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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 March 2023 (09.03.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/034408 A1

(51) International Patent Classification:
C12N 9/78 (2006.01) *C12N 5/0783* (2010.01)
A61K 35/17 (2015.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2022/042193

(22) International Filing Date:
31 August 2022 (31.08.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/238,927 31 August 2021 (31.08.2021) US
63/355,396 24 June 2022 (24.06.2022) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY PLATFORM

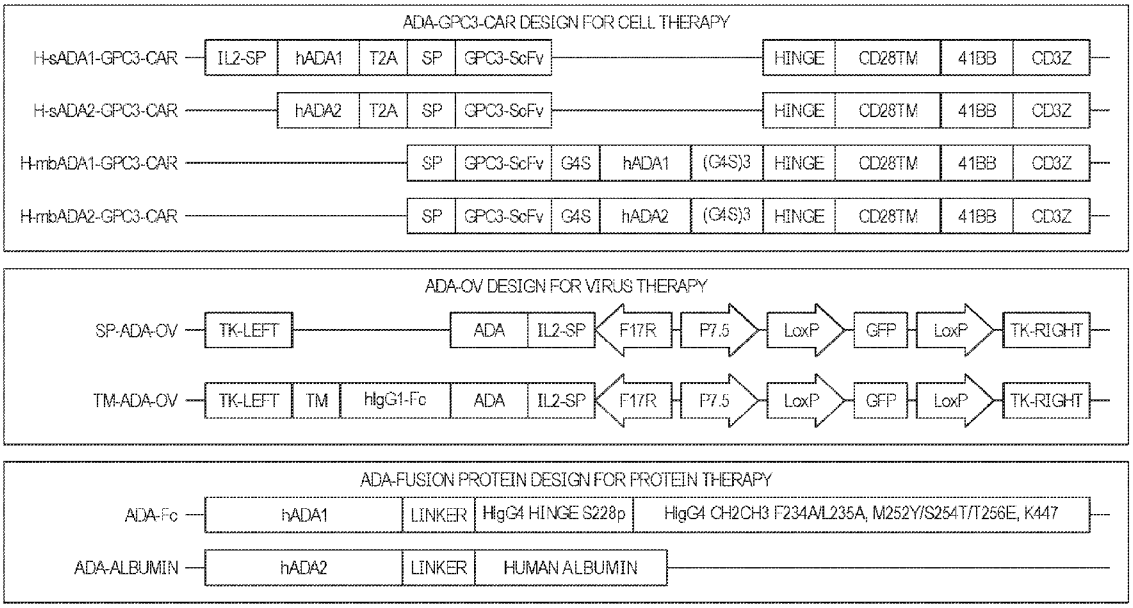


FIG. 1

(57) Abstract: The present application provides methods and compositions for treating cancers using a CAR T cell therapy platform. Also provided are methods and use of the CAR T cells for treating diseases and conditions, such as cancer, and in particular any disease or condition associated with elevated adenosine or other associate marker.

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EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY PLATFORM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from, and incorporates by reference the entire disclosures of, U.S. Provisional Patent Application No. 63/238,927 filed on August 31, 2021 and U.S. Provisional Patent Application No. 63/355,396 filed on June 24, 2022.

TECHNICAL FIELD

[0002] CAR T cell therapy has represented an exciting breakthrough in the treatment of patients with hematologic malignancies leading to the FDA approval of CAR T cell therapies for Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), lymphoma, and Multiple Myeloma (MM). However, the antitumor activity of CAR T cell therapy in solid tumors has been modest so far in clinical studies. The suboptimal efficacy of current CAR-T cell therapies for solid tumors is most likely due to i) immunosuppressive solid tumor microenvironment, ii) suboptimal T cell persistence in vivo post cell injection, and iii) suboptimal CAR-T cell trafficking *in vivo*. Thus, it is desirable to develop novel CAR-T cell therapy technology platforms that display enhanced cytotoxicity against tumors, specifically solid tumors.

BACKGROUND

[0003] A key but largely overlooked problem of CAR T cell therapy for tumors, specifically solid tumors, is nutrient competition between tumor cells and T cells in the nutrient-poor tumor microenvironment (TME). The TME represents a dramatic example of metabolic stress, wherein the high metabolic demands of cancer cells can restrict the function of CAR T cells through competition for nutrients (i.e., glucose) and by producing immunosuppressive metabolites (e.g., adenosine). Therefore, in order for CAR T cell therapy to be effective, a reliable source of nutrition is needed.

[0004] Inosine is a common component of food, and studies demonstrate that inosine has neuroprotective, cardioprotective and immunomodulatory effects. Although inosine has a weak binding affinity with adenosine receptor, earlier studies have shown that inosine

produces anti-inflammatory effects related to the activation of adenosine receptors, mainly the A2a and A3 receptor whose activation can contribute to the reduction of pro-inflammatory cytokines, and tissue protective effects from endotoxin-induced and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced inflammation. However, a small body of literature has showed opposing findings that inosine analogs can be proinflammatory and that A2AR signaling can sustain Th1 and anti-tumor immunity in mice. A recent study has showed that inosine is a key bacterial-derived metabolite acting through T cell-specific A2AR signaling to promote Th1 cell activation in a context-dependent manner. Specifically, in the presence of IFN-gamma, inosine strongly boosted Th1 differentiation of native T cells, whereas in the absence of IFN-gamma, inosine inhibited Th1 differentiation. Since adoptive T cell transfer and immune checkpoint inhibitors have shown the capability of converting a suppressive TME to a supportive TME, it is reasonable that inosine can amplify the antitumor efficacy of T-cell therapy or immune checkpoint inhibitors. In addition, an independent study has shown that inosine can strongly sensitize tumor cells to the cytolytic effect of immune cells by enhancing tumor immunogenicity.

[0005] Adenosine deaminase (ADA) is known to have anti-tumor activity. Several groups have developed ADA as cancer therapy. For example, a PEG-ADA2 (Adenosine deaminase 2) has been developed and has shown antitumor activities in mouse models. ADA's half-life is only several hours long, and PEG conjugation does not significantly increase the protein's half-life. Therefore, in order for ADA to serve as an effective cancer therapy, the half-life must be improved.

[0006] ADA decreases the level of adenosine, an immunosuppressive metabolite, by irreversibly converting it to inosine. ADA is a key enzyme of purine metabolism required for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. ADA1 and ADA2 are two ADA isoenzymes in humans. ADA1 is primarily involved in intracellular activity in most body cells, particularly lymphocytes. ADA1 deficiency in humans causes severe combined immune deficiency (SCID). ADA2 is the predominant form present in human blood plasma. ADA1 and ADA2 might play different roles in regulating immune responses independent of their ADA activity. Studies have shown that ADA1 has co-stimulatory effects on T cell-mediated immunity by binding to Teffs and CTLs that express CD26. In contrast, ADA2 binds to neutrophils, CD16+ monocytes, NK cells, B cells and

CD39+ Treg that do not express CD26. Recent studies have shown that in mice, PEGylated ADA2 inhibited tumor growth by targeting adenosine in an enzyme activity dependent manner and modulating immune responses. Additionally, ADA1 has a 100-fold higher affinity for adenosine than ADA2.

[0007] Adenosine signaling has emerged as a key immuno-metabolic checkpoint in tumors. ATP or NAD+ accumulate within the TME and can generate adenosine by ecto-nucelotidases including CD39, CD73, CD38, CD203a, ALP and PAP53-61. Studies have shown that adenosine enables tumor cells to escape immune-surveillance by suppressing the function of multiple potentially protective immune cells including T cells, DCs, NK cells, macrophages and neutrophils, while enhancing the activity of immunosuppressive cells such as MDSCs and Tregs. In addition, adenosine activates cancer associated fibroblasts and induces the formation of new blood vessels.

[0008] Several agents such as small molecules or mAbs targeting CD73 and CD38 to limit its production or targeting A2AR and A2BR to limit its binding to immune cells have been developed, and pre-clinical studies have demonstrated their anti-tumor activity, alone and in combination with other immunotherapies including immune checkpoint inhibitors and adoptive cell transfer. However, early phase clinical studies show that their antitumor effects are suboptimal. This can be explained by the fact that multiple ectonucleotidases contribute to extracellular adenosine production and adenosine binds to multiple receptors such as A2AR, A2BR and A3R to suppress antitumor immunity.

[0009] Although cure rates for several malignancies have significantly improved, the outcome for patients with advanced solid tumors remains grimly unchanged over the last decades, underscoring the need for new therapies. Oncolytic vaccinia viruses are an appealing addition to the current treatment options of solid tumors because of their safety and their potential to infect, replicate in, and lyse tumor cells. Although clinical studies have shown that intratumoral or intravenous injection of oncolytic vaccinia viruses was safe and can induce tumor lysis, the antitumor efficacy of the oncolytic vaccinia viruses is suboptimal and most tumors occurred. This is most likely due to the limited activation of antitumor T-cell responses within the tumor of oncolytic vaccinia virus and the immunosuppressive tumor environment. Therefore, an oncolytic vaccinia virus that 1) reverses immunosuppressive

tumor microenvironment, and 2) effectively supports T-cell function, will overcome the current limitations of oncolytic vaccinia viruses.

[00010] There is increasing evidence that T cells are able to control tumor growth and survival in cancer patients, both in early and late stages of the disease. For example, adoptive transfer of T cells has been demonstrated to effectively treat disseminated tumors including Hodgkin's Lymphoma, nasopharyngeal carcinoma, neuroblastoma and melanoma. However, tumor-specific T-cell responses are difficult to mount and sustain in cancer patients and are limited by numerous immune escape mechanisms of tumor cells selected during immunoediting. Therefore, it would be desirable to develop an alternative strategy to utilize T cells for cancer therapy with the ability of overcoming the immune escape mechanism of tumors.

[00011] The present application addresses the disadvantages that exist in current cancer therapies. Methods and compositions for treating cancers using an engineered immune cells (e.g., CAR-T cells, CAR-NK cells, CAR-NKT cells, macrophage) or a recombinant oncolytic virus encoding a adenosine deaminase are provided. The engineered immune cells or recombinant oncolytic virus encodes one or more other heterologous proteins. The present application also provides methods and uses of the engineered immune cells or oncolytic virus for treating diseases and conditions, such as cancer, and in particular any disease or condition associated with elevated adenosine or other associate marker.

SUMMARY OF THE INVENTION

[00012] An embodiment of the claimed invention is directed to an engineered cell comprising a nucleotide sequence encoding an Adenosine deaminase (ADA). In certain embodiments, the cell is T cell, natural killer cell, natural killer T cell, dendritic cell, macrophage, mesenchymal stem cell, and derivatives thereof.

[00013] An embodiment of the claimed invention is directed to a pharmaceutical composition comprising an engineered cell.

[00014] A further embodiment of the claimed invention is directed to a method of administering a pharmaceutical composition comprising an engineered cell to a patient in an amount effective for treatment of a cancer.

[00015] Another embodiment of the invention is directed to a recombinant oncolytic virus comprising a nucleotide sequence encoding an ADA, wherein the nucleotide sequence encoding the ADA is operably linked to a promoter.

[00016] An embodiment of the invention is directed to a recombinant protein comprising an amino acid sequence encoding an ADA.

BRIEF DESCRIPTION OF THE DRAWINGS

[00017] FIG. 1 shows the designs of conjugated ADA used in embodiments of the invention;

[00018] FIG. 2 shows a rationale for an CD26/ADA-CD3-scFv expressed CAR T cell therapy. CAR T cells are engineered to express a tumor-specific CAR, a membrane CD26, and a secreted bispecific ADA-CD3-scFv. These CAR T cells 1) target and kill specific tumor cells; 2) stimulate CAR T cells by ADA-CD3-scFv (CD3-scFv anchors ADA to T cells since the binding affinity of CD3 and CD3-scFv is higher than that of ADA-CD26, while CD3-scFv itself can activate T cells), inducing CAR T cell activation and migration, 3) release CAR T cells from adenosine-mediated immune suppression; and 4) provide inosine as alternative carbon source for CAR T cells, and enhance the anti-tumor activity of CAR T cell therapy;

[00019] FIG. 3 shows the construction of an HER2-targeted CD26/ADA-CD3-scFv expressed CAR retroviral vector and an CD26/ADA-CD3-scFv expressed retroviral vector;

[00020] FIG. 4 shows that Rv-CD26 transduced CAR T cells can resist the suppression of TGF-beta on CD26 expression, while CD26 expression of non-Rv-CD26-transduced CAR T cells were suppressed by TGF-beta;

[00021] FIGS. 5A and 5B show that Rv-CD26 transduced CAR T cells displayed enhanced migration capacity in a fluorescent migration assay (FIG. 5A) and a transwell migration assay (FIG. 5B);

[00022] FIG. 6 shows the mechanism of action of the ADA-CD3-scFv on the metabolic reprogrammed CAR-T cells: (A) CD3-scFv anchors ADA to T cells since CD3 and

CD3-scFv has higher binding affinity than that of CD26-ADA, while CD3-scFv can directly activate T cells through CD3; (B) ADA engages CD26, co-stimulating CAR T cells; and (C) ADA converts ADO to INO, overcoming ADO-mediated immunosuppression on CAR T cells while providing INO as alternative energy resources for CAR T cell survival and proliferation;

[00023] FIGS. 7A and 7B show the expression of ADA-CD3 (ADA-CD3-scFv) by the retroviral vector transduced HEK 293 T or Jurkat T cells: (FIG. 7A) western blotting of ADA-CD3-scFv using monoclonal Ab against ADA in HEK 293 T cells that were transduced with retroviral vector expressing ADA-CD3-scFv with or without a human IL2 signal peptide compared to non-transduced HEK 293 T cells, and (FIG. 7B) western blotting of ADA-CD3-scFv using monoclonal Ab against ADA in Jurkat T cells that were transduced with retroviral vector expressing ADA-CD3-scFv with a human IL2 signal peptide or a lentiviral vector expressing CRISPR ADA activation elements, compared to non-transduced Jurkat T cells;

[00024] FIGS. 8A, 8B and 8C show the expression of ADA-CD3-scFv (ADA-CD3): (FIG. 8A) ELISA measurement of ADA in the supernatant or cell lysate of Rv-ADA-CD3-IL2sp (signal peptide)-transduced, Rv-ADA-CD3 (no IL2 sp)-transduced or non-transduced HEK 293 T cells; (FIG. 8B) ELISA measurement of ADA in the supernatant of co-culture of Jurkat T cells with Rv-ADA-CD3-IL2sp (signal peptide)-transduced, Rv-ADA-CD3 (no IL2 sp)-transduced or non-transduced HEK 293 T cells at day 1 and day 4; and (FIG. 8C) flow analysis of ADA-CD3 on Jurkat T cells or Rv-CD26-transduced Jurkat T cells post co-culture with Rv-ADA-CD3-IL2sp (signal peptide)-transduced, Rv-ADA-CD3 (no IL2 sp)-transduced or non-transduced HEK 293 T cells;

[00025] FIGS. 9A, 9B and 9C show the enzyme activity of ADA (ADA1) by ADA enzyme activity assay: (FIG. 9A) ADA enzyme activity assay of co-culture of Jurkat T cells and Rv-ADA-CD3 (with IL2sp)-transduced or non-transduced HEK 293 T cells (Top: original reads; Bottom: non-transduced HEK 293 T cell background reads were subtracted); (FIG. 9B) ADA binding assay of Jurkat-Dual or Jurkat-Dual-CD26 T cell lines; and (FIG. 9C) ADA binding assay of Jurkat-NEFA or Jurkat-NFAT-CD26 T cells lines;

[00026] FIGS. 10A, 10B, 10C and 10D show T-cell activation using Jurkat NFAT reporter T cell line: (FIG. 10A) Jurkat NFAT reporter T cells were added with the supernatant of or the Rv-ADA or Rv-ADA-CD3 transduced HEK 293 T cell for 6 hours and subjected to luciferase assay; (FIG. 10B) Jurkat NFAT reporter T cells were added with the supernatant of or the Rv-ADA or Rv-ADA-CD3 transduced HEK 293 T cell for 24 hours and subjected to luciferase assay; (FIG. 10C) Jurkat NFAT reporter T cells were added with the supernatant of or the Rv-ADA-CD3 or Rv-ADA-CD3 (IL2sp) transduced HEK 293 T cell for 6 hours or 24 hours and subjected to luciferase assay; and (FIG. 10D) ADA-CD3 (IL2sp) expression level were calculated;

[00027] FIGS. 11A, 11B, 11C, 11D and 11E show the antitumor activity of ADA-CAR-T cells using in vitro LDH assay: (FIG. 11A) GPC3 CAR expression level of different groups of cells by flow analysis; (FIG. 11B, FIG. 11C, FIG. 11D and FIG. 11E) LDH assay of groups of CAR T cells against GPC-positive HepG2 or Huh7 liver tumor cell lines; and

[00028] FIGS. 12A, 12B, 12C and 12D show the results of an antitumor efficacy and toxicity study carried out in mice.

DETAILED DESCRIPTION

[00029] An embodiment of the invention is directed to an engineered CAR T cell that expresses a tumor-specific CAR and expresses ADA (FIG. 1 & 3). The engineered CAR T cell 1) targets and kills tumor-antigen specific tumor cells; 2) CAR T cells are co-stimulated by ADA through CD26 signaling, 3) CAR T cells are released from adenosine-mediated immune suppression; and 4) inosine is provided as an alternative carbon source for CAR T cells, enhancing the anti- tumor activity of CAR T cell therapy.

[00030] Studies have shown that extracellular adenosine in the TME has a marked suppressive effect on immune responses, suppressing T cell function and stabilizing immunosuppressive regulatory cells. Thus, ADA that irreversibly converts adenosine to inosine release CAR T cells from adenosine-driven immunosuppression, while providing an energy source for CAR T cells. In addition, ADA mediates effective co-stimulatory signals and promote T- cell proliferation independent of its enzyme activity. FIG. 1 sets forth examples of ADA-CAR T cell conjugates.

[00031] A schematic of an ADA-CD3-scFv/CD26 overexpressed metabolic reprogrammed (MR) CAR-T cell therapy platform is shown in FIG. 2. ADA-CD3-scFv/CD26-MRCAR T cells that express a tumor Ag-specific CAR and ADA-CD3-scFv/CD26 complex enhance the anti-tumor activity of CAR T cell therapy through a series of mechanisms: i) CAR molecule targets and direct T cells to kill tumor Ag-positive tumor cells; ii) ADA/CD26 complex releases the adenosine-mediated immune suppression by providing inosine as carbon source for CAR T cells by converting adenosine to inosine in the TME; and iii) CD26 mediates CAR T cell trafficking and remain bound to T cell surface via ADA. Such an ADA-CD3-scFv/CD26-MRCAR T cell has the potential to overcome current limitations on CAR-T cell therapy against solid tumors.

[00032] The ADA-CD3-scFv/CD26-MRCAR in FIG. 3 contains the following components: a chimeric antigen receptor containing a tumor antigen (e.g., HER2, GPC3) specific scFv (small chain variable fragment), a spacer, hinge, transmembrane domain (e.g., CD28 and/or 4-1BBL), CD3 zeta domain; ADA-CD3-scFv bispecific fusion protein, and CD26. These components were linked with the 2A sequence, or expressed in separate viruses (e.g., retroviral vector, lentiviral vector). Ecto-ADA is critical for protecting T cells from adenosine-mediated immune suppression, while cell free ADA is not. Thus, it is desirable to develop an effective strategy to anchor ADA on the surface of T cells. Rv-ADA T cells can express ~ng ADA/ml per 24 hours and the ADA expression by Rv-ADA T cells only increases by 20% compared to that of non-modified T cells. However, ADA concentration in human breast tumor tissue, normal tissue, or serum is 16.4-47.7ug/g, 11.5ug/g, or 160-242ng/ml respectively. Thus, ADA-armed T cell therapy is significantly limited by the ADA expression level of T cells. Anchoring ADA to the cell surface overcomes this limitation by engagement of CD26 on the surface of T cells.

[00033] In certain embodiments, the CAR comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID Nos: 1, 3, 5, 7, 9, 18, 20, 22, and 24. 10, 12, 14, and 16 2, 4, 6, 8, 9, 19, 21, 23, and 25 11, 13, 15, and 17

[00034] As shown in FIG. 2, CD3-scFv engages with CD3 on the infused CAR-T cells or endogenous T cells by anchoring ADA on T cell surface and promoting

ADA-CD26 engagement and activating T cells through CD3 signaling. When ADA engages with CD26, it activates the infused CAR-T cells or endogenous T cells. ADA converts adenosine to inosine, overcoming adenosine-mediated immune suppression on CAR-T cells and providing inosine as alternative energy resources for CAR-T cells.

[00035] FIG. 3 also shows a vector that contains ADA-CD3-scFv bispecific fusion protein and CD26 only. This vector can be used together with a vector that expresses a CAR against any type of tumor antigens to transduce human T cells to produce MRCAR T cells.

[00036] A retroviral vector system is used to express a HER2-specific CAR, a membrane CD26 and a secreted ADA-CD3-scFv (FIG. 3). A retroviral vector system was also used to express a membrane CD26 and a secreted ADA-CD3-scFv only (FIG. 3). T cell codon optimization was used to enhance transgene expression of ADA in human T cells. An optimized human IL2 signal peptide was added to express ADA by human T cells. Human peripheral monocytes were activated by plate-bound CD3 and CD28 antibodies for 2 days followed by virus transduction. Expression and secretion of ADA1 by T cells was confirmed by western blotting (FIG.7) and ELISA (FIG. 8). ADA expression effectively converted adenosine to inosine (FIG. 9). As shown in FIG.10, ADA-CD3 expression effectively activated human T cells (FIG.10). The in vitro LDH cytotoxicity assays showed that GPC3-specifici MR-CAR displayed enhanced cytotoxicity against GPC3-positive liver cancer cell lines such as HepG2 and Huh7 (FIG.11).

[00037] Overexpression of CD26 in CAR T cells is critical for CAR T cell therapy. As shown in FIG.4, in the presence of TGF-beta, the expression of CD26 on CAR T cells are reduced in 48 hours, suggesting the CD26 expression can be suppressed by tumor microenvironment. Rv-CD26 can restore the CD26 expression in CAR T cells. As shown in FIG.4, Rv-CD26 transduced T cells expressed CD26 at a higher level compared to non-modified T cells. In addition, Rv-CD26 transduced T cells resisted TGF-beta-mediated immunosuppression and retained the CD26 expression at high level. In addition, Rv-CD26 transduced T cells had enhanced migration capacity, as evidenced by in vitro florescent migration assay and transwell migration assay (FIG. 5).

[00038] A further embodiment of the invention is directed to an ADA-CAR T cell therapy in transplantable mouse tumor models. The results show the efficacy and antitumor properties of a CAR T cell therapy model. MR-CAR T cells displayed enhanced anti-tumor effects in two mouse models. In the first model, NSG mice were inoculated with $2x10^{e6}$ Huh7 HCC tumor cells on the right flank at day 0. At day 7, the Huh7 tumor bearing mice were treated through tail vein with PBS, $2x10^{e6}$ GPC3-CAR T cells, or $2x10^{e6}$ GPC3-ADA1-CD3-CD26-CAR (designated as GPC3-MR-CAR), followed by tumor monitoring with caliper (FIG. 12A) and body weight measurement (FIG. 12B). In the second model, NSG mice were inoculated with $2x10^{e6}$ A549 NSCLC tumor cells on the right flank at day 0. At day 7, the A549 tumor bearing mice were treated through tail vein with PBS, $2x10^{e6}$ HER2-CAR T cells, or $2x10^{e6}$ HER2-ADA1-CD3-CD26-CAR (designated as HER2-MR-CAR), followed by tumor monitoring with caliper (FIG. 12C) and body weight measurement (FIG. 12D). In both models, MR-CAR T cells showed enhanced anti-tumor activity. MR-CAR T cells significantly inhibit tumor growth in both Huh7 HCC and A549 NSCLC mouse models while native CAR T cells only moderately inhibit Huh7 or A549 tumor growth. In addition, no significant difference in body weight was observed in either Huh7 HCC or A549 NSCLC mouse model, indicating MR-CAR T cells did not induce toxicity in the mouse models. The promising results in the mouse models provide a good predictor of efficacy of the CAR T cell platform in human subjects.

[00039] Another embodiment of the invention is directed to an optimized ADA that displays enhanced ADA activity compared to native ADA. In certain embodiments, the optimized ADA is an ADA-Fc fusion protein that significantly prolongs ADA's half-life, and improves its antitumor activity (FIG.1). In other embodiments, the optimized ADA is an antibody conjugated ADA (FIG.1).

[00040] Another embodiment of the invention is directed to an engineered oncolytic virus that expresses ADA or ADA derivatives such as ADA-Fc (FIG.1). The engineered oncolytic virus that expresses ADA will infect and replicate in the tumor cells, and expresses ADA or ADA-Fc that can convert ADO to INO within tumor tissues, enhancing the antitumor efficacy of oncolytic virus.

[00041] In certain embodiments, the engineered oncolytic virus comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or

at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 5, 7, 9, 22, and 24.

WORKING EXAMPLES

[00042] CAR T cell generation

Material:

OKT3 (ortho Biotech)- 1mg/ml

CD28 (no azide (NA)/low endotoxin (NE) (Pharmingen #33740D) - 1mg/ml

Plate: Non-tissue culture treated 24-well plate (Falcon #1147)

Protocol:

Day 0:

1. Dilute antibodies to a final concentration of 1 ug/ml in sterile water (for example to coat 6 wells, add 3 uL of OKT3 and 3 uL of CD28 to 3 ml of sterile water)
2. Add 0,5 ml (= 0.5 ug of antibody) of the antibody solution/well in the 24 well plate; seal it with parafilm
3. Incubate at +4°C overnight

Day 1:

1. Resuspend 5 x 10^6 PBMC in 10 ml of complete RPMI (10% FCS)
2. Aspirate antibody solution and wash wells once with RPMI or DMEM complete
3. Aspirate media and plate 1x10^6 PBMC/ well in a final volume of 2 ml of complete media

Day 2:

1. Aspirate and discard about 1ml from each well and add back 1 ml of complete RPMI medium containing 200U/ml of IL-2
2. Coat a non-tissue culture treated 24-well plate with retronectin; seal it with parafilm
3. Place the retronectin coated plate at +4 ° C overnight.

Day 3:

1. Remove retronectin solution and wash wells once with RPMI or DMEM complete (please store retronectin at +4C in 15 cc tube; can be reused once within one month)

2. Add 500 - 700 ul of viral supernatant to well. Place plate into incubator for 30 min

3. Remove viral supernatant; repeat step 2

4. During incubation time prepare blasts at $5x10^5$ cells/cc with 400 U/cc IL-2

5. Remove viral supernatant. Add 1.5 cc of viral supernatant.

6. Add 500 ul of blasts ($2.5x10^5$ cells)

7. spin the plate at 1000 g for 30 minutes then put the plate back in the incubator (optional)

Day 5/6:

1. Remove 1 cc of media from wells; transfer cells of retronectin-coated plate into new well (by scraping); add 1 cc of complete media containing 100U/ml of IL-2

2. Feed with IL-2 (100U/ml) every 2-3 days. Freeze back or use in experiments with in 7 to 21 days.

MR-CAR resisted TGF-beta mediated suppression on CD26 expression on CAR T cells and displayed greater migration capacity.

[00043] Human PBMC was activated by CD3/CD28 antibodies followed by transduced with Rv-CD26 as described as above. The Rv-CD26 transduced T cells (Rv-CD26) or non-transduced T cells (NT) were cultured in the absence or presence of 5ng/ml, 10ng/ml or 20ng/ml of TGF-beta for 48 hours. The T cells were collected and stained with FITC-conjugated human CD26 specific antibody. The CD26 expression on T cells was then detected by flow analysis and showed as MFI (median fluorescence intensity). Rv-CD26 transduced human T cells expressed CD26 at a higher level compared to non-transduced T cells (FIG. 4, Rv-CD26 vs NT = MFI 1420 vs MFI 801) in the absence of TGF-beta. When NT T cells were cultured in the presence of TGF-beta (5ng/ml, 10ng/ml, or 20ng/ml) for 48 hours, CD26 expressions were decreased from MFI 801 to MFI 603, MFI 599, or MFI 626 individually, while CD26 expression on Rv-CD26-transduced T cells remain unchanged after 48 hours culture in the presence of TGF-beta (5ng/ml, 10ng/ml, or 20ng/ml). These results suggested that Rv-CD26 transduced CAR T cells can resist the suppression of TGF-beta on CD26 expression, while CD26 expression of non-Rv-CD26-transduced CAR T cells were suppressed by TGF-beta.

To investigate the migration capacity of the human T cells, in vitro transwell migration assay and fluorescent migration assay were conducted.

A. Transwell Cell Migration Assay Protocol (By counting the cell):

1. Grow enough cells (Rv-CD26-transduced HER2 CAR T cells or control HER2 CAR T cells) to perform a Cell Migration Assay in desired media and culture conditions.

2. Collect the cells and resuspend the cells in serum-free media and count the number of cells using a hemocytometer. Resuspend cells at 1.5 x 10^6 cells/mL in a serum-free media or make the cell as per your desired concentration.

3. Bottom Chamber: Add 235 µL of HER2-positive A549 cell supernatant (Chemoattractant) and 365 µL of serum free media to make it 600 µL to the bottom chamber for both NT (Non-Transduced) and CD26 Transduced HER2-CAR T cells.

4. Place the top chamber (Transwell, 5mm) carefully into the bottom chamber so that no air bubbles are trapped between the top and the bottom chamber.

5. Top Chamber: Add 300 µL (5,00000 cells) of cell suspension (both NT and Rv-CD26 transduced) to each well of the top chamber.

6. Carefully place the plate cover and incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-24 hours.

7. Count the cells using a haemocytometer at different time point (2, 4, 22/24 hrs).

B. Fluorescent Cell Migration Assay Protocol (By fluorescent intensity with Abcam transwell migration assay kit ab235696):

Standard Curve:

1. Each cell type requires a separate Standard Curve. Prepare a Standard Curve by adding 50 µL cell suspension (1.8 x 10^6 cells/mL, 90,000 cells or make it 50000 with 1 x 10^6 cells/ml).
2. Serially dilute the cells 1:1 in Wash Buffer and generate a Standard Curve of cells (90000, 45000, 22500, 11250, 5625, 2812, 1406 and 703) in 100 µL total volume in 96 well white plate.

3. As blank, use 100 µL of Wash Buffer.
4. Add 10 µL of Cell Dye to each well.
5. Incubate at 37°C for 1 hour.
6. Read the fluorescence at Ex/Em = 530/590 nm.
7. Plot the Standard Curve of Number of Cells vs RFU obtained.
8. Fit the data points using a linear trendline.

Count Migrated Cells:

1. Make the cell dye solution as desired depending on the number of wells. Add 100 µL Cell Dye to 1 mL Cell Dissociation Solution. Mix well.

2. Add 550 µL of the mix to each well of bottom chamber (after 22hrs, step A6). It can be after 2Hrs or 4Hrs too depending on experiment design.

3. Incubate at 37°C in CO_2 incubator for 60 minutes.

4. After incubation transfer 110 µL of mix from bottom chamber to the 96 well white plate. Read the plate at Ex/Em = 530/590 nm. Multiply the reading by 5 to account for the 5X higher volume in each well of the 24-well plate.

Data Analysis:

The number of cells migrated using the equation of the straight line obtained from the Standard Curve was calculated

[00044] As shown in FIG. 5, both fluorescent migration assay and transwell migration assay results showed that Rv-CD26 transduced HER2 CAR T cells displayed enhanced migration capacity compared to HER2 CAR T cells.

[00045] ADA-CD3-scFv expression by MR-CAR T cells is biologically functional

[00046] Western blotting was used to measure the expression of ADA-CD3 (scFv) in FIG. 7. Rv-ADA-CD3 (scFv) or Rv-ADA-CD3(scFv)-IL2sp (a human IL2 signal peptide was inserted to the 5' terminal of ADA-CD3 (scFv) in order to increase its

expression) was transduced into 293 T cells and cell lysates are subjected to western blotting using anti-ADA antibody (FIG. 7A). The results showed that Rv-ADA-CD3-IL2sp expressed ADA-CD3 at a higher level compared to Rv-ADA-CD3 (without IL2sp). Then Rv-ADA-CD3-IL2sp was compared to a lentiviral vector that expresses an ADA CRISPR activation element. Lv-ADA (CRISPR activation) virus or Rv-ADA-IL2sp were transduced into Jurkat T cells. Lv-ADA (CRPSPR activation) transduced Jurkat T cells were selected by puromycin. ADA expression was measured by western blotting using anti-ADA mAb at 7, 14, or 18 days of T cell culture. As shown in FIG.7B, Rv-ADA1-IL2sp expressed ADA at a higher level compared to Lenti-ADA activation. These results supported that Rv-ADA-CD3-IL2sp is an optimized strategy to express ADA-CD3 (scFv) in CAR T cells.

[00047] The secretion of ADA-CD3(scFv) by MRCAR T cells were measured by ELISA against human ADA. As shown in FIG. 8A, ADA in the supernatant or cell lysate of Rv-ADA-CD3-IL2sp (signal peptide)-transduced, Rv-ADA-CD3 (no IL2 sp)-transduced or non-transduced (NT) HEK 293 T cells were measured by ELISA. The results showed in both supernatant or cell lysate, Rv-ADA-CD3-scFv-IL2sp (ADA1-CD3-IL2sp) or Rv-ADA-CD3-scFv (no IL2sp, ADA1-CD3) expressed ADA-CD3. And Rv-ADA-CD3-IL2sp expressed a higher level of ADA-CD3 than Rv-ADA-CD3.

[00048] Then the Rv-ADA-CD3-IL2sp or Rv-ADA-CD3 transduced HEK 293 T cells were co-cultured with Jurkat T cells to mimic a stress condition for cell culture (increased cell density and cell contact) in order to confirm the ADA-CD3 expression. At day 1 or day 4 of the co-culture, the cell culture medium was collected and subjected to ELISA measurement of ADA. The results showed Rv-ADA-CD3-IL2sp induced higher ADA expression than NT or Rv-ADA-CD3. We observed that NT groups had ADA expression (FIG. 8B). This can be explained by background expression of ADA by Jurkat T or HEK 293 T cells.

[00049] Next, we investigated if the ADA-CD3 will engage CD3-expressed Jurkat T cells or Rv-CD26-transduced CD3/CD26 double positive CD26-Jurkat T cells. Rv-ADA-CD3-IL2sp transduced Jurkat T cells or CD26-Jurkat T cells were cultured for 24 hours and stained with PE-conjugated mAb against ADA and subjected to flow analysis of ADA expression. As shown in FIG. 8C, ADA level on Rv-ADA-CD3-transduced Jurkat T cells was higher than that of Jurkat T cells, indicating CD3-scFv anchored ADA-CD3 on the

surface of Jurkat T cells. The ADA level on Rv-ADA-CD3-transduced CD26-Jurkat T cells was further increased compared that on Rv-ADA-CD3-transduced Jurkat T cells, indicating the engagement of ADA and CD26 further enhanced the binding of ADA-CD3 with CD26-Jurkat T cells. Thus， these data suggested that our designed ADA-CD3 can be secreted by and engaged with T cells.

[00050] Next, the secreted ADA-CD3's enzyme activity was investigated. Since ADA-CD3 has high binding affinity with T cells and will engage on the surface of the T cells, the T cells were directly used to measure the ADA-CD3's enzyme activity. First, HEK 293 T cells were transduced with Rv-ADA-CD3 and seeded in 96 well plate at different density for overnight. After 24 hours, the cells were counted, and cell density was shown in FIG.9A. Then same amount of Jurkat T cells was added to the cell culture for 6 hours. The cell culture medium was removed, and the cells were directly subjected to ADA activity assay. As shown in FIG.9A, Rv-ADA-CD3 resulted in higher enzyme activity of converting ADO to INO at cell culture density of 10e5/well or 5x10e5/well compared to control group, indicating the ADA secretion is stress condition mediated (left: original reads; right: non-transduced HEK 293 T cell background reads were subtracted).

[00051] Next, we investigated the binding efficacy of ADA-CD3 on T cells. Jurkat-Dual (NF-kB &IRF/IFN), Jurkat-Dual transduced with Rv-CD26 (Jurkat-Dual-CD26), Jurkat-NFAT or Jurkat-NFAT transduced with Rv-CD26 (Jurkat-NFAT-CD26) reporter cell lines were co-cultured with Rv-ADA or Rv-ADA-CD3 (scFv) transduced 293 T cells for 24 hours. The cell culture medium was then used to measure ADA by ADA activity assay. ADA membrane binding efficiency was calculated using Rv-ADA-CD3-293T/CD26-Jurkat T as maximum and 293T/JurkatT W/O CD26 as background. The results showed that the secreted ADA-CD3 engaged with both Jurkat-Dual and CD26-Jurkat-Dual (FIG. 9B) or Jurkat-NFAT and CD26-Jurkat-NFAT (FIG. 9C) completely, while secreted ADA (without CD3-scFv) only engaged with CD26-Jurkat-Dual (FIG. 9B) or CD26-Jurkat-NFAT (FIG. 9C) at 40% efficacy of secreted ADA-CD3. Thus, these results demonstrated that ADA-CD3 improved engagement of ADA

[00052] Next, we investigated if the secreted ADA-CD3 (scFv) can activate human T cells since either CD3-scFv or ADA can provide stimulatory or co-stimulatory signal to human T cells. As shown in FIG. 10A and 10B, Rv-ADA (ADA1) or Rv-ADA-CD3 (ADA1-

CD3) was transduced into 293 T cells. 293 T cell culture medium (supernatant) or 293 T cells were added to the culture of NFAT-luciferase Jurkat reporter cell line. The cells were cultured for 6 hours (FIG. 10A) or 24 hours (FIG. 10B), and then cell culture medium were collected and subjected to luciferase assay. Only Rv-ADA-CD3 transduced 293 T cells induced T cell activation at 24 hours, indicating the secretion of ADA-CD3 is mediated by stress condition (cell culture at high cell density for 24 hours). We also compared Rv-ADA-CD3 and Rv-ADA-CD3-IL2sp. Jurkat NFAT reporter T cells were added with the supernatant of or the Rv-ADA-CD3 or Rv-ADA-CD3 (IL2sp) transduced HEK 293 T cell for 6 hours or 24 hours and subjected to luciferase assay. The results showed that Rv-ADA-CD3-IL2sp induced T cell activation more effectively than Rv-ADA-CD3. ADA-CD3 (IL2sp) expression level were calculated as shown in FIG. 10D. Rv-ADA-CD3 or Rv-ADA-CD3-IL2sp can induce ADA-CD3 expression at 0.3 ug/ml or >0.5ug/ml at 24 hours individually.

LDH cytotoxicity assay of MR-CAR T cells

LDH assay protocol

Day 1: In 96 well U-bottom cell culture plate, tumor cells (1×10e4) were co-cultured with effector cells CAR-T and NT-T (non-transduced T cells as negative control) in 200ul DMEM medium at a gradient E:T ratio as below for 4 h. Maximum load (positive control) well will be added with 20 ul of lysis buffer and culture for 30 minutes.

E:T ratio	CAR-T (Effector)	Tumor cell (Target)
1:1	1 × 10e4	1 × 10e4
5:1	5 × 10e4	1 × 10e4
10:1	1 × 10e5	1 × 10e4
20:1	2 × 10e5	1 × 10e4
Maximum load (positive control)	20 ul of lysis buffer	1 × 10e4

After 4 hours: Detection of the lactate dehydrogenase (LDH) was conducted as specified by the manufacturer using Enzo LDH cytotoxicity EST assay (Cat# ENZ-KIT157)

1. The plate will be centrifuge at 250 x g for 2 minutes to precipitate the cells.
2. Transfer 100 ul of cell supernatant to each well of a new flat-bottom optically clear 96-well plate.
3. Add 100ul of the working solution to each well.
4. Protect the plate from light and incubate it at room temperature for 30 minutes.
5. Add 50 ul of the stop solution to each well.
6. Measure the absorbance at 490 nm by a microplate reader

The specific lysis was calculated using the following formula:

(experimental-spontaneous release)/(maximum load-spontaneous release) x100%

[00053] As shown in FIG. 11, the CAR T cells were co-cultured with HepG2 or Huh7 liver cancer cells and subjected to LDH assay as above. FIG.11B and FIG.11C showed that, at 4 hours of co-culture, MR-CAR (GPC3-ADA1-CD3-CD26) T cells displayed enhanced cytotoxicity against HepG2 (FIG.11B) or Huh7 (FIG.11C) at E:T ratio of 20:1 compared to that of GPC3-CAR T cells, while no significant difference were observed at E:T ration of 1:1. This is likely due to that the expression and secretion of ADA-CD3 (scFv) needs time. As a result, at the E:T ratio of 1:1, MR-CAR displayed a significantly enhanced cytotoxicity against HepG2 (FIG.11D) or Huh7 (FIG.11E) at either 18 hours or 24 hours of co-culture of MR-CAR T cells and the tumor cells. These results indicated that MR-CAR T cells displayed enhanced cytotoxicity against specific tumor cells in vitro.

<u>In vivo mouse studies of MR-CAR T cell therapy</u>

[00054] 6 weeks old NSG mice were purchased from Jax laboratory, and each group has 5 mice. To investigate the effects of MR-CAR T cell therapy in liver cancer, groups of NSG mice were first subcutaneously inoculated with 2x10e6 Huh7 HCC tumor cells in 200ul medium on the right flank at day 0. At day 7, the Huh7 tumor bearing mice were treated through tail vein with 200ul PBS, 2x10e6 GPC3-CAR T cells in 200ul medium, or 2x10e6 GPC3-ADA1-CD3-CD26-CAR T cells in 200ul medium (designated as GPC3-MR-CAR). The mice were monitored every 2 or 3 days. Tumor size were measure with

caliper and the tumor size was calculated as: tumor size = L x W x W /2 (L: length of the tumor, W: width of the tumor). The mice body weight was also measured to monitor the toxicity of MR-CAR T cell therapy.

[00055] To investigate the effects of MR-CAR T cell therapy in lung cancer, groups of NSG mice were first subcutaneously inoculated with 2x10e6 A549 NSCLC tumor cells in 200ul medium on the right flank at day 0. At day 7, the A549 tumor bearing mice were treated through tail vein with 2x10e6 HER2-CAR T cells, or 2x10e6 HER2-ADA1-CD3-CD26-CAR T cells in 200ul medium (designated as HER2-MR-CAR) or no treatment as control (NT). The tumor size and body weight were measured as described above.

[00056] In both models, MR-CAR T cells showed enhanced anti-tumor activity. MR-CAR T cells significantly inhibit tumor growth in both Huh7 HCC or A549 NSCLC mouse models while original CAR T cells only moderately inhibit Huh7 or A549 tumor growth. In addition, no significant difference in body weight was observed in both Huh7 HCC and A549 NSCLC mouse models, indicating MR-CAR T cells didn't induce toxicity in these mouse models.

[00057] Sequence Listings: The sequence listings listed below are part of the instant disclosure.

[00058] **HER2-scFv-CD28TM-CD3zeta-ADA1-CD26 (SEQ ID NO:1)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMRRMQL
LLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIALPANTAE
GLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGVVYVEV
RYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSILCCMR
HQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKSGIHRT

VHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICPWSSYL
TGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTEEEFKR
LNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLGSGATNFSLLKQAGDVEE
NPGPMKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNT
YRLKLYSLRWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPD
GQFILLEYNYVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAY
VWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFL
AYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTN
ATSIQITAPASMLIGDHYLCDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWN
CLVARQHIEMSTTGWVGRFRPSEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFI
TKGTWEVIGIEALTSDYLYYISNEYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERC
QYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMP
SKKLDFIILNETKFWYQMILPPHFDKSKKYPLLLDVYAGPCSQKADTVFRLNWATYL
ASTENIIVASFDGRGSGYQGDKIMHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIA
IWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNL
DHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWY
TDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

[00059] **HER2-scFv-CD28TM-CD3zeta-ADA2-CD26 (SEQ ID NO:2)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMLVDGPS
ERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEELANERL
MTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLHDIGIVT
MDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRVQNVTE
FDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRSMQEF
YEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKIIYSDH
RSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAKDGV
KLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDIPIEV
CPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFMGIG
GMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATKGSGATNFS
LLKQAGDVEENPGPMKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKT
YTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFG
HSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTW
SPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITDWVYEEEVFSAYSAL
WWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVNPTVKFFVV
NTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERISLQWLRRIQNYSVMDICD
YDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDGNSFYKIISNEEGYRHICYF

QIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPGGRNLYKIQLSDYTKVTCL
SCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRVLEDNSALDK
MLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKYPLLLDVYAGPCSQKADTVF
RLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRLGTFEVEDQIEAARQFSKM
GFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYM
GLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQISKALVDVG
VDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

[00060] **HER2-scFv-CD28TM-CD3zeta-ADA1 (SEQ ID NO:3)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTS
VGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDF
TFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKF
WVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY
APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDY
MNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL
GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMRR
MQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIALPAN
TAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGVVY
VEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSILCC
MRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKSGIH
RTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICPWSS
YLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTEEEF
KRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNL

[00061] **HER2-scFv-CD28TM-CD3zeta-ADA2 (SEQ ID NO:4)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMLVDGPS
ERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEELANERL
MTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLHDIGIVT
MDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRVQNVTE
FDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRSMQEF
YEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKIIYSDH
RSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAKDGV

KLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDIPIEV
CPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFMGIG
GMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKR WDKFIADVATK

[00062] **IL2SP-ADA1-CD26 (SEQ ID NO:5)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIAL
PANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGV
VYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSI
LCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKS
GIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICP
WSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTE
EEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLGSGEGRGSLLTCG
DVEENPGPMKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDY
LKNTYRLKLYSLRWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYS
ISPDGQFILLEYNYVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHK
LAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNG
TFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSS
VTNATSIQITAPASMLIGDHYLCDVTWATQERISLQWLRRIQNYSVMDICDYDESSG
RWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKD
CTFITKGTWEVIGIEALTSDYLYYISNEYKGMPGGRNLYKIQLSDYTKVTCLSCELNP
ERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNV
QMPSKKLDFIILNETKFWYQMILPPHFDKSKKYPLLLDVYAGPCSQKADTVFRLNWA
TYLASTENIIVASFDGRGSGYQGDKIMHAINRRLGTFEVEDQIEAARQFSKMGFVDN
KRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPE
DNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQA
MWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

[00063] **ADA2-CD26 (SEQ ID NO:6)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKE
ELANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHL
HDIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRK
RVQNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDY
VFRSMQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPE
FIGIKIIYSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEA
LMIPAKDGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRT
YSWKKDIPIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLS
YDFYEVFMGIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIAD
VATKGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTIITVPVVLLNKG
TDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVFNAEYGNSS
VFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYDLNKRQLITE
ERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITDW
VYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYP
KAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERISLQ
WLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDGN
SFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPGG
RNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSS
VNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKY

PLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINR
RLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIA
VAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTA
DDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQC
FSLP

[00064] **IL2SP-ADA1 (SEQ ID NO:7)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIAL
PANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGV
VYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSI
LCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKS
GIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICP
WSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTE
EEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNL

[00065] **ADA2 (SEQ ID NO:8)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEE
LANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLH
DIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRV
QNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRS
MQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKII
YSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAK
DGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDI
PIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFM
GIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATK

[00066] **CD26 (SEQ ID NO:9)**

MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLK
LYSLRWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFIL
LEYNYVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNN
DIYVKIEPNLPSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQF
NDTEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQI
TAPASMLIGDHYLCDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVAR
QHIEMSTTGWVGRFRPSEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTW
EVIGIEALTSDYLYYISNEYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVS
FSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFI
ILNETKFWYQMILPPHFDKSKKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENII
VASFDGRGSGYQGDKIMHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWS
YGGYVTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRN
STVMSRAENFKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDH
GIASSTAHQHIYTHMSHFIKQCFSLP

[00067] **HER2-scFv-ADA1-CD28TM-CD3zeta-CD26 (SEQ ID NO:10)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRSSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIALPANTA
EGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGVVYVE
VRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSILCCM
RHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKSGIHR
TVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICPWSSY
LTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTEEEFKR
LNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSDPKFWVLVVVGGVLA
CYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R

[00068] **HER2-scFv-ADA2-CD28TM-CD3zeta-CD26 (SEQ ID NO:11)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRSSMLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKM
MRLGGRLVLNTKEELANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFN
ILRMMPKGAALHLHDIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSE
KCSKWILLEDYRKRVQNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTIS
GLIHYAPVFRDYVFRSMQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQE
VAQKFVETHPEFIGIKIIYSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDT
GHSLHDYKEALMIPAKDGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFA
LSKHPAVRTYSWKKDIPIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPA
MFGAKGLSYDFYEVFMGIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKR
WDKFIADVATKSSDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM
NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

[00069] **HER2-scFv-CD28TM-CD3zeta-ADA1-CD28TM-CD3zeta-CD26 (SEQ ID NO:12)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNW
VKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDM
ATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLS

TSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSG
PDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPD
PKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKH
YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
LHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVE
ENPGPMRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGR
RRGIALPANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEM
KAKEGVVYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDF
GVKARSILCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQA
YQEAVKSGIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQE
NMHFEICPWSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMT
KRDMGFTEEEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSDP
KFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY
QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAA
LVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQ
ENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTA
SYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITW
TGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDE
SLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLC
DVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGR
FRPSEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYL
YYISNEYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLR
CSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQ
MILPPHFDKSKKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSG
YQGDKIMHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMV
LGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAEN
FKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQ
HIYTHMSHFIKQCFSLP

[00070] **HER2-scFv-CD28TM-CD3zeta-ADA2-CD28TM-CD3zeta-CD26 (SEQ ID NO:13)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNW
VKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDM
ATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLS
TSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSG
PDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPD
PKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKH
YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
LHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG

MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVE
ENPGPMRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGR
RRGIALPANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEM
KAKEGVVYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDF
GVKARSILCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQA
YQEAVKSGIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQE
NMHFEICPWSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMT
KRDMGFTEEEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSDP
KFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY
QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAA
LVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQ
ENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTA
SYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITW
TGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDE
SLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLC
DVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGR
FRPSEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYL
YYISNEYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLR
CSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQ
MILPPHFDKSKKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSG
YQGDKIMHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMV
LGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAEN
FKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQ
HIYTHMSHFIKQCFSLP

[00071] **ADA1-CD28TM-CD3zeta-CD26 (SEQ ID NO:14)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIA
LPANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKE
GVVYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKA
RSILCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEA
VKSGIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHF
EICPWSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDM
GFTEEEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSDPKFWVL
VVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP
RDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTIITV
PVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVF
NAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYD
LNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDI
IYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPK
TVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWA
TQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEP
HFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNE
YKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGL
PLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPH

FDKSKKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDK
IMHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSG
VFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEY
LLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHM
SHFIKQCFSLP

[00072] **ADA2-CD28TM-CD3zeta-CD26 (SEQ ID NO:15)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEE
LANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLH
DIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRV
QNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRS
MQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKII
YSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAK
DGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDI
PIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFM
GIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATKSSDPKF
WVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY
APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTII
TVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNIL
VFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIY
DLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKED
IIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPK
TVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWAT
QERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHF
TLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYK
GMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY
TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKS
KKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAI
NRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCG
IAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGT
ADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHF
IKQCFSLP

[00073] **ADA1-CD28TM-CD3zeta (SEQ ID NO:16)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIAL
PANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGV
VYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSI
LCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKS
GIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICP
WSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTE
EEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSDPKFWVLVVVG
GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA
YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR

[00074] **ADA2-CD28TM-CD3zeta (SEQ ID NO:17)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEE
LANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLH
DIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRV
QNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRS
MQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKII
YSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAK
DGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDI
PIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFM
GIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATKSSDPKF
WVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY
APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
RRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[00075] **HER2-scFv-CD28TM-CD3zeta-ADA1-CD3-scFv-CD26 (SEQ ID NO:18)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMRRMQ
LLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIALPANTA
EGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGVVYVE
VRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSILCCM
RHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKSGIHR
TVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICPWSSY
LTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTEEEFK
RLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSGGGGSDIKLQQSGAE
LARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFK
DKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSG
GGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTS
PKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGA
GTKLELKSGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTIITVPVVLL
NKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVFNAEYG
NSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYDLNKRQLI
TEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITD
WVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYP
KAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERISLQ
WLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDGNS
FYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPGGR

NLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSV
NDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKYPL
LLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRLG
TFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAP
VSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNV
HFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

[00076] **HER2-scFv-CD28TM-CD3zeta-ADA2-CD3-scFv-CD26 (SEQ ID NO:19)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNW
VKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDM
ATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLS
TSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSG
PDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCP
DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL
STATKDTYDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
LHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVE
ENPGPGSGEGRGSLLTCGDVEENPGPMLVDGPSERPALCFLLLAVAMSFFGSALSID
ETRAHLLLKEKMMRLGGRLVLNTKEELANERLMTLKIAEMKEAMRTLIFPPSMHFF
QAKHLIERSQVFNILRMMPKGAALHLHDIGIVTMDWLVRNVTYRPHCHICFTPRGI
MQFRFAHPTPRPSEKCSKWILLEDYRKRVQNVTEFDDSLLRNFTLVTQHPEVIYTNQ
NVVWSKFETIFFTISGLIHYAPVFRDYVFRSMQEFYEDNVLYMEIRARLLPVYELSG
EHHDEEWSVKTYQEVAQKFVETHPEFIGIKIIYSDHRSKDVAVIAESIRMAMGLRIKF
PTVVAGFDLVGHEDTGHSLHDYKEALMIPAKDGVKLPYFFHAGETDWQGTSIDRNI
LDALMLNTTRIGHGFALSKHPAVTYSWKKDIPIEVCPISNQVLKLVSDLRNHPVATL
MATGHPMVISSDDPAMFGAKGLSYDFYEVFMGIGGMKADLRTLKQLAMNSIKYST
LLESEKNTFMEIWKKRWDKFIADVATKSSGGGGSDIKLQQSGAELARPGASVKMSC
KTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSS
TAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGSGGGGSGG
GGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV
ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKSGSG
EGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATAD
SRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVFNAEYGNSSVFLENSTF
DEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQ
WVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNGITDWVYEEEVFS
AYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVNPT
VKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERISLQWLRRIQNY
SVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDGNSFYKIISNEE
GYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPGGRNLYKIQLS
DYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRV
LEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKYPLLLDVYAG
PCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRLGTFEVED
QIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWE
YYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQ

SAQIS KALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

[00077] **HER2-scFv-CD28TM-CD3zeta-ADA1-CD3-scFv (SEQ ID NO:20)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMRRMQL
LLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIALPANTAE
GLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGVVYVEV
RYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSILCCMR
HQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKSGIHRT
VHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICPWSSYL
TGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTEEEFKRL
NINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSGGGGSDIKLQQSGAELA
RPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDK
ATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGG
GSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPK
RWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGT
KLELKS

[00078] **HER2-scFv-CD28TM-CD3zeta-ADA2-CD3-scFv (SEQ ID NO:21)**

MDWIWRILFLVGAATGAHSEVQLQQSGPELKKPGETVKISCKASGYPFTNYGMNWV
KQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSEDMAT
YFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQSHKFLSTSV
GDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRFTGSGSGPDFT
FTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLEPKSCDKTHTCPPCPDPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMN
MTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPMLVDGPS
ERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEELANERL
MTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLHDIGIVT
MDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRVQNVTE
FDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRSMQEF
YEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKIIYSDH
RSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAKDGV
KLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDIPIEV

CPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFMGIG
GMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKRWDKFIADVATKSSGGGGSDI
KLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYT
NYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGT
TLTVSSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWY
QQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSS
NPLTFGAGTKLELKS

[00079] **ADA1-CD3-scFv-CD26 (SEQ ID NO:22)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIAL
PANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGV
VYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSI
LCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKS
GIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICP
WSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTE
EEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSGGGGSDIKLQQS
GAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQ
KFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTV
SSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKS
GTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLT
FGAGTKLELKSGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAALVTIITVPV
VLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQENNILVFNA
EYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASYDIYDLNK
RQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIIYNG
ITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQYPKTVRV
PYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERIS
LQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPSEPHFTLDG
NSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPG
GRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSS
VNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKYP
LLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRL
GTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVA
PVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADD
NVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIA
SSTAHQHIYTHMSHFIKQCFSLP

[00080] **ADA2-CD3-scFv-CD26 (SEQ ID NO:23)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEE
LANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLH
DIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRV
QNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRS
MQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKII
YSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAK
DGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDI
PIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFM
GIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATKSSGGG
GSDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPS
RGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYW

GQGTTLTVSSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYM
NWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEADAATYYCQQ
WSSNPLTFGAGTKLELKSGSGEGRGSLLTCGDVEENPGPMKTPWKVLLGLLGAAAL
VTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSLRWISDHEYLYKQEN
NILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNYVKQWRHSYTASY
DIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTG
KEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSFYSDESLQ
YPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVT
WATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS
EPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN
EYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGL
PLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHF
DKSKKYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKI
MHAINRRLGTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGV
FKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLL
IHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFI
KQCFSLP

[00081] **ADA1-CD3-scFv (SEQ ID NO:24)**

MRRMQLLLLIALSLALVTNSMAQTPAFDKPKVELHVHLDGSIKPETILYYGRRRGIAL
PANTAEGLLNVIGMDKPLTLPDFLAKFDYYMPAIAGCREAIKRIAYEFVEMKAKEGV
VYVEVRYSPHLLANSKVEPIPWNQAEGDLTPDEVVALVGQGLQEGERDFGVKARSI
LCCMRHQPNWSPKVVELCKKYQQQTVVAIDLAGDETIPGSSLLPGHVQAYQEAVKS
GIHRTVHAGEVGSAEVVKEAVDILKTERLGHGYHTLEDQALYNRLRQENMHFEICP
WSSYLTGAWKPDTEHAVIRLKNDQANYSLNTDDPLIFKSTLDTDYQMTKRDMGFTE
EEFKRLNINAAKSSFLPEDEKRELLDLLYKAYGMPPSASAGQNLSSGGGGSDIKLQQS
GAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQ
KFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTV
SSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKS
GTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLT
FGAGTKLELKS

[00082] **ADA2-CD3-scFv (SEQ ID NO:25)**

MLVDGPSERPALCFLLLAVAMSFFGSALSIDETRAHLLLKEKMMRLGGRLVLNTKEE
LANERLMTLKIAEMKEAMRTLIFPPSMHFFQAKHLIERSQVFNILRMMPKGAALHLH
DIGIVTMDWLVRNVTYRPHCHICFTPRGIMQFRFAHPTPRPSEKCSKWILLEDYRKRV
QNVTEFDDSLLRNFTLVTQHPEVIYTNQNVVWSKFETIFFTISGLIHYAPVFRDYVFRS
MQEFYEDNVLYMEIRARLLPVYELSGEHHDEEWSVKTYQEVAQKFVETHPEFIGIKII
YSDHRSKDVAVIAESIRMAMGLRIKFPTVVAGFDLVGHEDTGHSLHDYKEALMIPAK
DGVKLPYFFHAGETDWQGTSIDRNILDALMLNTTRIGHGFALSKHPAVRTYSWKKDI
PIEVCPISNQVLKLVSDLRNHPVATLMATGHPMVISSDDPAMFGAKGLSYDFYEVFM
GIGGMKADLRTLKQLAMNSIKYSTLLESEKNTFMEIWKKRWDKFIADVATKSSGGG
GSDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSR
GYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWG
QGTTLTVSSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMN
WYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAAT
YYCQQWSSNPLTFGAGTKLELKS

[00083] **CD28-TM (SEQ ID NO:26)**
DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRS

[00084] **CD3-zeta (SEQ ID NO:27)**
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

[00085] **G4S linker (SEQ ID NO:28)**
GGGGSGGGGSGGGGS

[00086] **CD3-scFv (SEQ ID NO:29)**
DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRG
YTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQ
GTTLTVSSGGGGSGGGGSGGGGSDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNW
YQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWS
SNPLTFGAGTKLELKS

[00087] **T2A sequence (SEQ ID NO:30)**
GSGEGRGSLLTCGDVEENPGP

[00088] **P2A sequence (SEQ ID NO: 31)**
GSGATNFSLLKQAGDVEENPGP

[00089] **IL2 leader sequence (SEQ ID NO:32)**
MRRMQLLLLIALSLALVTNS

[00090] The term "substantially" is defined as largely but not necessarily wholly what is specified, as understood by a person of ordinary skill in the art. In any disclosed embodiment, the terms "substantially", "approximately", "generally", and "about" may be substituted with "within [a percentage] of" what is specified, where the percentage includes 0.1, 1, 5, and 10 percent.

[00091] The foregoing outlines features of several embodiments so that those skilled in the art may better understand the aspects of the disclosure. Those skilled in the art should appreciate that they may readily use the disclosure as a basis for designing or modifying other processes and structures for carrying out the same purposes and/or achieving the same advantages of the embodiments introduced herein. Those skilled in the art should also realize that such equivalent constructions do not depart from the spirit and scope of the disclosure, and that they may make various changes, substitutions, and alterations herein without departing from the spirit and scope of the disclosure. The scope of the invention

should be determined only by the language of the claims that follow. The term "comprising" within the claims is intended to mean "including at least" such that the recited listing of elements in a claim are an open group. The terms "a", "an", and other singular terms are intended to include the plural forms thereof unless specifically excluded.

CLAIMS

What is claimed is:

1. An engineered cell comprising a nucleotide sequence encoding an Adenosine deaminase (ADA).

2. The cell of claim 1, wherein the cell is T cell, natural killer cell, natural killer T cell, dendritic cell, macrophage, mesenchymal stem cell, and derivatives thereof.

3. The cell of any one of the preceding claims, wherein the cell comprises chimeric Antigen Receptor (CAR).

4. The cell of any one of the preceding claims, wherein the CAR comprises (which may or may not be in the following order, N to C terminus) an antigen binding domain, ADA, an extracellular linking domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain.

5. The cell of any one of the preceding claims, wherein the ADA is a protein having Adenosine deaminase activity.

6. The cell of any one of the preceding claims, wherein the ADA is a human ADA or a derivative thereof.

7. The cell of any one of the preceding claims, wherein the ADA is a human ADA 1.

8. The cell of claim 7, wherein ADA 1 is a secreted protein.

9. The cell of claim 8, wherein the ADA 1 comprises an optimized human IL2 signal peptide operably linked to the ADA, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

10. The cell of claim 9, wherein the CAR comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, 9, 18, 20, 22, and 24.

11. The cell of claim 7, wherein ADA 1 is a membrane bound protein.

12. The cell of claim 11, wherein the CAR comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 10, 12, 14, and 16.

13. The cell of any one of the preceding claims, wherein the ADA is a human ADA 2.

14. The cell of claim 7, wherein ADA 2 is a secreted protein.

15. The cell of claim 14, wherein the ADA 2 comprises a natural or artificial signal peptide operably linked to the ADA2, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

16. The cell of claim 15, wherein the CAR comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, 9, 19, 21, 23, and 25.

17. The cell of claim 7, wherein ADA 2 is a membrane bound protein.

18. The cell of claim 17, wherein the CAR comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 11, 13, 15, and 17.

19. The cell of any of the preceding claims further comprising human CD26 or its derivates.

20. A pharmaceutical composition comprising the engineered cell of any one of the preceding claims and a pharmaceutically acceptable carrier.

21. A method of treatment, wherein the method comprises administering the engineered cells of any one of the preceding claims to a patient in an amount effective for treatment of a cancer.

22. A recombinant oncolytic virus comprising a nucleotide sequence encoding an ADA, wherein the nucleotide sequence encoding the ADA is operably linked to a promoter.

23. The oncolytic virus of claim 22, wherein said oncolytic virus is a virus selected from the group consisting of: vaccinia virus, reovirus, Seneca Valley virus (SVV), vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), herpes simplex virus (HSV), morbillivirus virus, retrovirus, influenza virus, Sinbis virus, poxvirus, measles virus, cytomegalovirus (CMV), lentivirus, adenovirus, coxsackievirus, and derivatives thereof.

24. The oncolytic virus of claim 23, wherein said oncolytic virus is a poxvirus.

25. The oncolytic virus of claim 24, wherein said poxvirus is a vaccinia virus.

26. The oncolytic virus of claim 25, wherein the vaccinia virus is of a strain selected from the group consisting of Dryvax, Lister, M63, LIVP, Tian Tan, Modified Vaccinia Ankara, New York City Board of Health (NYCBOH), Dairen, Ikeda, LC16M8, Tashkent, IHD-J, Brighton, Dairen I, Connaught, Wyeth, Copenhagen, Western Reserve, Elstree, CL, Lederle-Chorioallantoic, AS, and derivatives thereof.

27. The recombinant oncolytic virus of claim 25, wherein the virus is vaccinia virus Western Reserve.

28. The recombinant oncolytic virus of claim 22-25, wherein the oncolytic virus is Talimogene Laherparepvec.

29. The recombinant oncolytic virus of claim 22-25, wherein the virus is a reovirus.

30. The recombinant oncolytic virus of claim 22-25, wherein the virus is an adenovirus having an E1 and E2 deletion.

31. The oncolytic virus of claim 22-30, wherein the ADA is a protein having Adenosine deaminase activity.

32. The oncolytic virus of claim 22-30, wherein the ADA is a human ADA or a derivative thereof.

33. The oncolytic virus of claim 22-30, wherein the ADA is ADA1.

34. The oncolytic virus of claim 33, wherein ADA1 is a secreted protein.

35. The oncolytic virus of claim 34, wherein signal peptide is an optimized human IL2 signal peptide, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

36. The oncolytic virus of claim 35, wherein the oncolytic virus comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 5, 7, 9, 22, and 24.

37. The oncolytic virus of claim 32, wherein ADA1 is a membrane bound protein

38. The oncolytic virus of claim 36, wherein ADA1 is linked to a human IgG1 hinge, CH2CH3 domain, and a transmembrane domain.

39. The oncolytic virus of claim 36, wherein signal peptide is an optimized human IL2 signal peptide.

40. The oncolytic virus of claim 36, wherein the oncolytic virus comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID Nos: 14, and 16.

41. The oncolytic virus of claim 22-31, wherein the ADA is ADA2.

42. The oncolytic virus of claim 40, wherein ADA2 is a secreted protein, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

43. The oncolytic virus of claim 40, wherein the ADA comprises a natural or an artificial signal peptide operably linked to the ADA.

44. The oncolytic virus of claim 22-31, wherein ADA2 is a membrane bound protein

45. The oncolytic virus of claim 43, wherein the ADA is linked to a human IgG1-Fc domain and a transmembrane domain.

46. The oncolytic virus of any of the preceding claims further comprising CD26.

47. A pharmaceutical composition comprising the recombinant oncolytic virus of claim 21-44 and a pharmaceutically acceptable carrier.

48. A method of treatment, wherein the method comprises administering the recombinant oncolytic virus of claim 45 to a patient in an amount effective for treatment of a cancer.

49. A recombinant protein comprising an amino acid sequence encoding an ADA.

50. The recombinant protein of claim 47, wherein the ADA is a protein having Adenosine deaminase activity.

51. The recombinant protein of claim 47-48, wherein the ADA is a human ADA or a derivative thereof.

52. The recombinant protein of claim 47-49, wherein the ADA is ADA1, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

53. The recombinant protein of claim 50, wherein the ADA is linked to a linking domain, human IgG4 hinge, and a human IgG4 CH2CH3 domain or a derivative thereof.

54. The recombinant protein of claim 51, wherein the human IgG4 hinge has a mutation S228P.

55. The recombinant protein of claim 51, wherein the human IgG4 CH2Ch3 domain has mutations F234A, L235A, M252Y, S254T, T256E, K447del.

56. The recombinant protein of claim 50-53, wherein the protein comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID Nos: 7.

57. The recombinant protein of claim 47, wherein the ADA is ADA2, and a linker sequence and a human CD3 specific ligand, antibody, scFv, or its derivates.

58. The recombinant protein of claim 55, wherein the ADA is linked to a linking domain and a human albumin.

59. The recombinant protein of claim 56, wherein the protein comprises an amino acid sequence having at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID Nos: 8.

60. A pharmaceutical composition comprising the recombinant protein of claim 47-57 and a pharmaceutically acceptable carrier.

61. A method of administering the recombinant protein of claim 58 to a patient in an amount effective for treatment of a cancer.

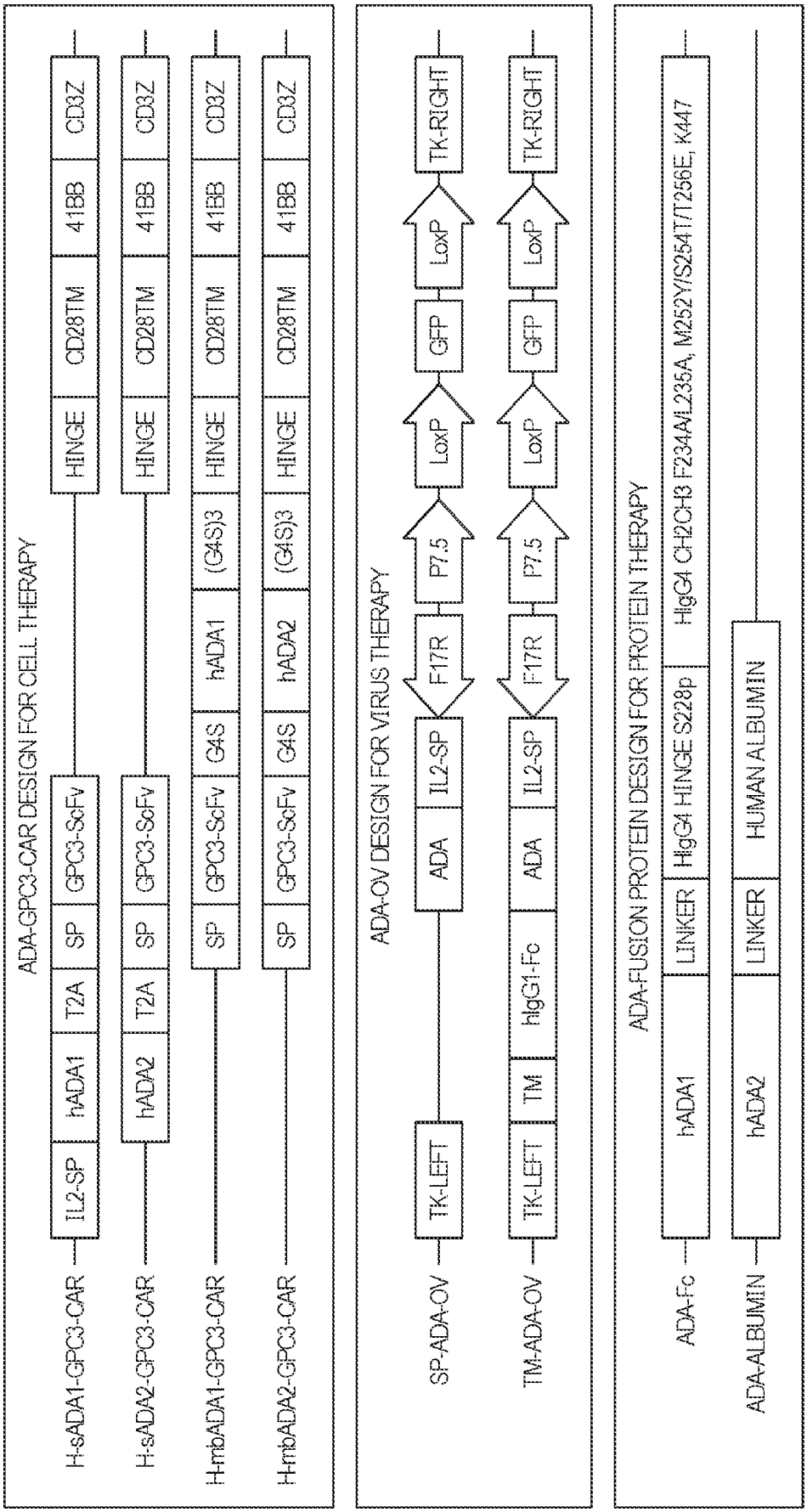

ADA-GPC3-CAR DESIGN FOR CELL THERAPY
H-sADA1-GPC3-CAR
IL2-SP
hADA1
T2A
SP
GPC3-ScFv
HINGE
CD28TM
41BB
CD3Z
H-sADA2-GPC3-CAR
hADA2
T2A
SP
GPC3-ScFv
HINGE
CD28TM
41BB
CD3Z
H-mbADA1-GPC3-CAR
SP
GPC3-ScFv
G4S
hADA1
(G4S)3
HINGE
CD28TM
41BB
CD3Z
H-mbADA2-GPC3-CAR
SP
GPC3-ScFv
G4S
hADA2
(G4S)3
HINGE
CD28TM
41BB
CD3Z
ADA-OV DESIGN FOR VIRUS THERAPY
SP-ADA-OV
TK-LEFT
ADA
IL2-SP
F17R
P7.5
LoxP
GFP
LoxP
TK-RIGHT
TM-ADA-OV
TK-LEFT
TM
HIgG1-Fc
ADA
IL2-SP
F17R
P7.5
LoxP
GFP
LoxP
TK-RIGHT
ADA-FUSION PROTEIN DESIGN FOR PROTEIN THERAPY
ADA-Fc
hADA1
LINKER
HIgG4 HINGE S228p
HIgG4 CH2CH3 F234A/L235A, M252Y/S254T/T256E, K447
ADA-ALBUMIN
hADA2
LINKER
HUMAN ALBUMIN

FIG. 1

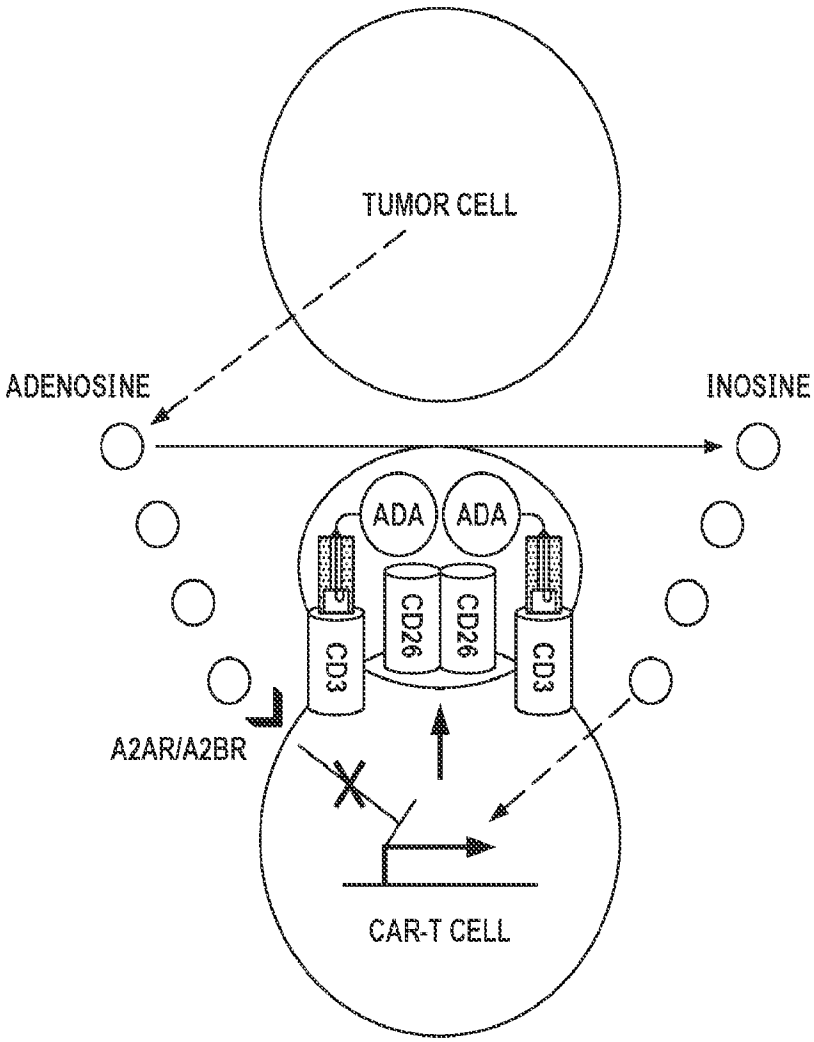

TUMOR CELL
ADENOSINE
INOSINE
ADA
ADA
CD26
CD26
CD3
CD3
A2AR/A2BR
CAR-T CELL

FIG. 2

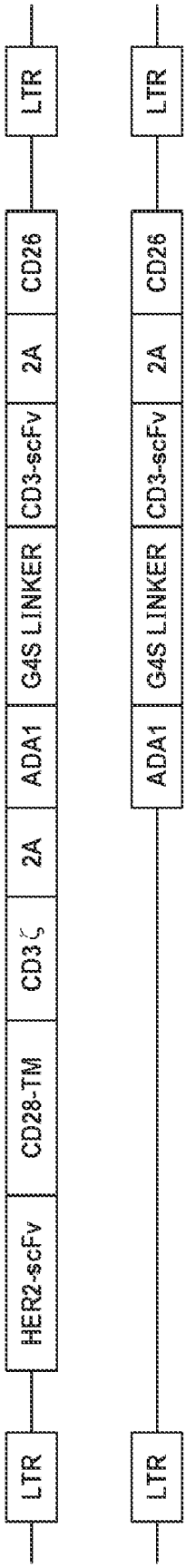

LTR
HER2-scFv
CD28-TM
CD3ζ
2A
ADA1
G4S LINKER
CD3-scFv
2A
CD26
LTR
LTR
ADA1
G4S LINKER
CD3-scFv
2A
CD26
LTR

FIG. 3

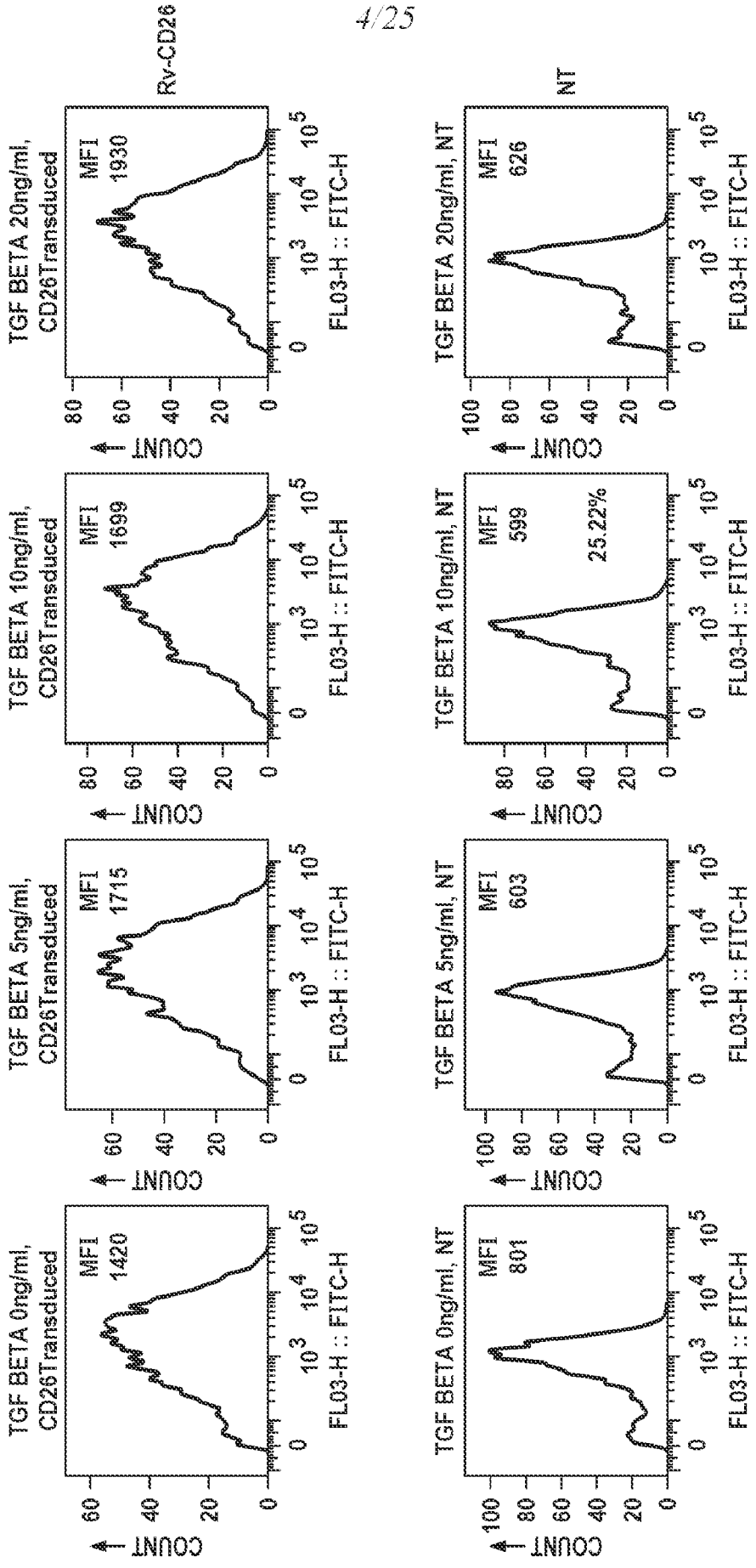

Rv-CD26
NT
TGF BETA 0ng/ml, CD26Transduced
MFI 1420
TGF BETA 5ng/ml, CD26Transduced
MFI 1715
TGF BETA 10ng/ml, CD26Transduced
MFI 1699
TGF BETA 20ng/ml, CD26Transduced
MFI 1930
TGF BETA 0ng/ml, NT
MFI 801
TGF BETA 5ng/ml, NT
MFI 603
TGF BETA 10ng/ml, NT
MFI 599
25.22%
TGF BETA 20ng/ml, NT
MFI 626
COUNT
FL03-H :: FITC-H

FIG. 4

FIG. 5A

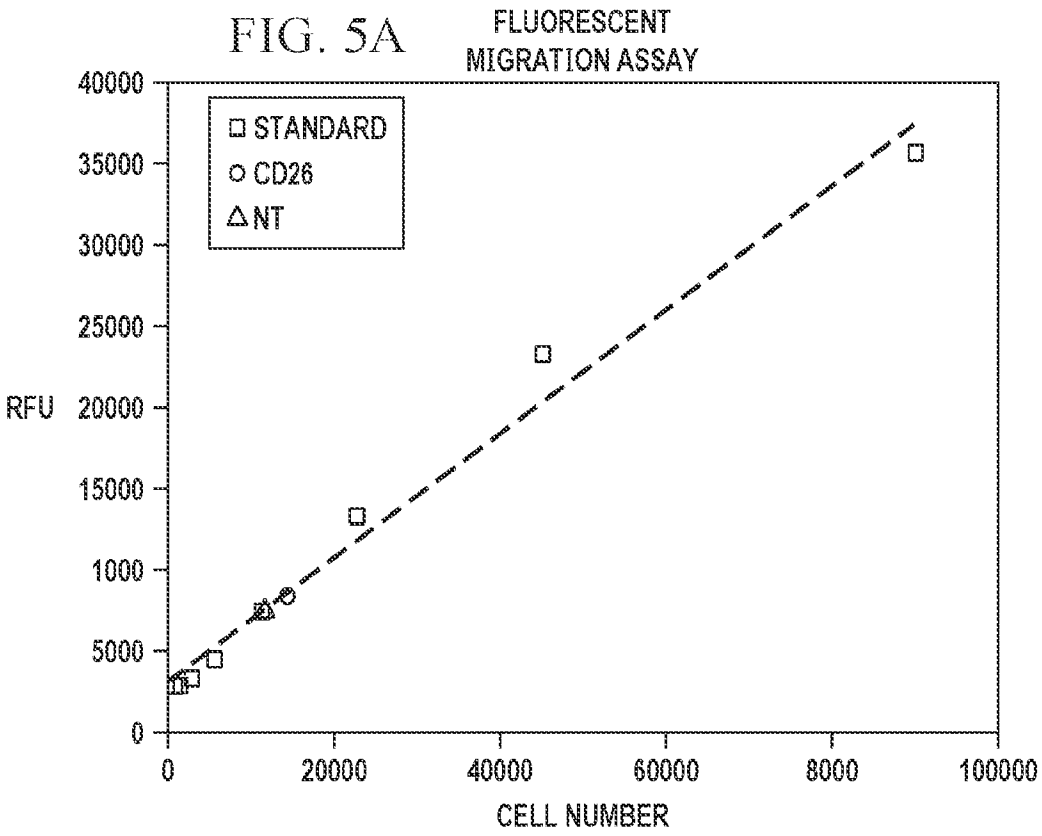
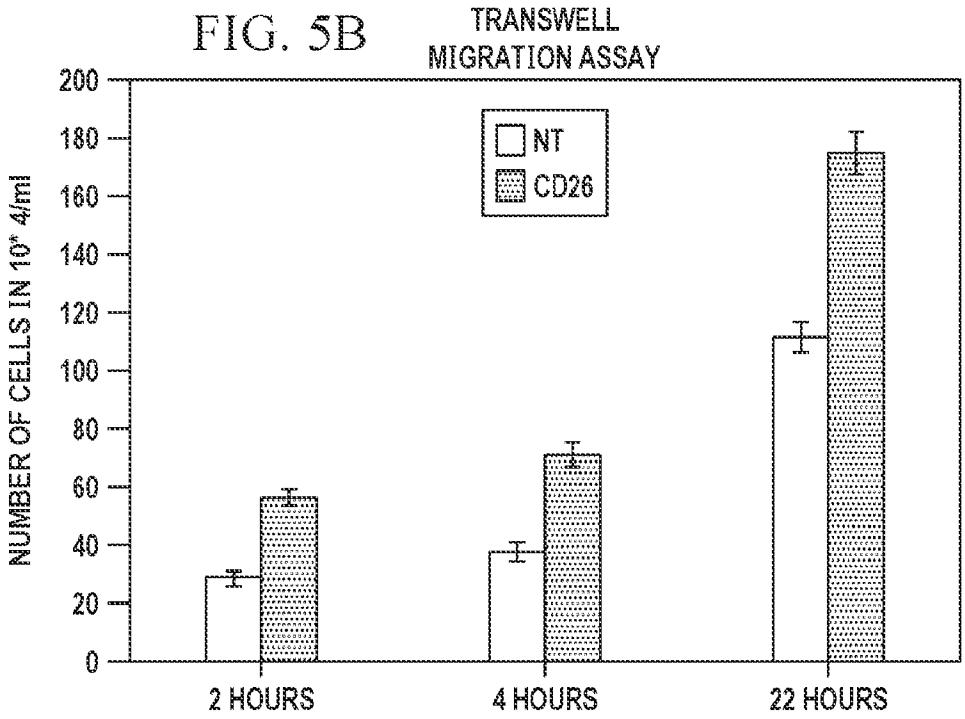

FLUORESCENT
MIGRATION ASSAY
STANDARD
CD26
NT
RFU
40000
35000
30000
25000
20000
15000
1000
5000
0
0
20000
40000
60000
8000
100000
CELL NUMBER

FIG. 5B


TRANSWELL
MIGRATION ASSAY
NT
CD26
NUMBER OF CELLS IN 10^4/ml
200
180
160
140
120
100
80
60
40
20
0
2 HOURS
4 HOURS
22 HOURS

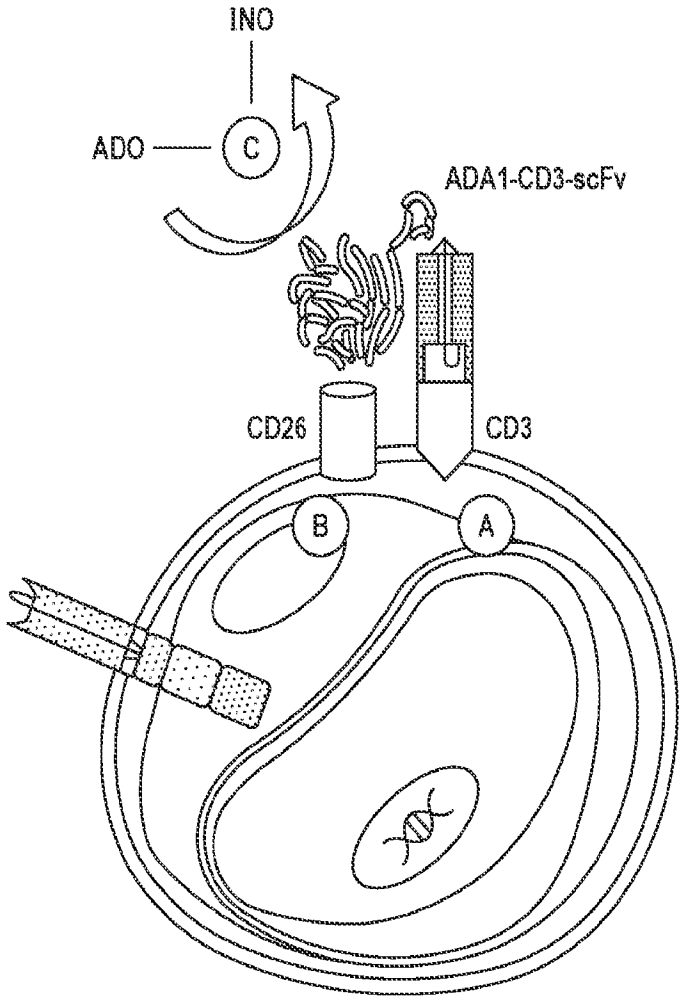


FIG. 6

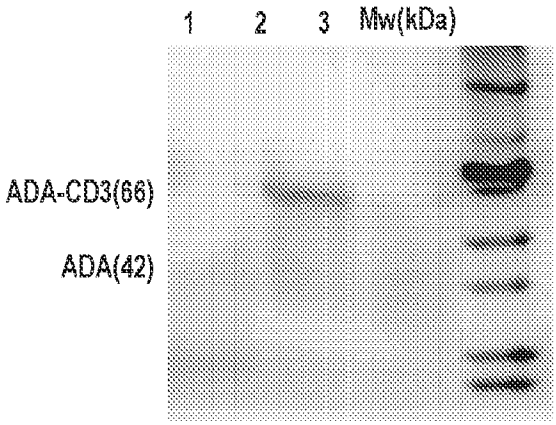
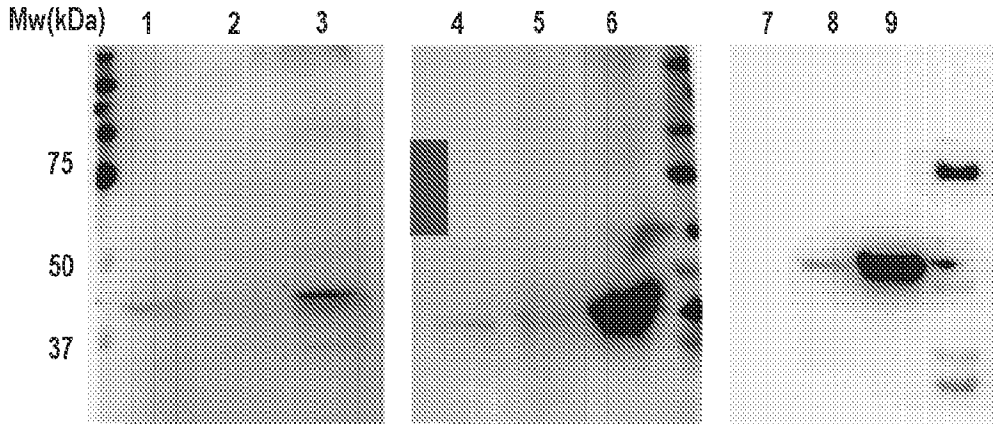


FIG. 7A

1. HEK293T CELL NT
2. HEK293T CELL Rv-ADA-CD3-IL2sp
3. HEK293T CELL
Rv-ADA-CD3(WITHOUT IL2sp)



1. JURKAT CELL NT
2. JURKAT CELL LENTI-ADA ACTIVATION (7 DAYS)
3. JURKAT CELL Rv-ADA-IL2sp (7 DAYS)
4. JURKAT CELL NT
5. JURKAT CELL LENTI-ADA ACTIVATION (14 DAYS)
6. JURKAT CELL Rv-ADA-IL2sp (14 DAYS)
7. JURKAT CELL NT
8. JURKAT CELL LENTI-ADA ACTIVATION (18 DAYS)
9. JURKAT CELL Rv-ADA-IL2sp (18 DAYS)

FIG. 7B

FIG. 8A

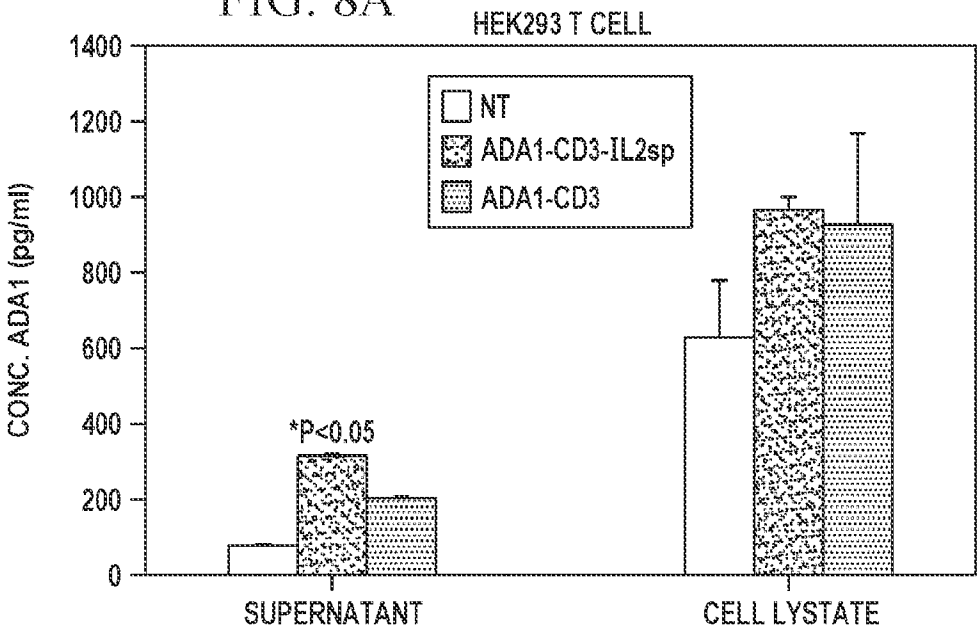
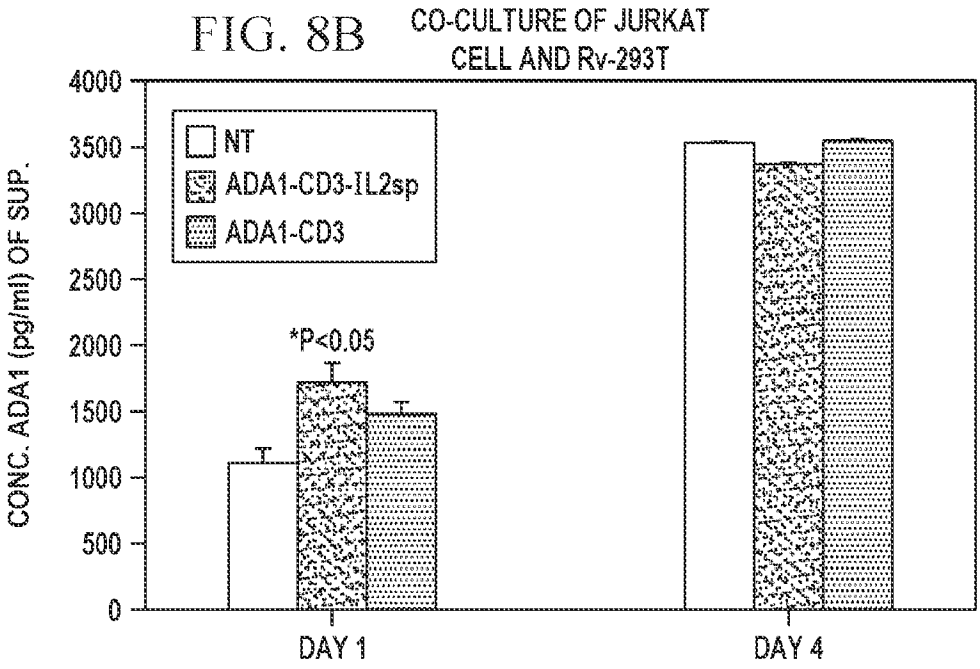

HEK293 T CELL
NT
ADA1-CD3-IL2sp
ADA1-CD3
CONC. ADA1 (pg/ml)
1400
1200
1000
800
600
400
200
0
*P<0.05
SUPERNATANT
CELL LYSTATE

FIG. 8B


CO-CULTURE OF JURKAT CELL AND Rv-293T
NT
ADA1-CD3-IL2sp
ADA1-CD3
CONC. ADA1 (pg/ml) OF SUP.
4000
3500
3000
2500
2000
1500
1000
500
0
*P<0.05
DAY 1
DAY 4

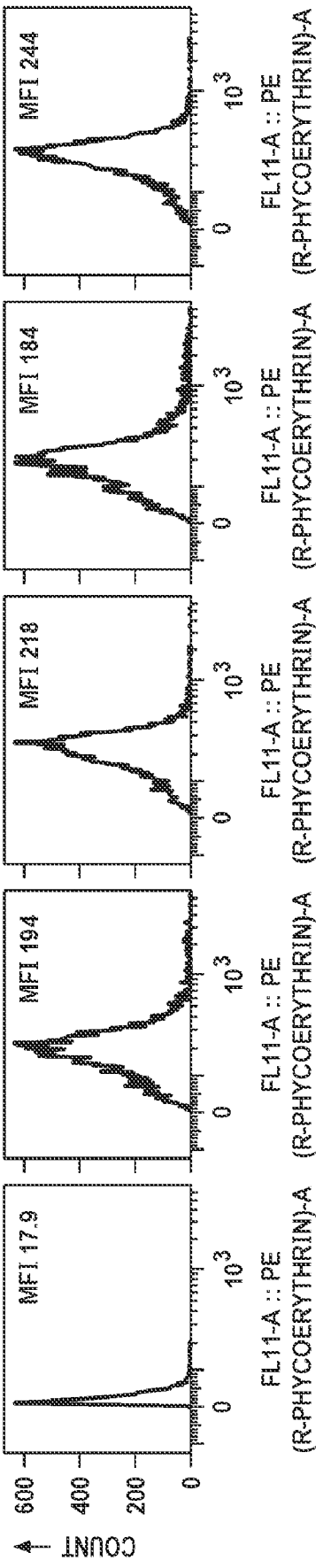
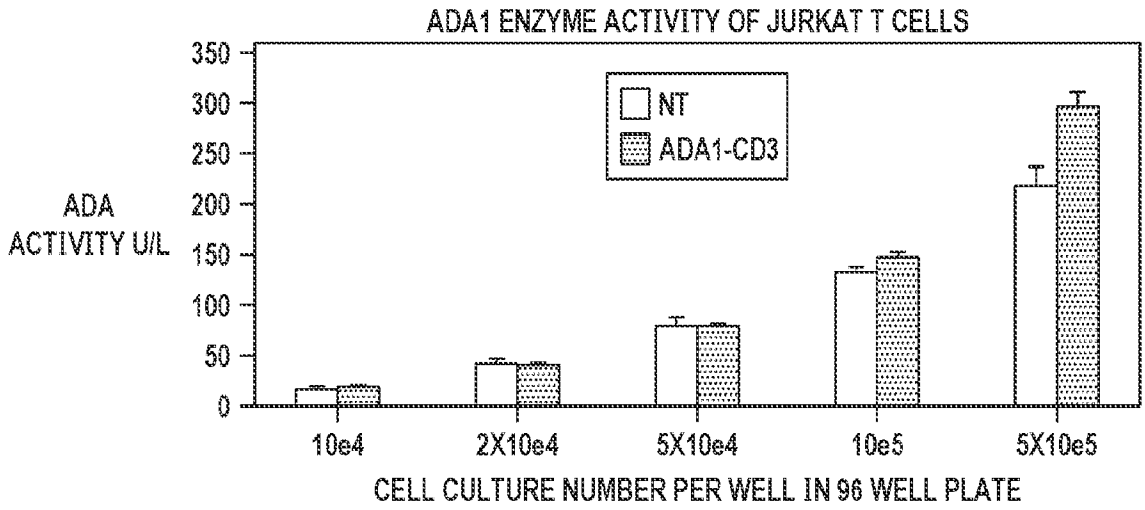

NEGATIVE CONTROL
MFI 17.9
JURKAT
MFI 194
ADA-CD3/JURKAT
MFI 218
CD26-JURKAT
MFI 184
ADA-CD3/CD26-JURKAT
MFI 244
COUNT
600
400
200
0
0
10^3
FL11-A :: PE
(R-PHYCOERYTHRIN)-A

FIG. 8C


ADA1 ENZYME ACTIVITY OF JURKAT T CELLS
ADA ACTIVITY U/L
350
300
250
200
150
100
50
0
NT
ADA1-CD3
10e4
2X10e4
5X10e4
10e5
5X10e5
CELL CULTURE NUMBER PER WELL IN 96 WELL PLATE

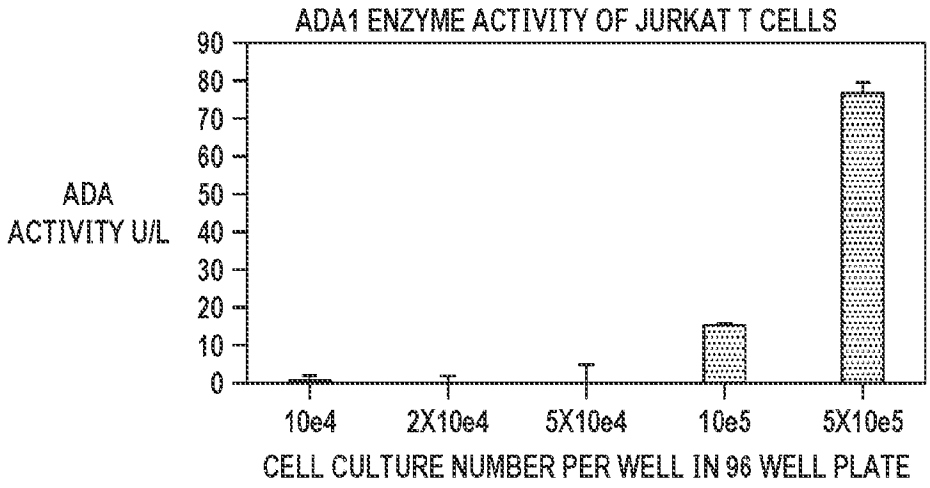

ADA1 ENZYME ACTIVITY OF JURKAT T CELLS
ADA ACTIVITY U/L
90
80
70
60
50
40
30
20
10
0
10e4
2X10e4
5X10e4
10e5
5X10e5
CELL CULTURE NUMBER PER WELL IN 96 WELL PLATE

FIG. 9A

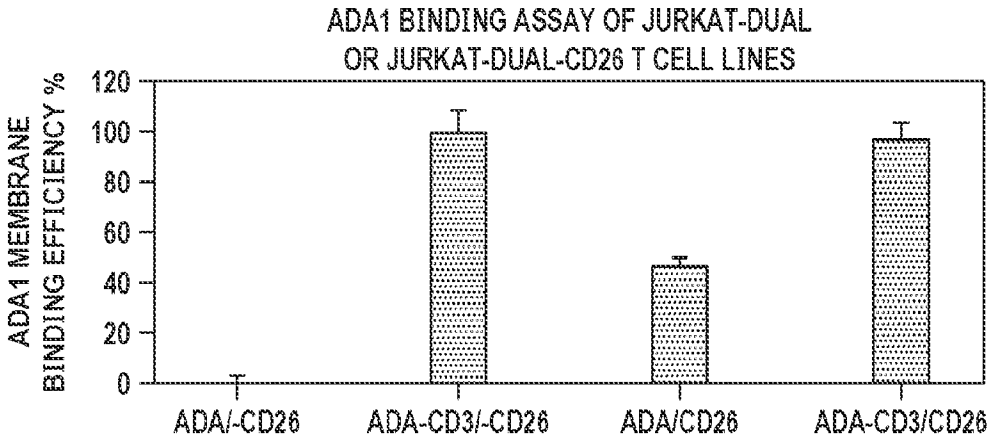

ADA1 BINDING ASSAY OF JURKAT-DUAL
OR JURKAT-DUAL-CD26 T CELL LINES
ADA1 MEMBRANE
BINDING EFFICIENCY %
120
100
80
60
40
20
0
ADA/-CD26
ADA-CD3/-CD26
ADA/CD26
ADA-CD3/CD26

FIG. 9B

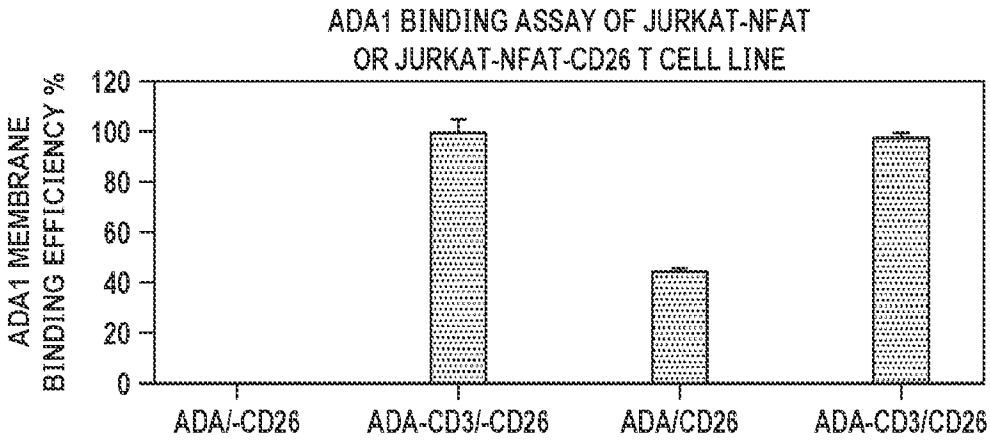

ADA1 BINDING ASSAY OF JURKAT-NFAT
OR JURKAT-NFAT-CD26 T CELL LINE
ADA1 MEMBRANE
BINDING EFFICIENCY %
120
100
80
60
40
20
0
ADA/-CD26
ADA-CD3/-CD26
ADA/CD26
ADA-CD3/CD26

FIG. 9C

FIG. 10A

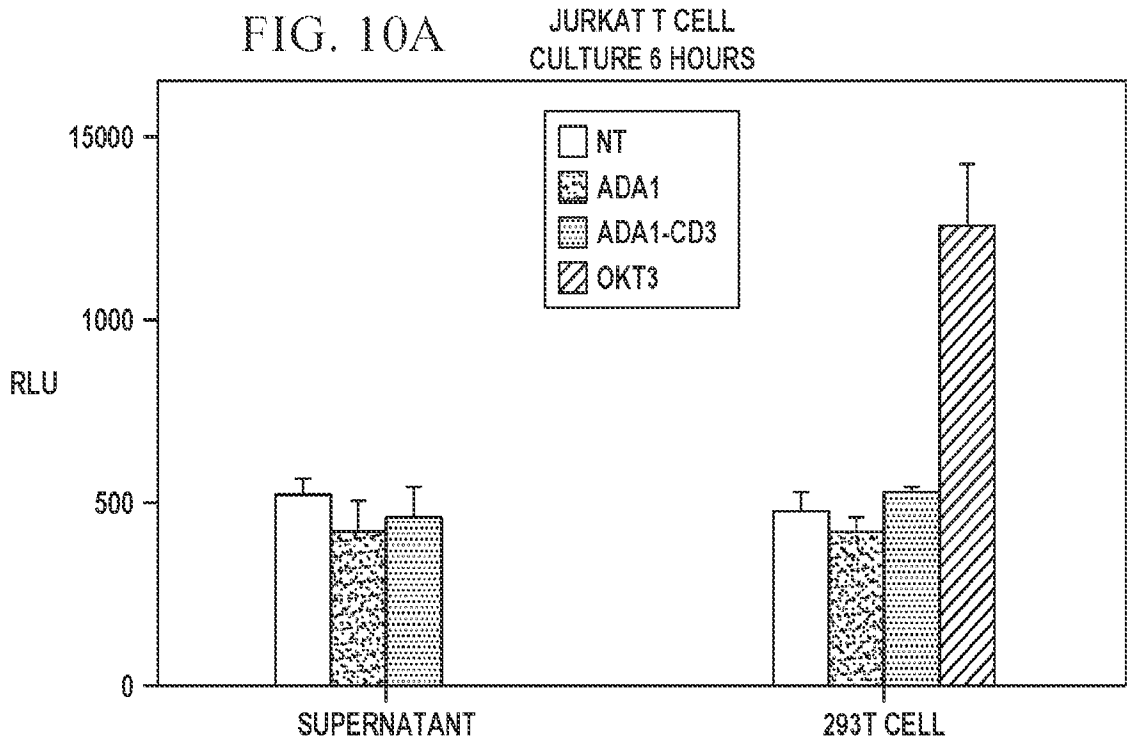

JURKAT T CELL
CULTURE 6 HOURS
NT
ADA1
ADA1-CD3
OKT3
15000
1000
500
0
RLU
SUPERNATANT
293T CELL

FIG. 10B

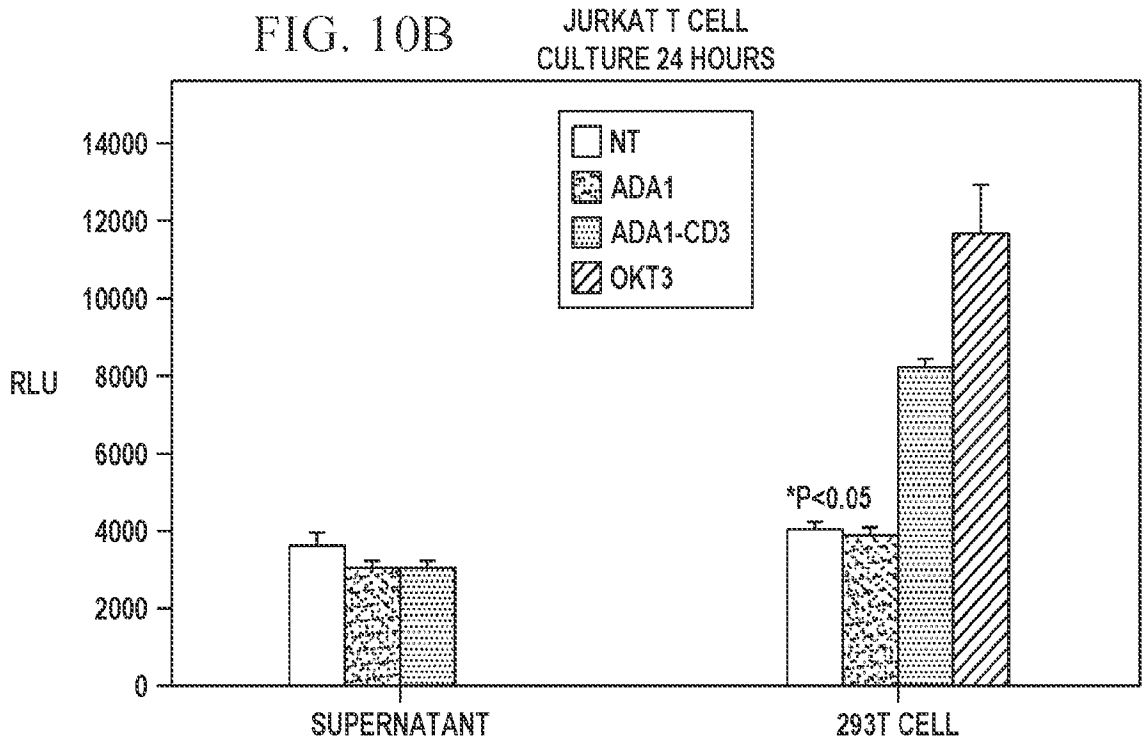

JURKAT T CELL
CULTURE 24 HOURS
NT
ADA1
ADA1-CD3
OKT3
RLU
14000
12000
10000
8000
6000
4000
2000
0
*P<0.05
SUPERNATANT
293T CELL

FIG. 10C

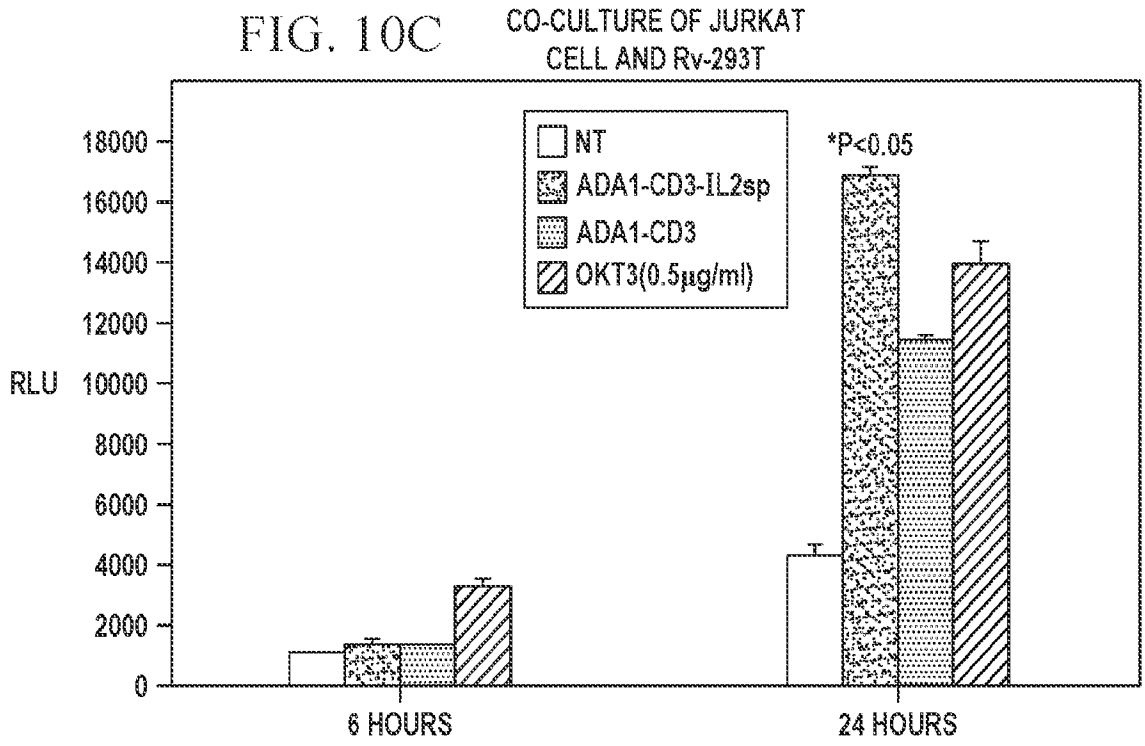
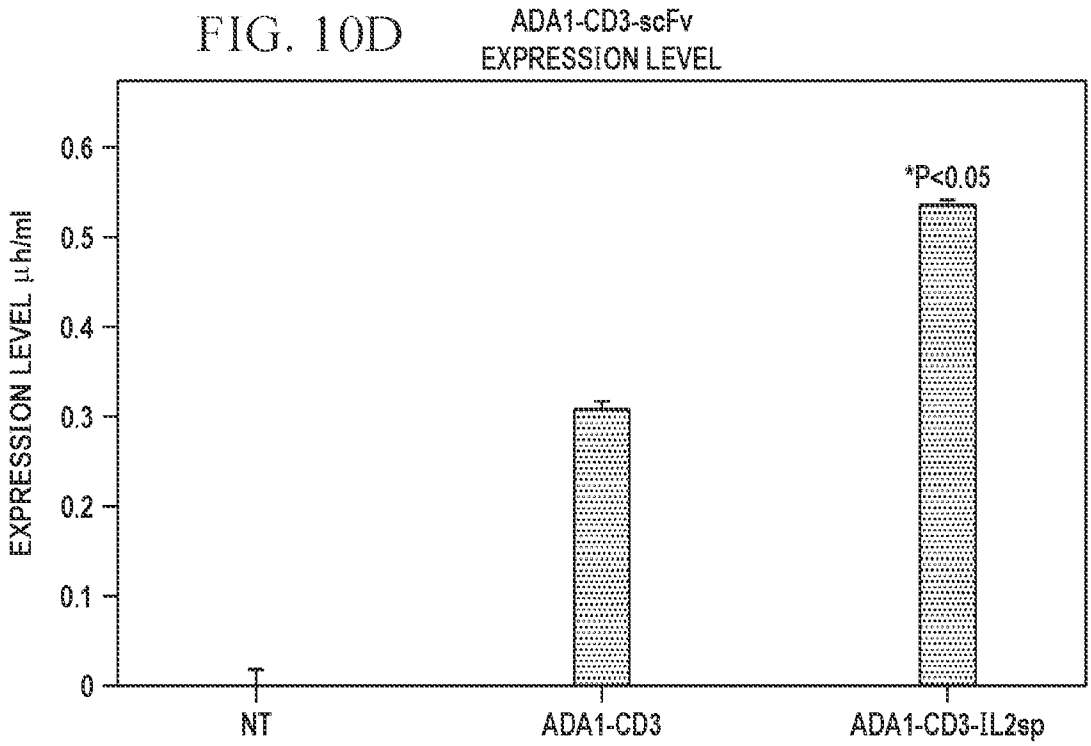

CO-CULTURE OF JURKAT CELL AND Rv-293T
NT
ADA1-CD3-IL2sp
ADA1-CD3
OKT3(0.5μg/ml)
*P<0.05
RLU
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
6 HOURS
24 HOURS

FIG. 10D


ADA1-CD3-scFv
EXPRESSION LEVEL
EXPRESSION LEVEL μh/ml
0.6
0.5
0.4
0.3
0.2
0.1
0
*P<0.05
NT
ADA1-CD3
ADA1-CD3-IL2sp

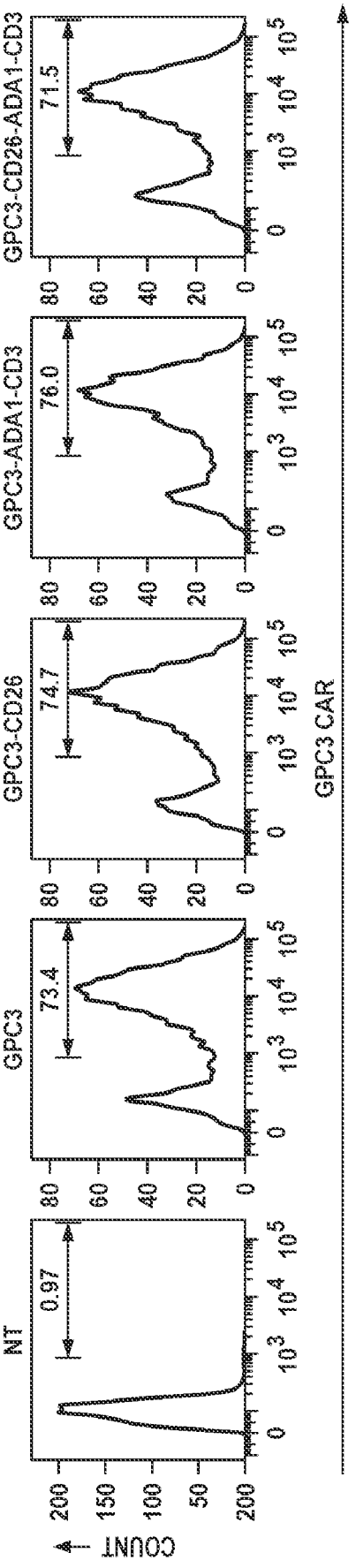

NT
GPC3
GPC3-CD26
GPC3-ADA1-CD3
GPC3-CD26-ADA1-CD3
0.97
73.4
74.7
76.0
71.5
COUNT
GPC3 CAR

FIG. 11A

FIG. 11B

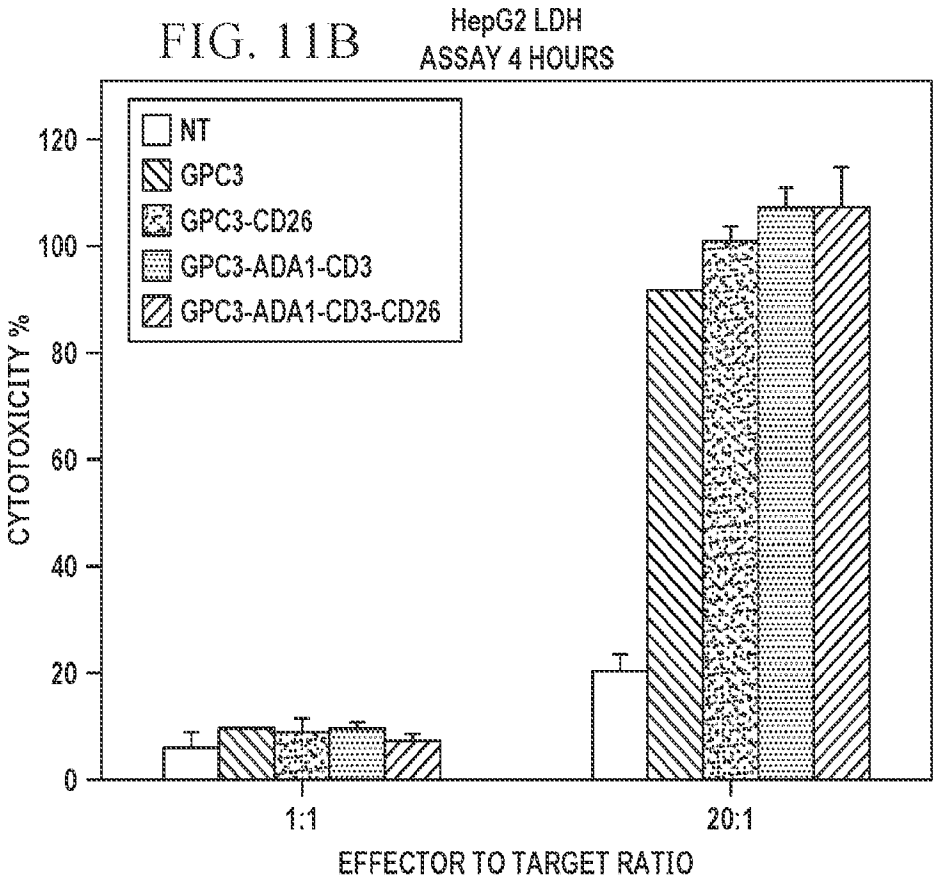

HepG2 LDH
ASSAY 4 HOURS
NT
GPC3
GPC3-CD26
GPC3-ADA1-CD3
GPC3-ADA1-CD3-CD26
CYTOTOXICITY %
0
20
40
60
80
100
120
1:1
20:1
EFFECTOR TO TARGET RATIO

FIG. 11C

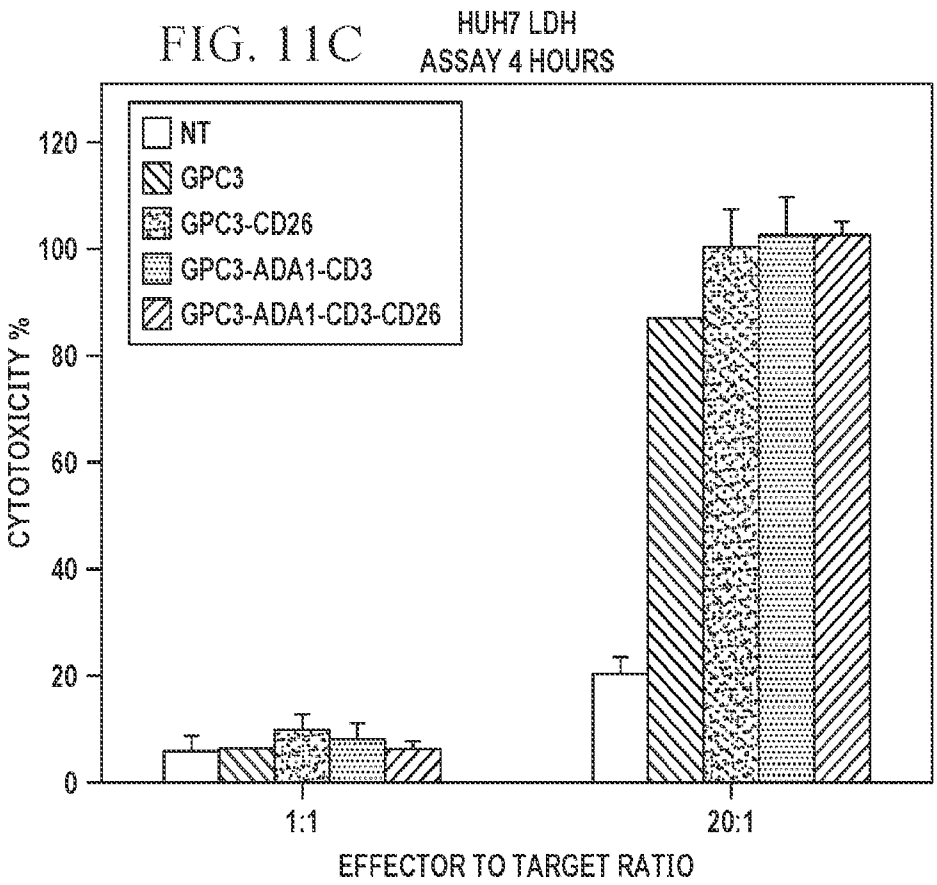

HUH7 LDH
ASSAY 4 HOURS
NT
GPC3
GPC3-CD26
GPC3-ADA1-CD3
GPC3-ADA1-CD3-CD26
CYTOTOXICITY %
0
20
40
60
80
100
120
1:1
20:1
EFFECTOR TO TARGET RATIO

FIG. 11D

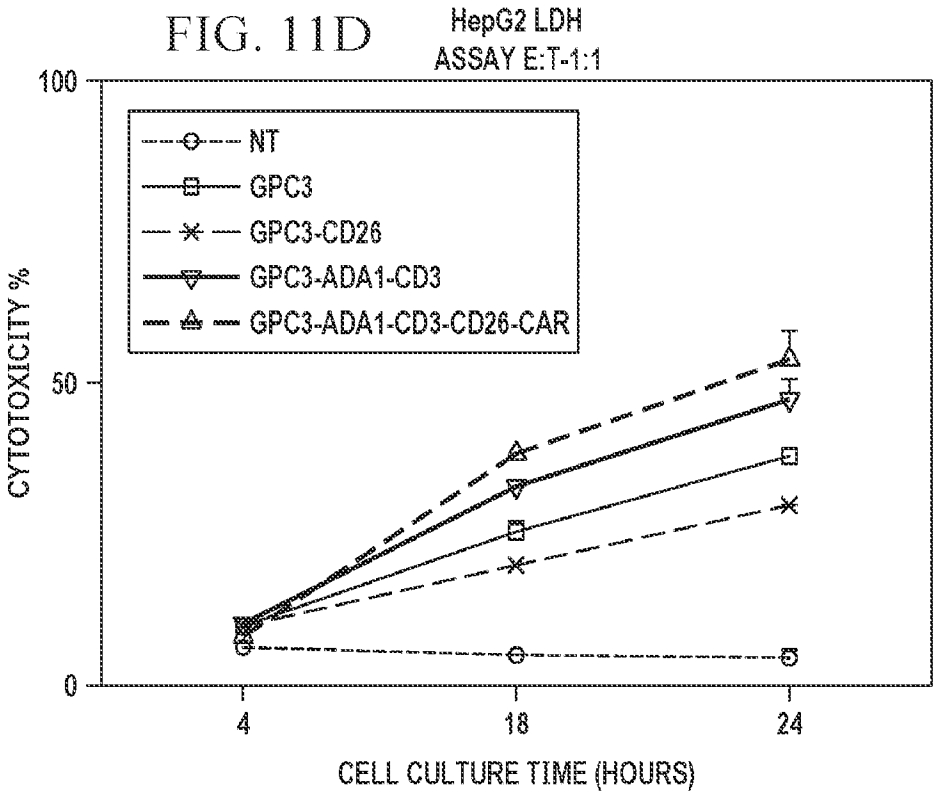

HepG2 LDH
ASSAY E:T-1:1
100
50
0
CYTOTOXICITY %
NT
GPC3
GPC3-CD26
GPC3-ADA1-CD3
GPC3-ADA1-CD3-CD26-CAR
4
18
24
CELL CULTURE TIME (HOURS)

FIG. 11E

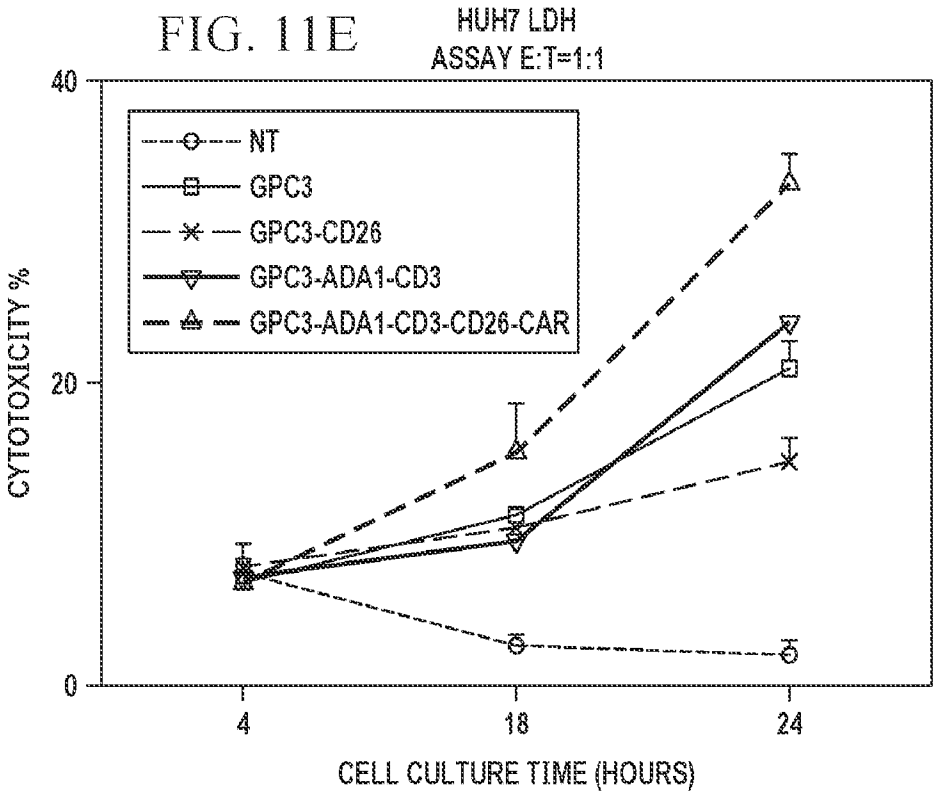

HUH7 LDH
ASSAY E:T=1:1
NT
GPC3
GPC3-CD26
GPC3-ADA1-CD3
GPC3-ADA1-CD3-CD26-CAR
CYTOTOXICITY %
0
20
40
4
18
24
CELL CULTURE TIME (HOURS)

FIG. 12A

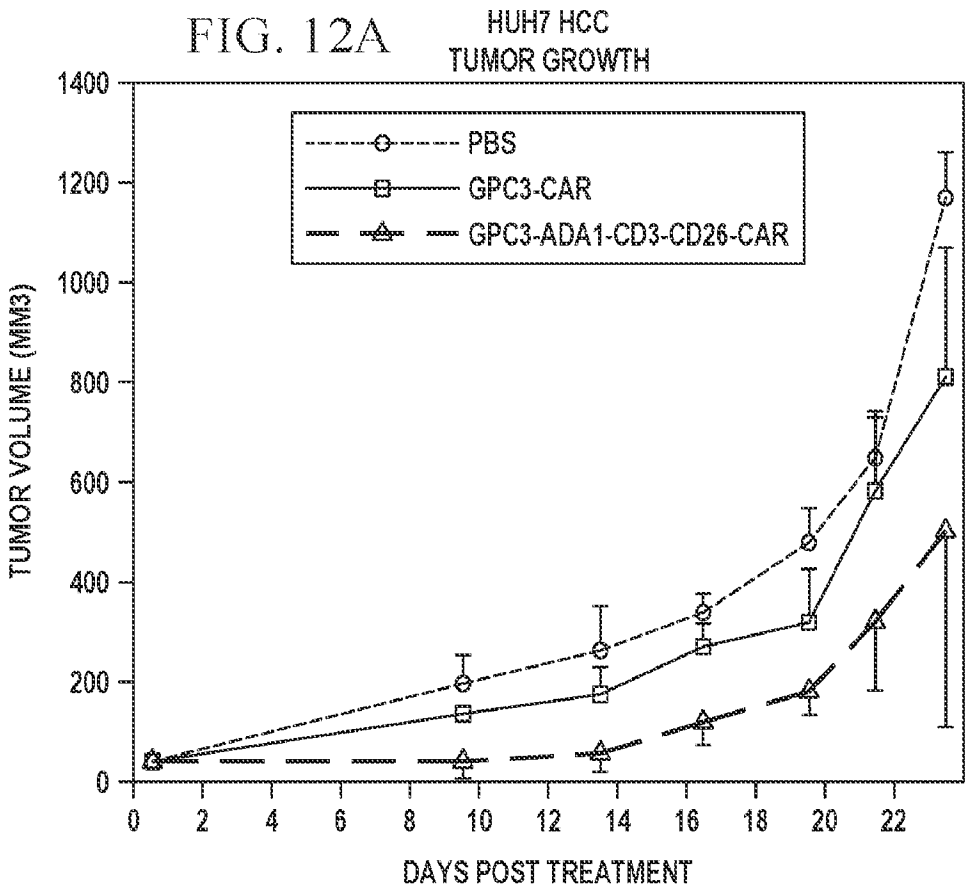

HUH7 HCC
TUMOR GROWTH
PBS
GPC3-CAR
GPC3-ADA1-CD3-CD26-CAR
TUMOR VOLUME (MM3)
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
22
DAYS POST TREATMENT

FIG. 12B

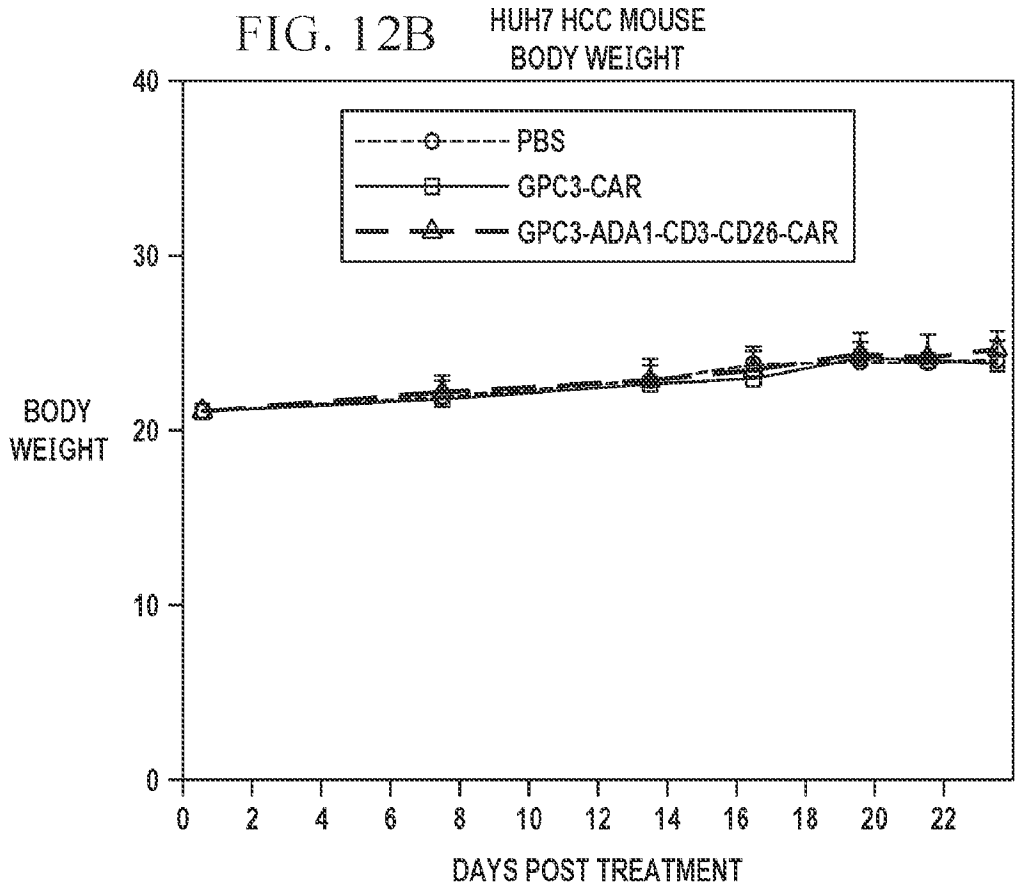

HUH7 HCC MOUSE
BODY WEIGHT
PBS
GPC3-CAR
GPC3-ADA1-CD3-CD26-CAR
BODY WEIGHT
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
DAYS POST TREATMENT

FIG. 12C

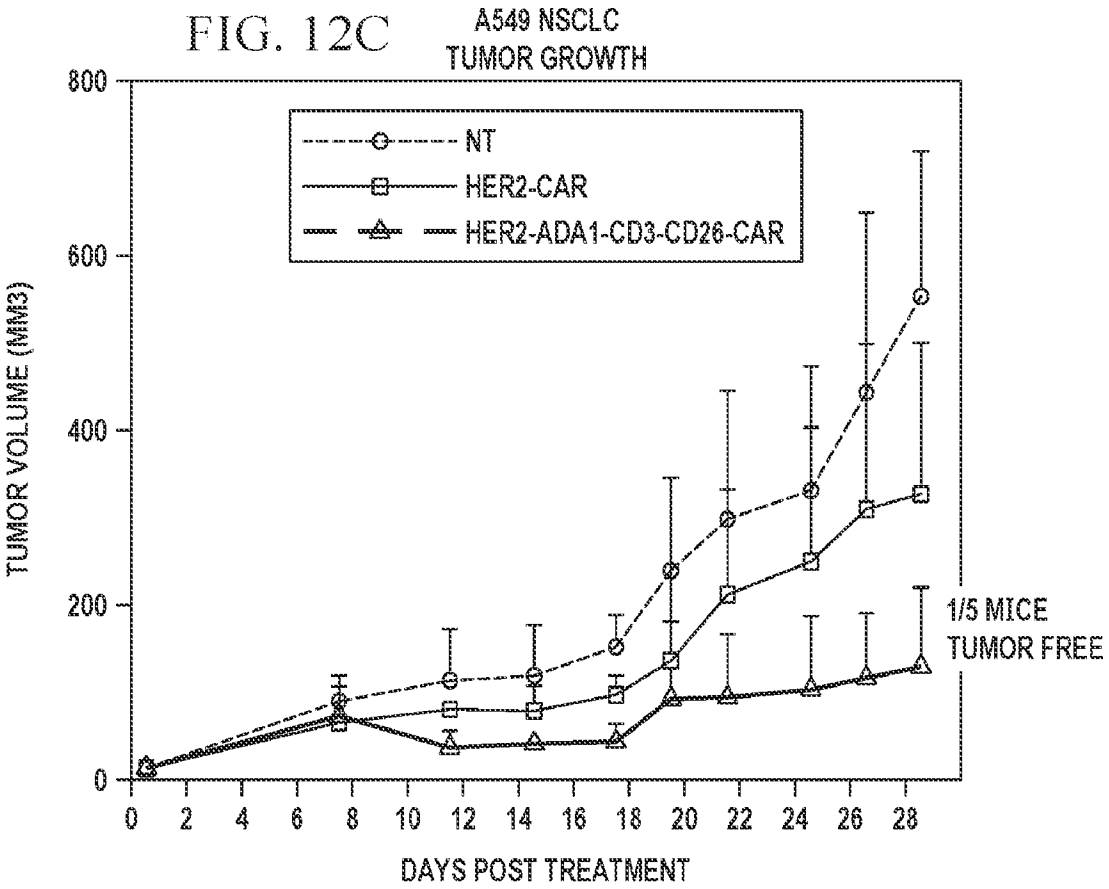
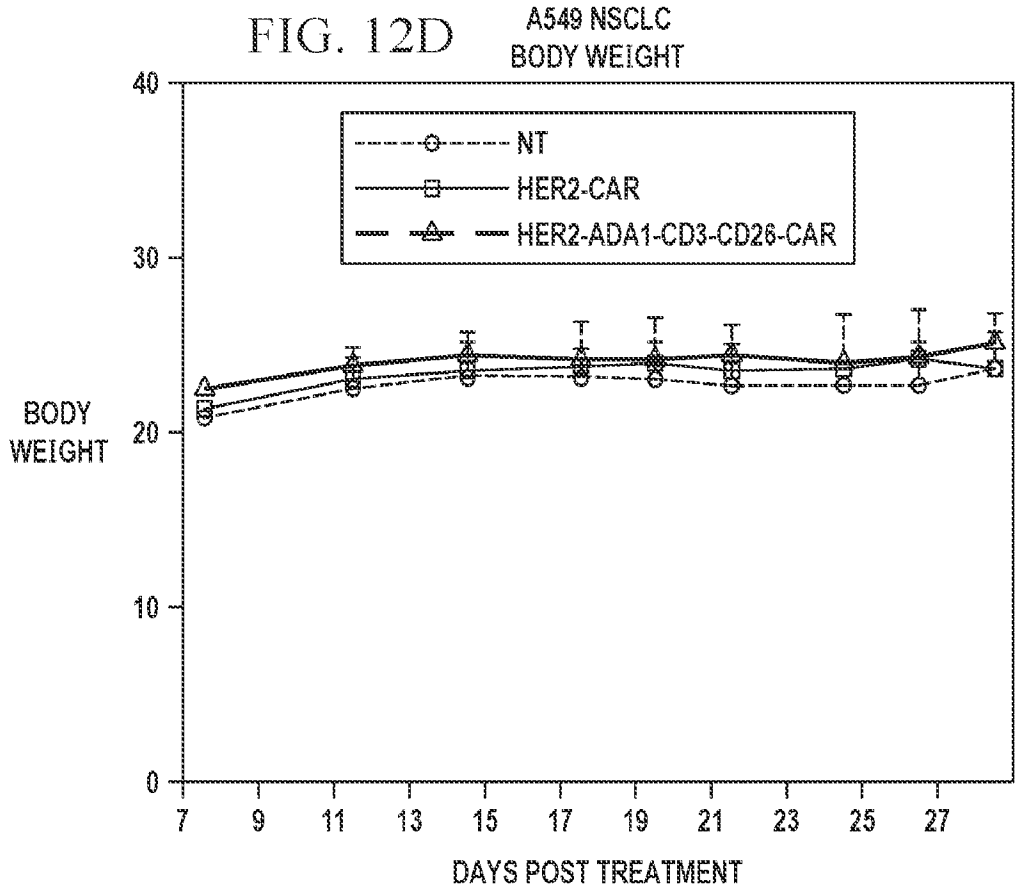

A549 NSCLC
TUMOR GROWTH
NT
HER2-CAR
HER2-ADA1-CD3-CD26-CAR
TUMOR VOLUME (MM3)
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS POST TREATMENT
1/5 MICE
TUMOR FREE

FIG. 12D


A549 NSCLC
BODY WEIGHT
NT
HER2-CAR
HER2-ADA1-CD3-CD26-CAR
BODY WEIGHT
40
30
20
10
0
7
9
11
13
15
17
19
21
23
25
27
DAYS POST TREATMENT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/42193

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 9/78, A61K 35/17, C12N 5/0783 (2022.01)
ADD. A61P 35/00 (2022.01)

CPC - INV. C12N 9/78, C12Y 305/04004, A61K 35/17, C12N 5/0636, C07K 2319/01
ADD. A61P 35/00, C12N 2510/00, C12N 2310/20

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/120982 A2 (AUTOLUS LIMITED) 18 June 2020 (18.06.2020) pg 4 ln 13, pg 34 ln 24-32, claims 1, 12-14.	1-3
A	WO 2020/168122 A1 (BEAM THERAPEUTICS, INC.) 20 August 2020 (20.08.2020) pg 2 ln 24-25, claim 1, claim 47.	1-3
A	US 2018/0216095 A1 (HALOZYME, INC.) 2 August 2018 (02.08.2018) claims 31-35.	1-3

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2022	JAN 13 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/42193

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/42193

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

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

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

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

3. ☒ Claims Nos.: 4-21, 28-48, 51-52, 56, 59-61
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
----continued on Extra Sheet-----

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-3

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/42193

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-3, drawn to an engineered cell comprising a nucleotide sequence encoding an Adenosine deaminase (ADA).

Group II: Claims 22-27, drawn to a recombinant oncolytic virus comprising a nucleotide sequence encoding an ADA, wherein the nucleotide sequence encoding the ADA is operably linked to a promoter.

Group III: Claims 49-50, 53-55, 57-58, drawn to a recombinant protein comprising an amino acid sequence encoding an ADA.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of an engineered cell comprising a nucleotide sequence encoding an Adenosine deaminase (ADA), not required by Groups II or III.

Group II has the special technical feature of a recombinant oncolytic virus comprising a nucleotide sequence encoding an ADA, wherein the nucleotide sequence encoding the ADA is operably linked to a promoter, not required by Groups I or III.

Group III has the special technical feature of a recombinant protein comprising an amino acid sequence encoding an ADA, not required by Groups I or II.

Common Technical Feature:

Groups I-III share the common technical feature of the enzyme, adenosine deaminase (ADA), or a nucleic acid encoding it.

However, said common technical feature does not represent a contribution over the prior art, and is disclosed by US 8,071,741 B2 to Filpula et al. (hereinafter "Filpula").

As to the common technical feature, Filpula discloses the recombinant enzyme, adenosine deaminase (ADA), and a nucleic acid encoding it (claims 1-2; 1. "An isolated recombinant adenosine deaminase comprising the polypeptide of SEQ ID NO:1, or an isolated recombinant adenosine deaminase variant polypeptide of SEQ ID NO:1, wherein the polypeptide variant of SEQ ID NO:1 comprises one or more amino acid substitutions selected from the group consisting of: Gin in place of Lys198; Ala in place of Thr245; and Arg in place of Gly351. 2. The recombinant adenosine deaminase of claim 1 that is encoded by the DNA molecule of SEQ ID NO: 2").

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I-III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning claim 50: Claim 50 is written to depend from claim 47, but claim 47 does not have antecedent precedence to "recombinant protein". For the purposes of the International Search & Opinion, claim 50 is interpreted to depend from claim 49, which does have antecedent precedent to "recombinant protein".

Note concerning claim 57: Claim 57 is written to depend from claim 47, but claim 47 does not have antecedent precedence to "recombinant protein". For the purposes of the International Search & Opinion, claim 57 is interpreted to depend from claim 49, which does have antecedent precedent to "recombinant protein".

Item 4 (continued): Claims 4-21, 28-48, 51-52, 56, 59-61 are dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).