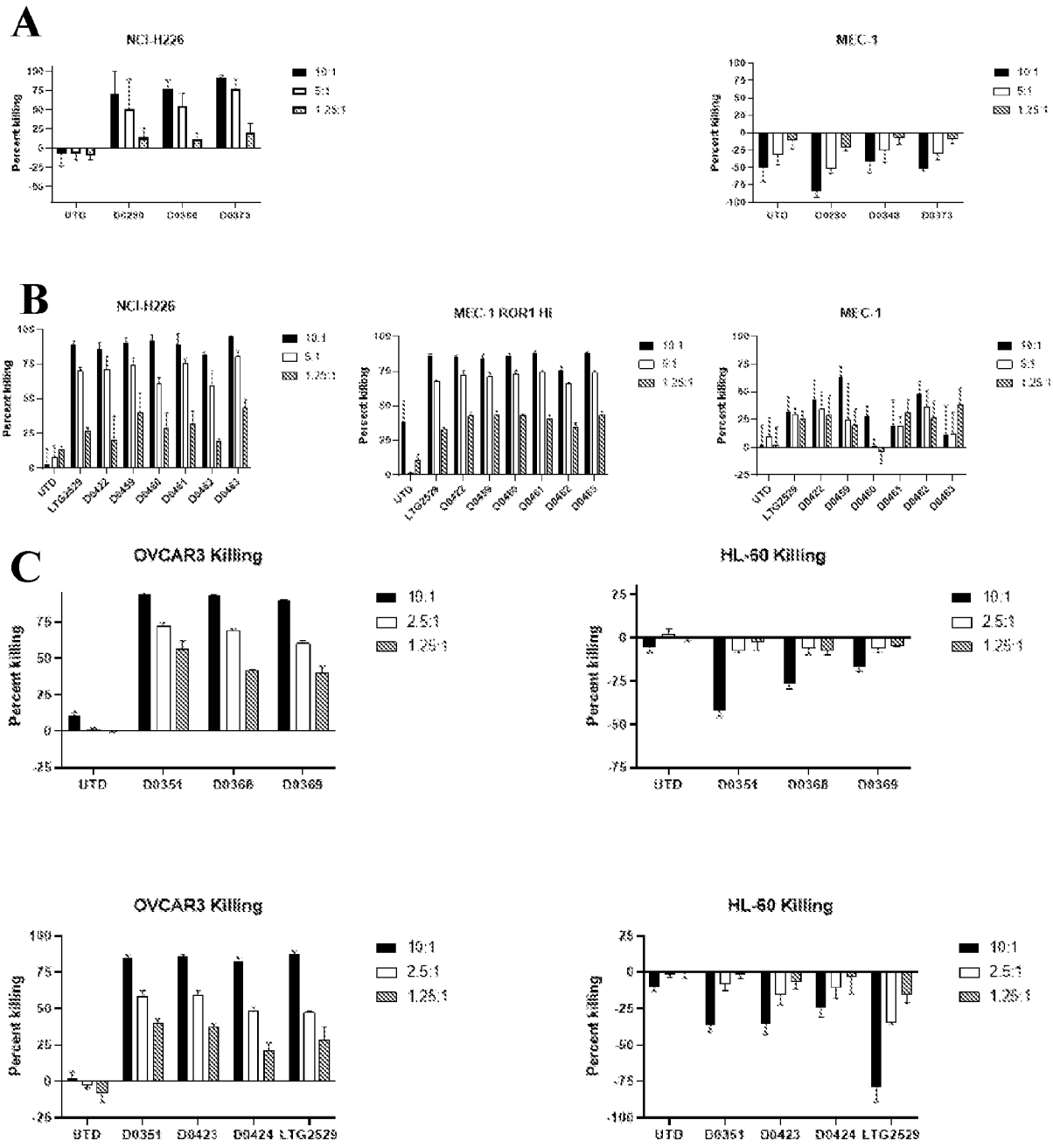
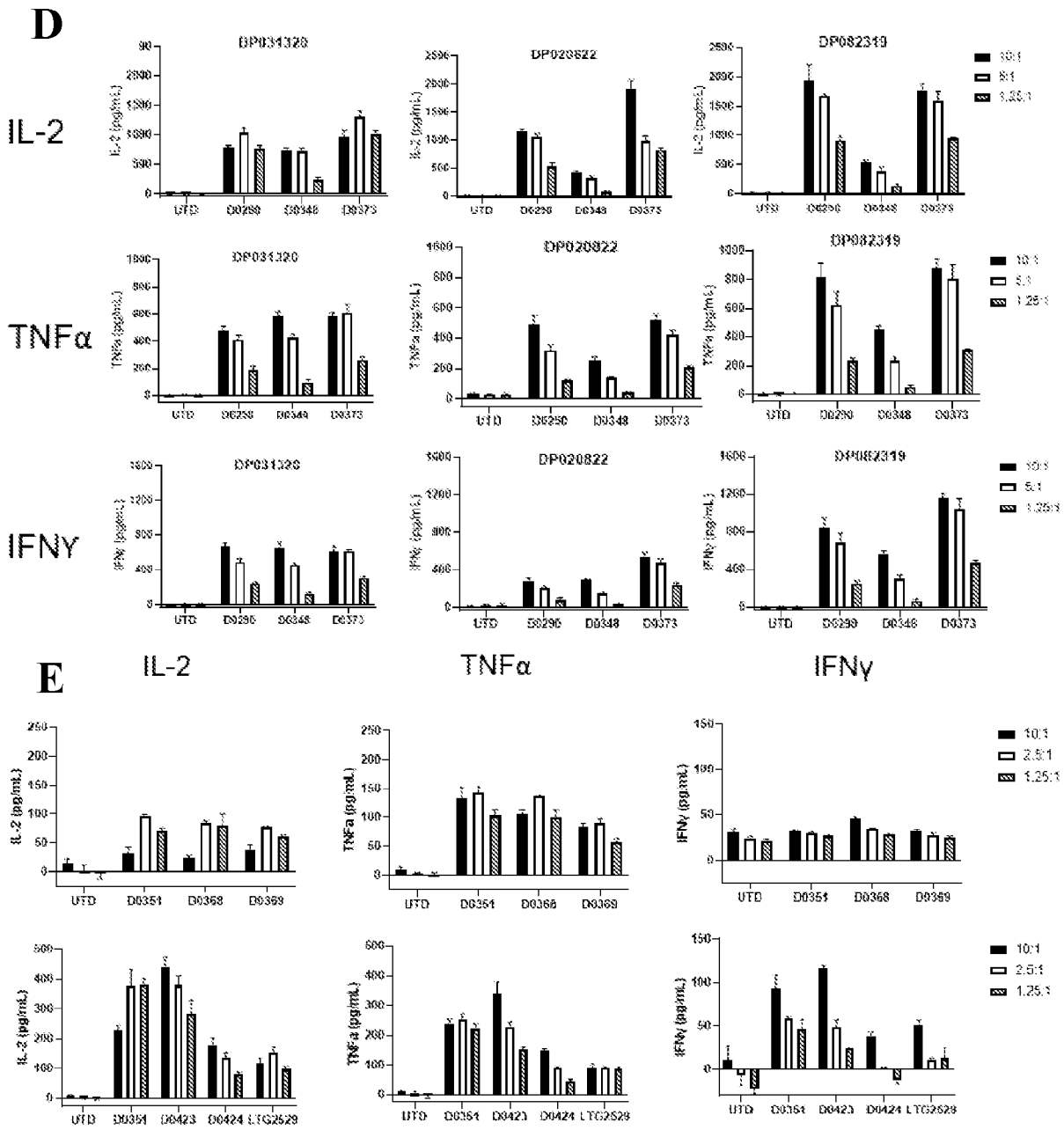

UTD
D0351
D0368
D0369
CAR FMO-D0351
CAR+%
93.5%
81.3%
82.4%
D0423
D0424
LTG2529
CAR FMO-LTG2529
CAR+%
80.5%
44.7%
29.7%
CD4 VB
FolR1 CAR AF647

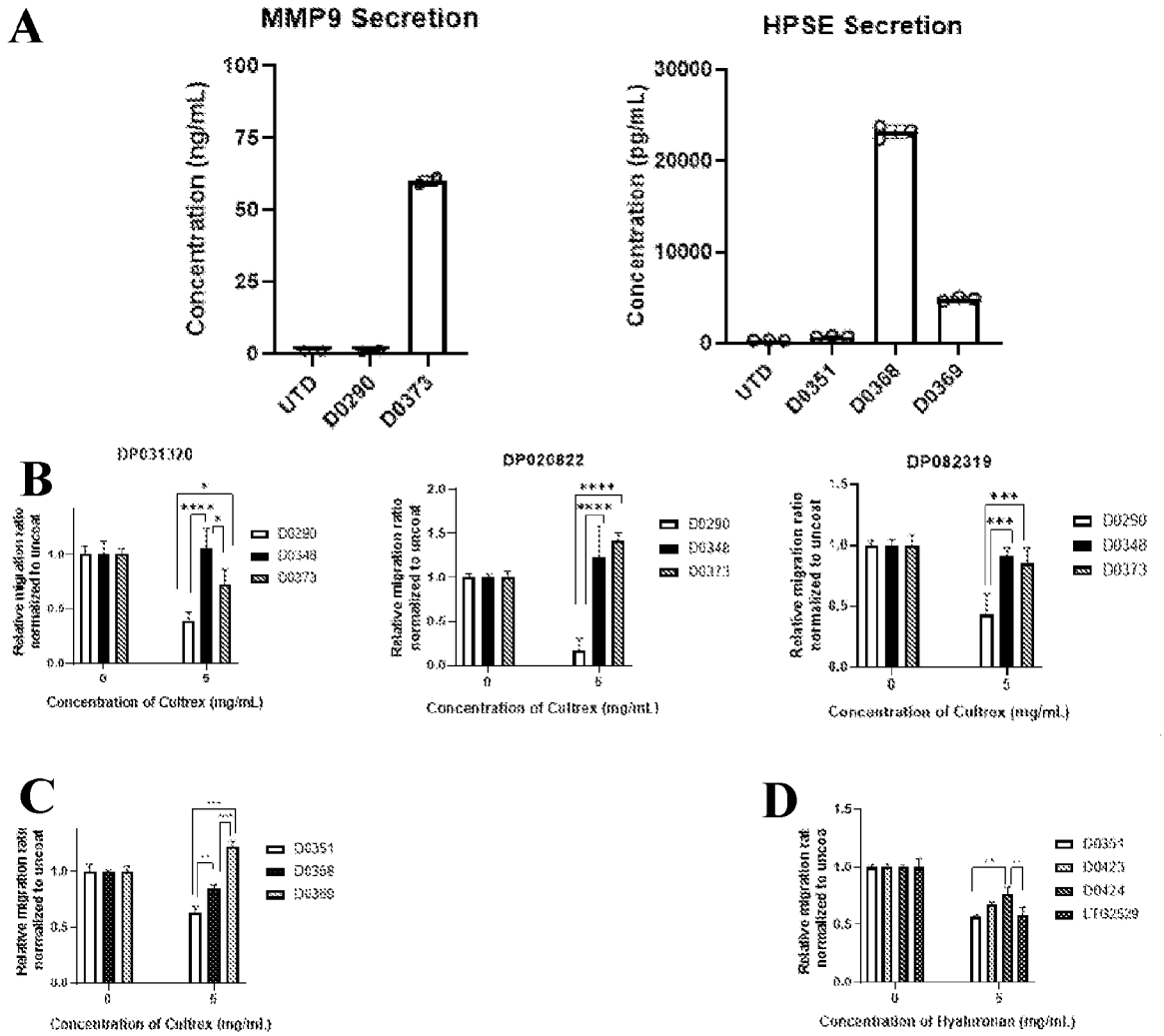
FIGURE 17 (2/2)



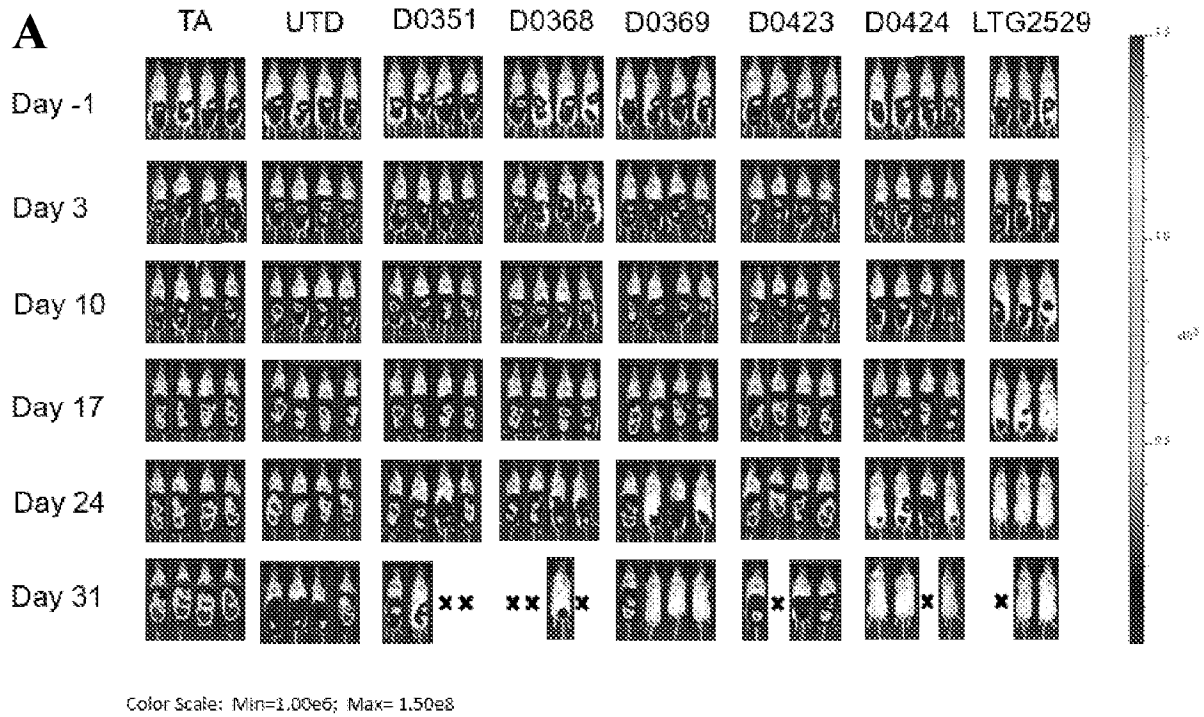
FIGURES 18A-18C (1/2)



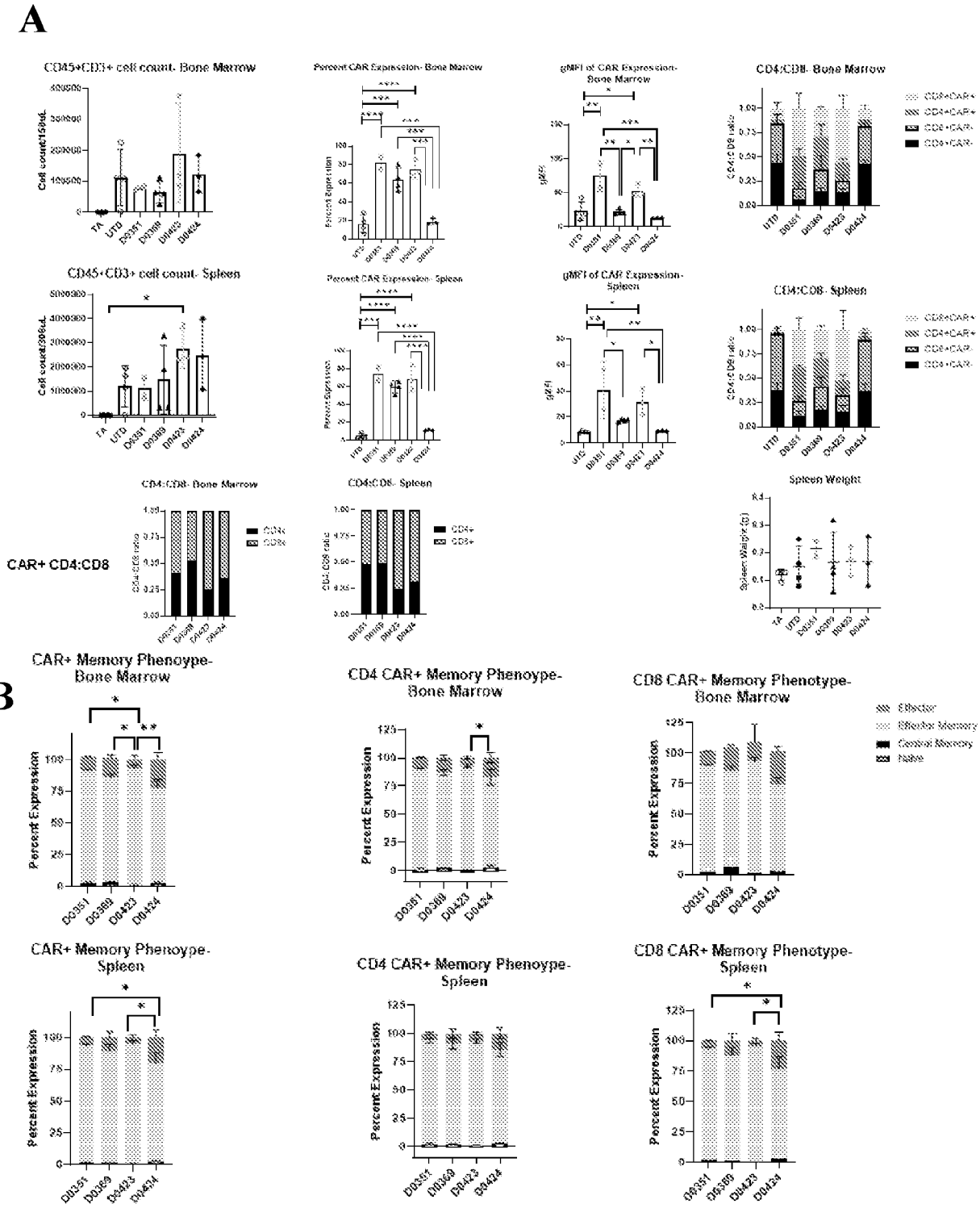
FIGURES 18D-18E (2/2)



FIGURES 19A-19D

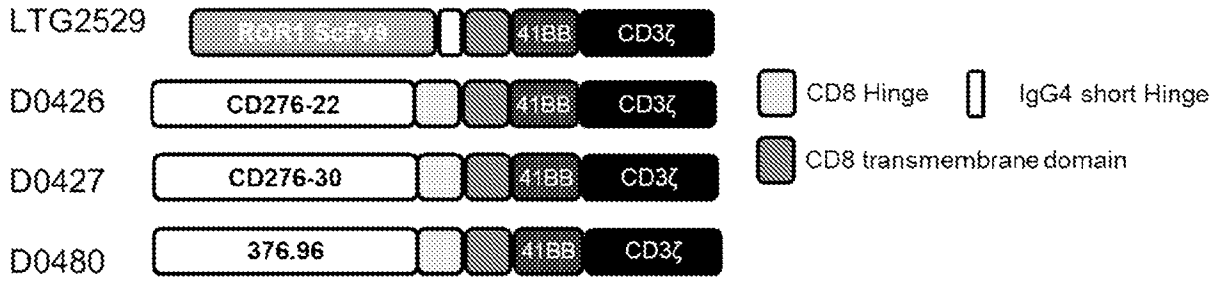


FIGURES 20A-20C

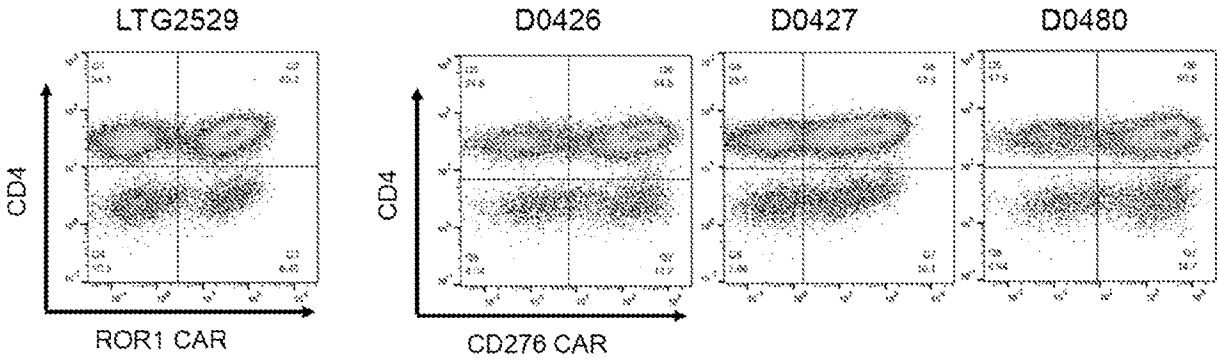


FIGURES 21A-21B

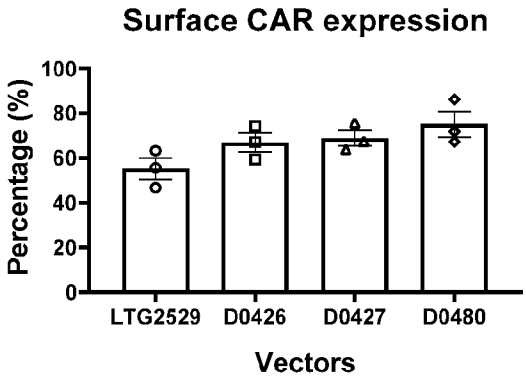
A



B

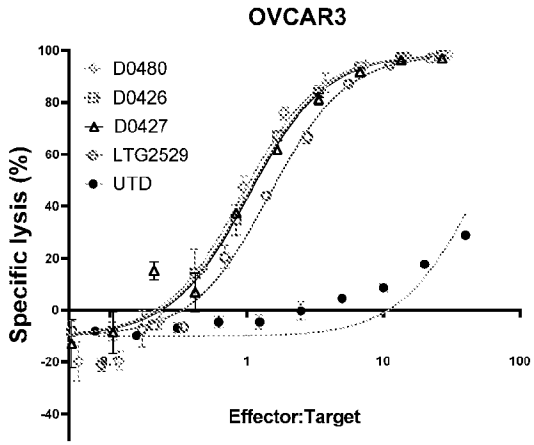


C

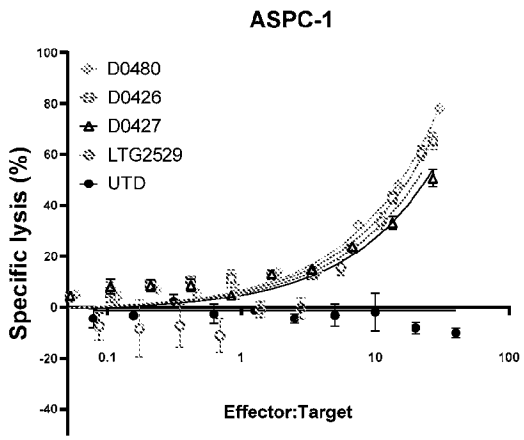


FIGURES 22A-22C

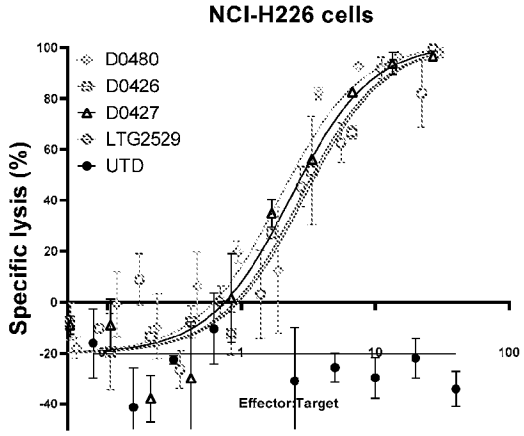
A



B

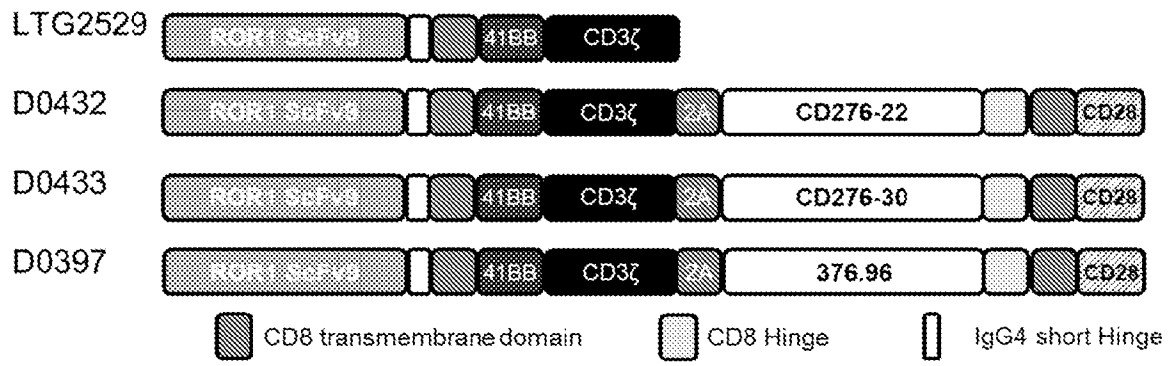


C

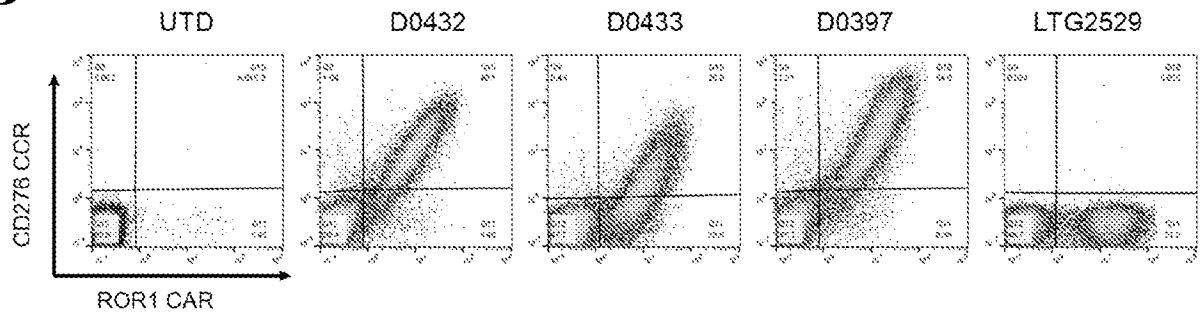


FIGURES 23A-23C

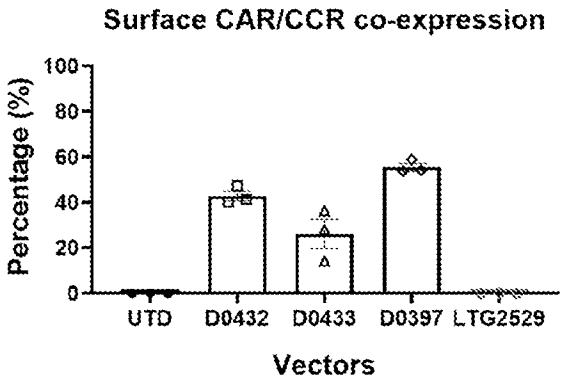
A



B

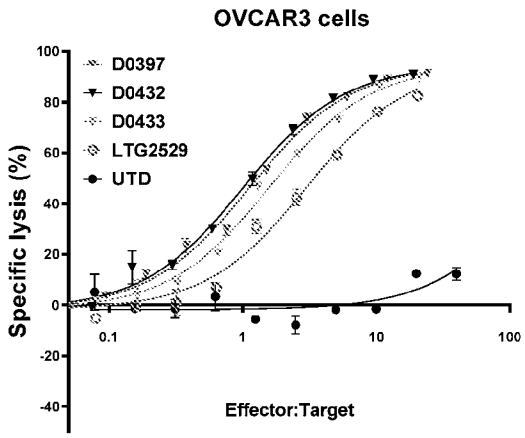


C

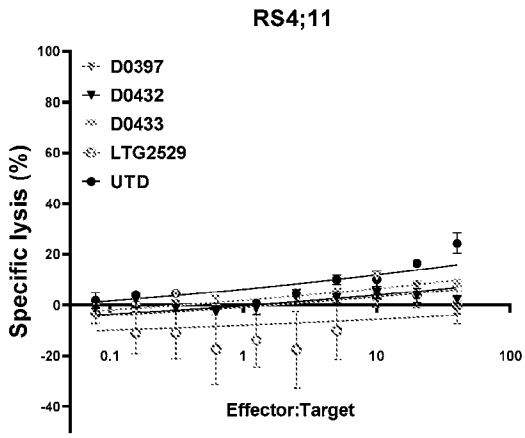


FIGURES 24A-24C

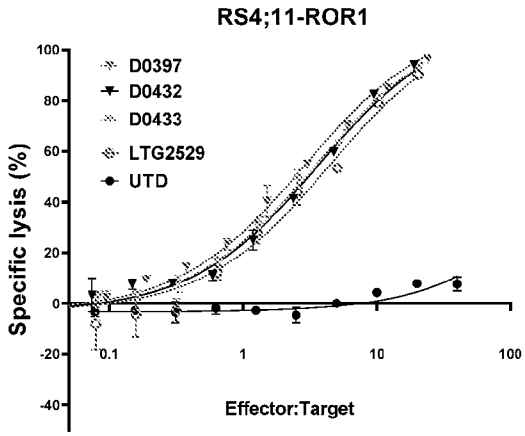
A



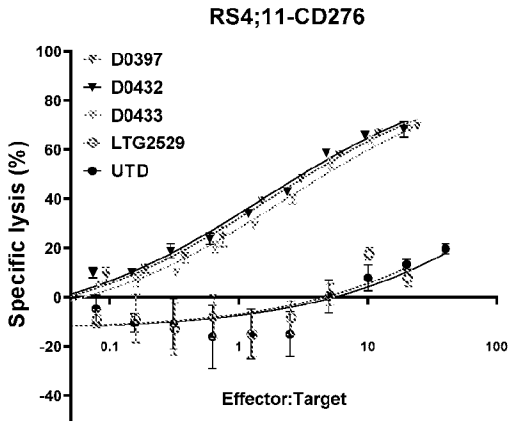
B



C

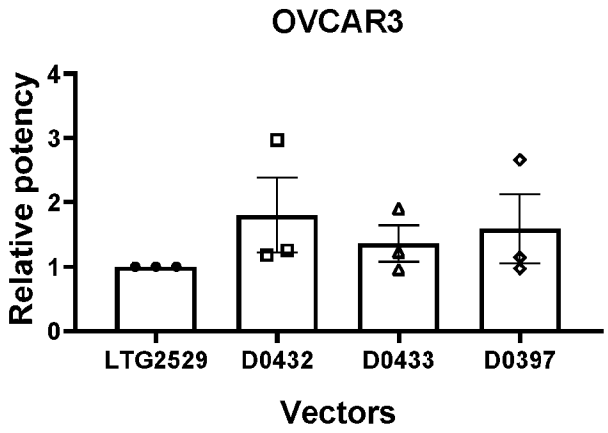


D

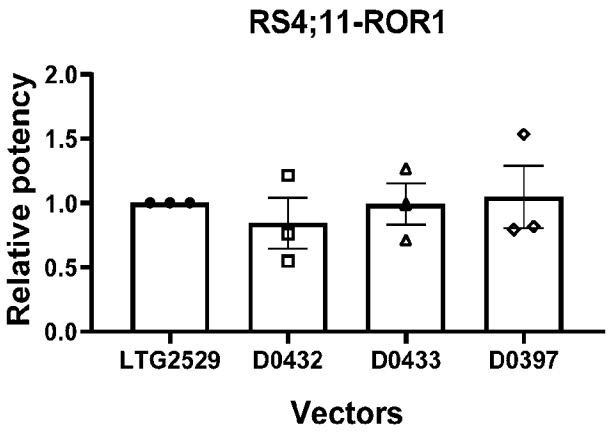


FIGURES 25A-25D

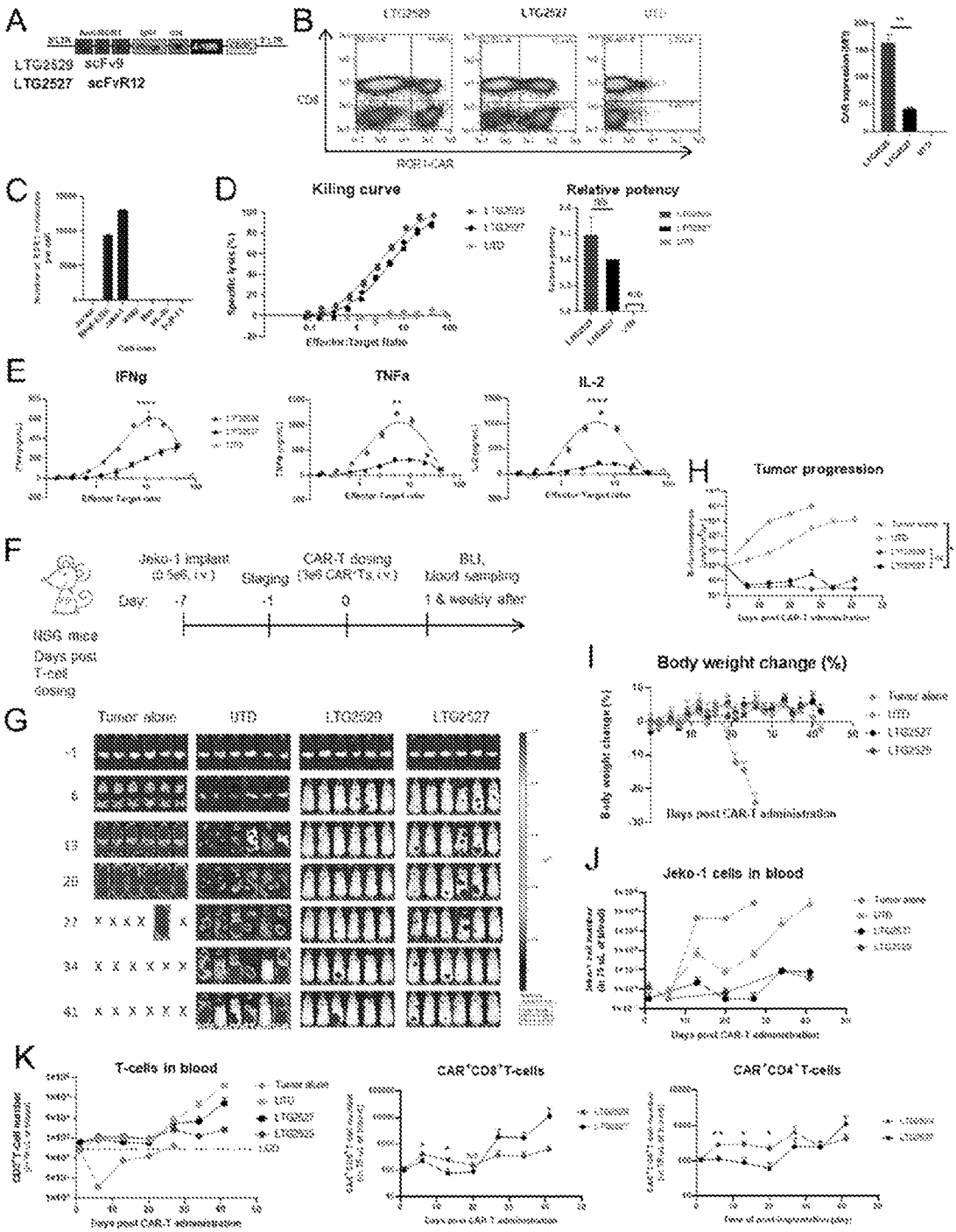
A



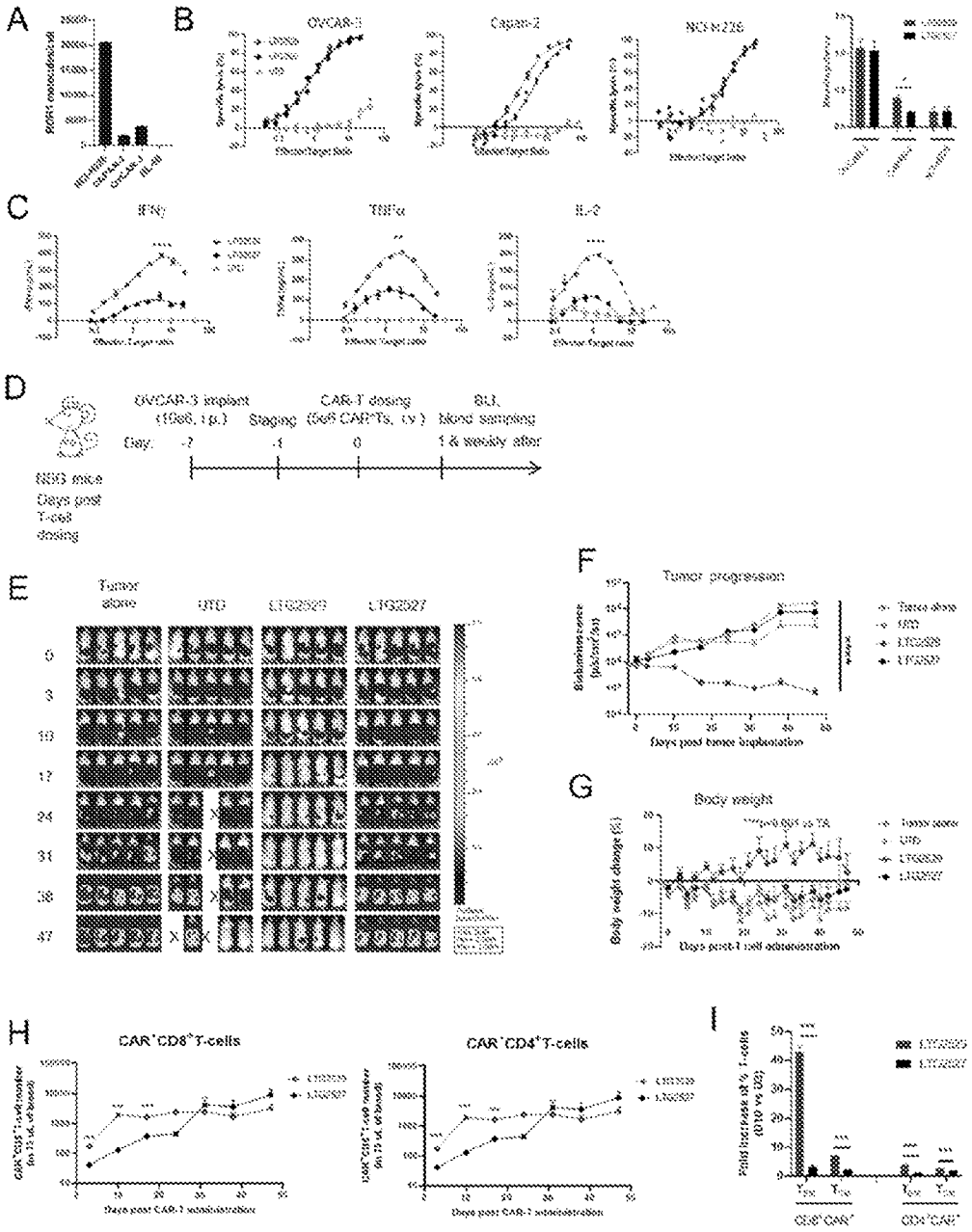
B



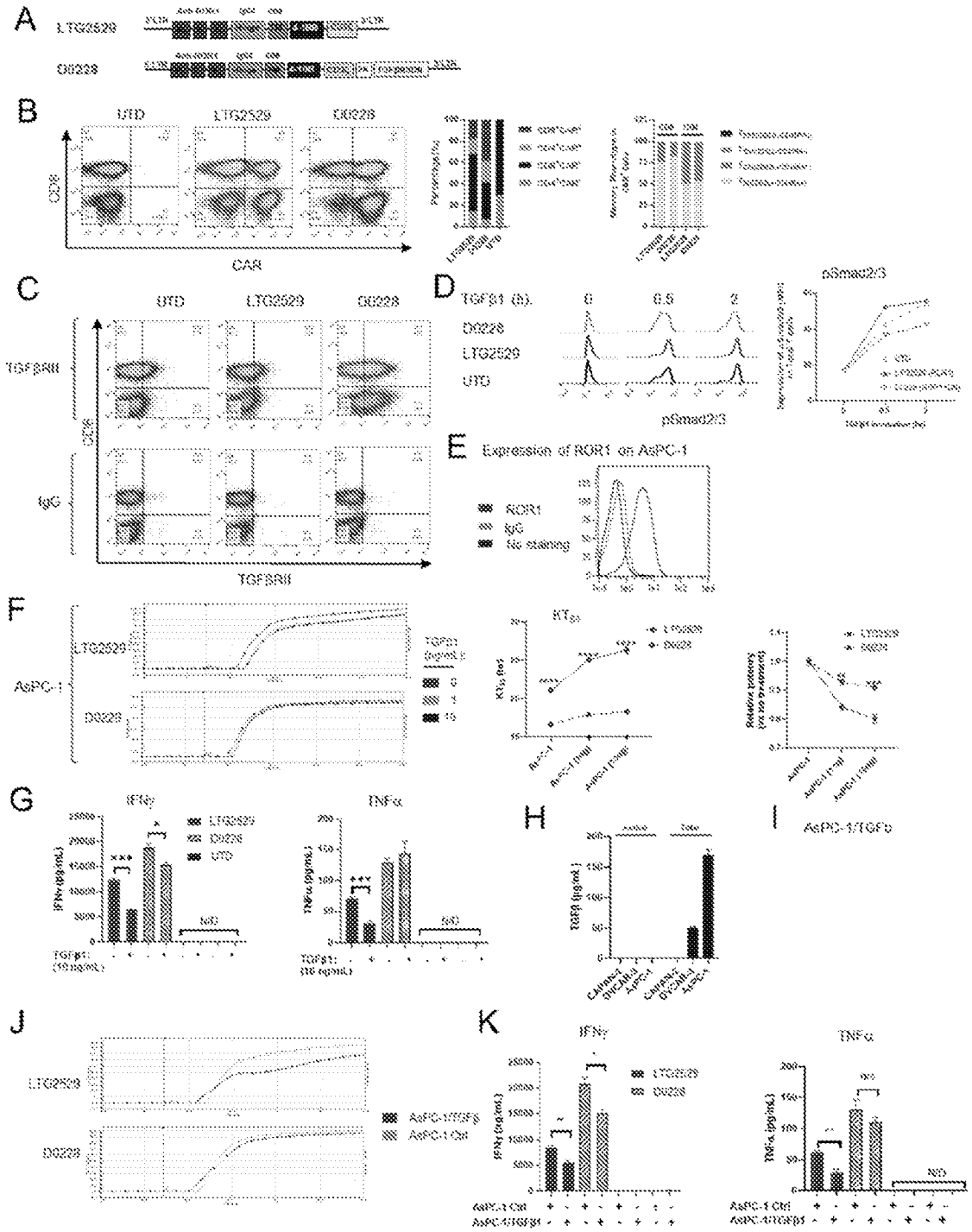
FIGURES 26A-26B



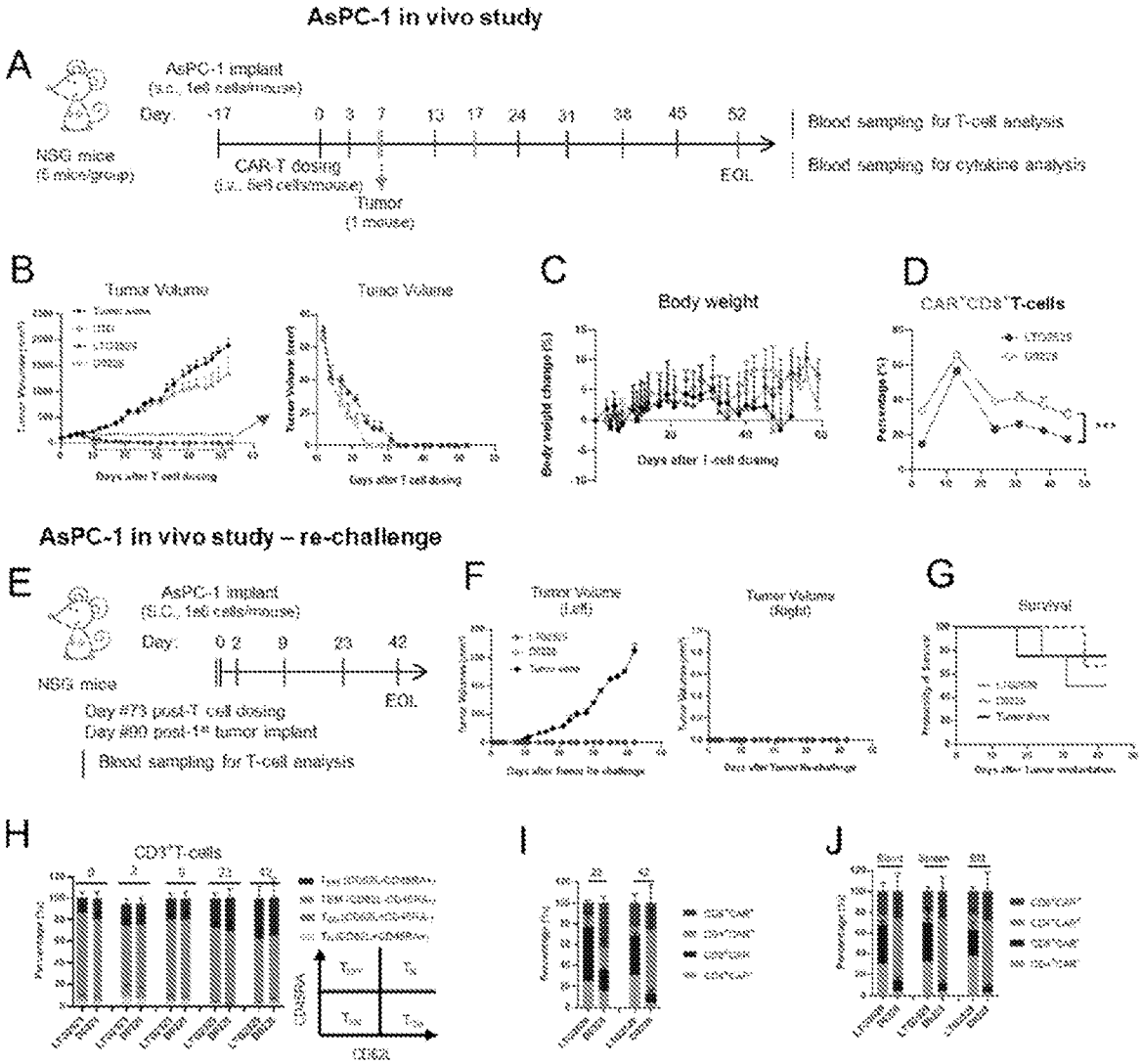
FIGURES 27A-27K



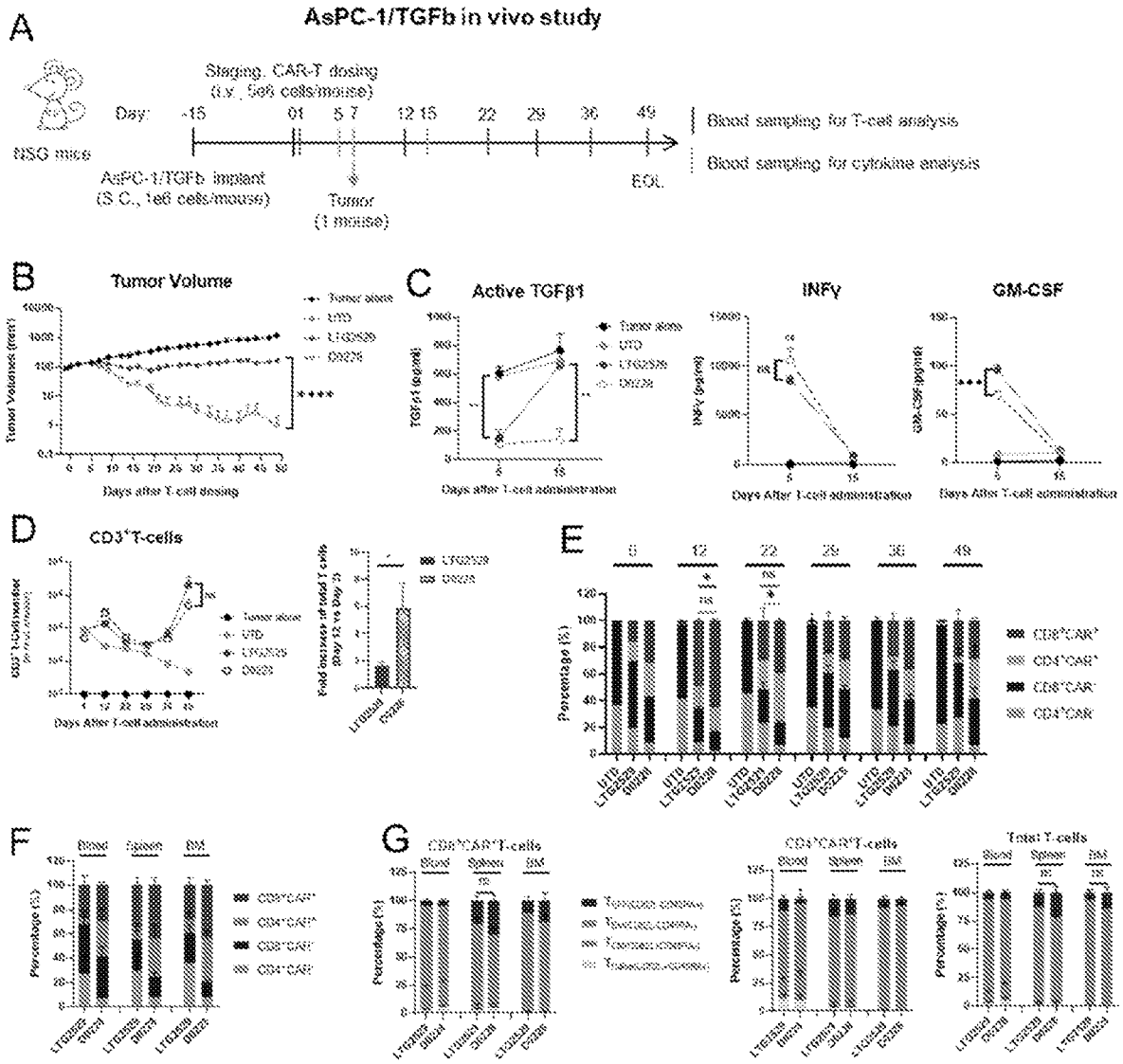
FIGURES 28A-28I



FIGURES 29A-29K



FIGURES 30A-30J



FIGURES 31A-31G