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(71) Applicants: **THE CALIFORNIA INSTITUTE FOR BIOMEDICAL RESEARCH** [US/US]; 11119 North Torrey Pines Road, Suite 100, La Jolla, California 92037 (US). **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, La Jolla, California 92037 (US).

(72) Inventors: **WANG, Feng**; 6151 Citracado Circle, Carlsbad, California 92009 (US). **PINKERTON, Stephanie A.**; 3535 Monair Drive, Unit D, San Diego, California 92117 (US). **LIU, Tao**; 8020 Camino Jonata, San Diego, California 92122 (US). **WANG, Rongsheng E.**; 9605 Genesee Avenue, Apt. C2, San Diego, California 92121 (US). **SCHULTZ, Peter G.**; 1650 La Jolla Rancho Road, La Jolla, California 92037 (US).

(74) Agent: **HARDT, Ingo H.**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (US).

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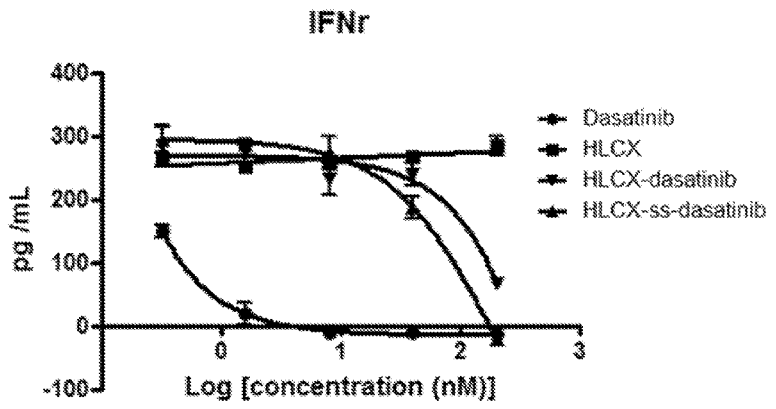
(54) Title: ANTIBODY DRUG CONJUGATES FOR THE TREATMENT OF IMMUNE CONDITIONS

FIG. 14A



(57) Abstract: Disclosed herein are antibody kinase inhibitor conjugates. The antibody kinase inhibitor conjugates may be used to treat conditions such as autoimmune diseases and cancers.

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ANTIBODY DRUG CONJUGATES FOR THE TREATMENT OF IMMUNE CONDITIONS

BACKGROUND OF THE INVENTION

[001] Antibody-drug conjugates (ADCs) are immunotherapeutic agents that allow the targeted delivery of potent cytotoxic agents to cancer cells. There is a need for novel ADC agents that have the ability to target many different cells and disease pathways. Currently, the NIH estimates that 23.5 million Americans suffer from various autoimmune diseases, with an annual direct health care cost of 100 billion dollars.

SUMMARY OF THE INVENTION

[002] Provided herein are novel ADCs, their methods of construction, and uses thereof. In certain embodiments the novel ADCs comprise a chimeric antibody. In certain embodiments, the chimeric antibody may further comprise an immunoglobulin heavy–chain complementarity determining region (CDR) or fragment thereof, of an antibody specific for a cell surface antigen, engrafted into the CDR of a different immunoglobulin. In certain embodiments, the chimeric antibody may be conjugated to a small molecule that acts as an inhibitor or activator of a disease relevant protein or signal transduction pathway. In certain embodiments, the disease relevant protein may be a protein kinase. In certain embodiments, the small molecule may be a kinase inhibitor. In certain other embodiments, the chimeric antibody may target the cell surface antigens expressed on immune cells. In certain embodiments, the immune cells are T cells. In certain embodiments the cell surface antigen is CD184 (also known as CXCR4). In certain other embodiments, the disease being treated is an autoimmune disorder.

[003] Disclosed herein are antibody kinase inhibitor conjugates that interact with a cell surface molecule on a target cell comprising: an antibody or antibody fragment; and a kinase inhibitor, wherein the antibody or antibody fragment is attached to the kinase inhibitor. The antibody or antibody fragment may bind the cell surface molecule on the target cell. The kinase inhibitor may inhibit a kinase expressed by a hematopoietic cell. The hematopoietic cell may be selected from a lymphocyte, a B cell, a T cell, a monocyte and a macrophage. The hematopoietic cell may be a T cell. The kinase inhibitor may be a modulator of a T cell activity. The kinase inhibitor may inhibit a kinase that modulates an immune and/or inflammatory activity. The kinase inhibitor may inhibit a kinase downstream of a protein selected from: an epidermal growth factor receptor (EGFR), a vascular endothelial growth factor receptor (VEGFR), a platelet derived growth factor receptor (PDGFR), a hepatocyte growth factor receptor (HGFR), and a mast/stem cell growth factor receptor (SCFR). The kinase inhibitor may inhibit a Src kinase. The Src kinase may be selected from Src, Yes, Fyn, Fgr, Lck, Hck, Blk, Lyn, and Frk. The kinase inhibitor may inhibit a tyrosine kinase. The

kinase inhibitor may inhibit a kinase selected from: an Abelson murine leukemia viral oncogene homolog 1 (Abl), a breakpoint cluster region protein- Abelson murine leukemia viral oncogene homolog 1 fusion (Bcr-Abl), a Src kinase, an anaplastic lymphoma kinase (ALK), a spleen tyrosine kinase (Syk), a Bruton's tyrosine kinase (BTK), a janus kinase (JAK), and a RET tyrosine kinase. The kinase inhibitor may inhibit a kinase selected from Abl and BCR-Abl. The kinase inhibitor may be a non-peptide small molecule. The kinase inhibitor may be selected from afatinib, axitinib, bosutinib, cetuximab, crizotinib, dasatinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, mubritinib, nilotinib, pazopanib, pegaptanib, ruxolitinib, sorafenib, sunitinib, SU6656, tofacitinib, vandetanib, and vemurafenib. The kinase inhibitor may be dasatinib. The antibody or antibody fragment may bind a cell surface molecule on a target cell. The antibody kinase inhibitor may comprise a targeting peptide, wherein the targeting peptide interacts with the cell surface molecule on the target cell. The targeting peptide may be attached to the antibody or antibody fragment. The targeting peptide may be selected from a peptide that is conformationally constrained, a peptide comprising a beta strand, a peptide comprising a beta-hairpin, a peptide that can bind a deep ligand binding pocket of the cell surface molecule, and combinations thereof. The target cell may be a hematopoietic cell. The hematopoietic cell may be selected from a lymphocyte, a B cell, a T cell, a monocyte, and a macrophage. The hematopoietic cell may be a T cell. The cell surface molecule may be a T cell antigen. The T cell antigen may be selected from CD3, CD4, CD71, CD69, CD25, and CXCR4. The cell surface molecule may be CXCR4. The antibody or antibody fragment may be selected from a rabbit antibody, a rodent antibody, an avian antibody, a simian antibody, a human antibody, a humanized antibody, a chimeric antibody, a bovine antibody, and fragments thereof, and combinations thereof. The antibody or antibody fragment may comprise a bovine ultralong complementary determining region or portion thereof. The antibody or antibody fragment may bind the cell surface molecule with a K_d value equal to or less than about 2 nM. The targeting peptide may bind the cell surface molecule with a K_d value equal to or less than about 2 nM. The antibody kinase inhibitor conjugate may further comprise a linker, wherein the linker attaches the kinase inhibitor to the antibody or antibody fragment. The linker may be uncleavable. The linker may be cleavable. The linker may comprise a disulfide bond. The linker may be selectively cleaved inside the cell. The linker may comprise a peptide that increases solubility.

[004] Further disclosed herein are antibody drug conjugates that interact with a cell surface molecule on a benign immunomodulatory cell comprising: an antibody or antibody fragment that interacts with the cell surface molecule; and a drug that modulates an immune activity of the benign immunomodulatory cell. The benign immunomodulatory cell may be selected from a lymphocyte, a B cell, a T cell, a monocyte and a macrophage. The benign immunomodulatory cell may be a T cell.

The drug may be a non-peptide small molecule. The drug may be a kinase inhibitor. The drug may be dasatinib. The antibody drug conjugate may further comprise a targeting peptide, wherein the targeting peptide interacts with the cell surface molecule on the target cell. The targeting peptide may be selected from a peptide that is conformationally constrained, a peptide comprising a beta strand, a peptide comprising a beta-hairpin, and a peptide that can bind a deep ligand binding pocket of the cell surface molecule. The cell surface molecule may be a T cell antigen. The T cell antigen may be selected from CD3, CD4, CD71, CD69, CD25, and CXCR4. The cell surface molecule may be CXCR4. The antibody or antibody fragment may be selected from a rabbit antibody, a rodent antibody, an avian antibody, a simian antibody, a human antibody, a humanized antibody, a chimeric antibody, a bovine antibody, and fragments thereof, and combinations thereof. The antibody or antibody fragment may comprise a bovine ultralong complementary determining region or portion thereof. The antibody or antibody fragment may bind the cell surface molecule with a K_d value equal to or less than about 2 nM. The targeting peptide may bind the cell surface molecule with a K_d value equal to or less than about 2 nM. The antibody drug may further comprise a linker, wherein the linker attaches the drug to the antibody or fragment thereof. The linker may be uncleavable. The linker may be cleavable. The linker may comprise a disulfide bond. The linker may be selectively cleaved inside the cell. The linker may comprise a peptide that increases solubility. The antibody-drug conjugate may have an immunosuppressive activity.

[005] Disclosed herein are methods of treating a condition in a subject in need thereof comprising administering of any one of the antibody kinase inhibitor conjugate or the antibody drug conjugate disclosed herein. The condition may be an immune condition. The condition may be an autoimmune disease. The condition may be an inflammatory condition. The condition may not be a cancer.

[006] Further disclosed herein are uses of any one of the antibody kinase inhibitor conjugates or the antibody drug conjugates disclosed herein in the manufacture of a medicament for the treatment of an autoimmune disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[007] **FIG.1** A representative flow cytometric analysis of CD3 and CXCR4 antigen levels on human T cells purified from healthy human donors.

[008] **FIG. 2A** shows a crystal structure of trastuzumab Fab (PDB code: 1N8Z). CDR3H of trastuzumab is the darker line shown within the dashed square, and the side chains of Arg98 and Asp108 are marked.

[009] **FIG. 2B** shows a graphic representation of anti-CXCR4 anti-body(HLCX) design. A disulfide cross-linked CXCR4-specific beta-hairpin peptide (red) stabilized by an anti-parallel β-stranded linker (blue) was engineered onto the CDR3H loop of trastuzumab.

[010] **FIG. 2C** shows a flow cytometry histogram showing the binding of HLCX to CXCR4-expressing Jurkat T cells.

[011] **FIG. 2D** shows a flow cytometry histogram showing the binding of HLCX to CXCR4-negative MDA-MB435 cells.

[012] **FIG. 2E** shows a flow cytometry histogram showing the binding of HLCX to and Her2-transfected MDA-MB435 cells (E).

[013] **FIG. 2F** shows a measurement of binding affinity between HLCX and human CXCR4 by Tag-lite HTRF binding assay.

[014] **FIG. 3A** shows a characterization of anti-CXCR4 antibody HLCX by SDS-PAGE analysis with or without DTT reduction.

[015] **FIG. 3B** shows a characterization of anti-CXCR4 antibody HLCX ESI-MS analysis of HLCX light chain (LC).

[016] **FIG. 3C** shows a characterization of anti-CXCR4 antibody HLCX ESI-MS analysis of HLCX deglycosylated heavy chain (HC).

[017] **FIG. 4** shows flow cytometric analysis of Her2 antigen levels on breast cancer cell line MDA-MB-435 as well as the Her2-transfected MDA-MB-435.

[018] **FIG. 5A** shows flow cytometry analysis of HLCX binding to HEK cells (low expression of CXCR4).

[019] **FIG. 5B** shows flow cytometry analysis of HLCX binding to CXCR4-transfected HEK cells.

[020] **FIG. 5C** shows flow cytometry analysis of HLCX binding to SJSA1A cells (CXCR4++).

[021] **FIG. 5D** shows flow cytometry analysis of HLCX binding to R7T1 cells (CXCR4–).

[022] **FIG. 5E** shows flow cytometry analysis of HLCX binding to 3T3L1 cells (CXCR4+).

[023] **FIG.6A** shows ESI-MS spectra of HLCX conjugate with Alexa Fluor 488 dye-NHS ester. Antibody conjugates were treated with PNGase F (New England Biolab) to remove N-glycans and with 10 mM DTT to afford reduced light chains (LC). All signal peaks are expected, with on average ~25% dye conjugation per light chain and at least ~1.5 equivalent conjugation per heavy chain, making it in total ~ four equivalent of dyes per antibody.

[024] **FIG.6B** shows ESI-MS spectra of HLCX conjugate with Alexa Fluor 488 dye-NHS ester. Antibody conjugates were treated with PNGase F (New England Biolab) to remove N-glycans and with 10 mM DTT to afford reduced heavy chains (HC). All signal peaks are expected, with on average ~25% dye conjugation per light chain and at least ~1.5 equivalent conjugation per heavy chain, making it in total ~ four equivalent of dyes per antibody.

[025] **FIG. 7A** shows confocal microscopy of internalization of anti-CXCR4 antibodies labeled with Alexa Fluor-488. Human T cells were incubated with 50 nM HLCX-AF488 for 30 min at 37° C. Cells were then fixed, stained with Hoechst dye (blue, nucleus), Alexa Fluor 594-conjugated wheat germ agglutinin (red, membrane), and imaged with a Leica 710 confocal microscope. Bar = 2 μm.

[026] **FIG. 7B** shows confocal microscopy of internalization of anti-CXCR4 antibodies labeled with Alexa Fluor-488. Human T cells were incubated with 50 nM HLCX-AF488 for 30 min at 4° C. Cells were then fixed, stained with Hoechst dye (blue, nucleus), Alexa Fluor 594-conjugated wheat germ agglutinin (red, membrane), and imaged with a Leica 710 confocal microscope. Bar = 2 μm.

[027] **FIG. 7C** shows confocal microscopy of internalization of anti-CXCR4 antibodies labeled with Alexa Fluor-488. Human T cells were incubated with 50 nM 12G5-AF488 at 37° C. Cells were then fixed, stained with Hoechst dye (blue, nucleus), Alexa Fluor 594-conjugated wheat germ agglutinin (red, membrane), and imaged with a Leica 710 confocal microscope. Bar = 2 μm.

[028] **FIG. 7D** shows confocal microscopy of internalization of anti-CXCR4 antibodies labeled with Alexa Fluor-488. Human T cells were incubated with 50 nM HLCX-AF488 at 37° C in the presence of 1 μM HLCX. Cells were then fixed, stained with Hoechst dye (blue, nucleus), Alexa Fluor 594-conjugated wheat germ agglutinin (red, membrane), and imaged with a Leica 710 confocal microscope. Bar = 2 μm.

[029] **FIG. 8A** shows ESI-MS spectra of untreated 12G5 LC conjugate with Alexa Fluor 488 dye-NHS ester.

[030] **FIG. 8B** shows ESI-MS spectra of antibody conjugates treated with PNGase F (New England Biolab) and DTT to afford reduced light chains. All signal peaks are expected, with on average one equivalent of dye conjugation per light chain and close to one equivalent conjugation per heavy chain, making it in total ~ four equivalent of dyes per antibody.

[031] **FIG. 8C** shows ESI-MS spectra of untreated 12G5 conjugate HC with Alexa Fluor 488 dye-NHS ester.

[032] **FIG. 8D** shows ESI-MS spectra of 12G5 conjugate treated with PNGase F (New England Biolab) and DTT to afford reduced heavy chains (HC). All signal peaks are expected, with on

average one equivalent of dye conjugation per light chain and close to one equivalent conjugation per heavy chain, making it in total ~ four equivalent of dyes per antibody.

[033] FIG. 9A shows *in vitro* inhibition of Lck kinase activity by Dasatinib parent compound, serially diluted and incubated with Lck in the presence of 50 μM ATP for three hours. ATP consumption was then measured and normalized to reflect the percent of inhibition. The potency of dasatinib is 0.022 nM, EC_{50}.

[034] FIG. 9B shows *in vitro* inhibition of Lck kinase activity by Dasatinib derivative 3, serially diluted and incubated with Lck in the presence of 50 μM ATP for three hours. ATP consumption was then measured and normalized to reflect the percent of inhibition. The potency of dasatinib derivative (0.064 nM, EC_{50}) is similar as that of dasatinib (0.022 nM, EC_{50}).

[035] FIG. 9C shows a lack of in vitro inhibition of Lck kinase activity by DMSO control, serially diluted and incubated with Lck in the presence of 50 μM ATP for three hours.

[036] FIG. 10 SDS-PAGE of HLCX conjugates including HLCX-dasatinib (non-cleavable ADC) and HLCX-ss-dasatinib (cleavable ADC).

[037] FIG 11A shows ESI-MS spectra of HLCX conjugates with dasatinib non-cleavable linker. ADC samples were treated with PNGase F (New England Biolab) to remove N-glycans and treated with 10 mM DTT to afford reduced light chains. All signal peaks are expected, with on average 50% drug conjugation per light chain and one equivalent conjugation per heavy chain, making it in total ~ three equivalent of drugs per antibody.

[038] FIG 11B shows ESI-MS spectra of HLCX conjugates with dasatinib cleavable linker. ADC samples were treated with PNGase F (New England Biolab) to remove N-glycans and treated with 10 mM DTT to afford reduced light chains. All signal peaks are expected, with on average 50% drug conjugation per light chain and one equivalent conjugation per heavy chain, making it in total ~ three equivalent of drugs per antibody.

[039] FIG 11C shows ESI-MS spectra of HLCX conjugates with dasatinib non-cleavable linker. ADC samples were treated with PNGase F (New England Biolab) to remove N-glycans and treated with 10 mM DTT to afford reduced heavy chains. All signal peaks are expected, with on average 50% drug conjugation per light chain and one equivalent conjugation per heavy chain, making it in total ~ three equivalent of drugs per antibody.

[040] FIG 11D shows ESI-MS spectra of HLCX conjugates with dasatinib cleavable linker. ADC samples were treated with PNGase F (New England Biolab) to remove N-glycans and treated with 10 mM DTT to afford reduced heavy chains. All signal peaks are expected, with on average 50%

drug conjugation per light chain and one equivalent conjugation per heavy chain, making it in total ~ three equivalent of drugs per antibody.

[041] **FIG 12** shows flow cytometry analysis of CD69 (top row) and CD25 (top row) activation markers on stimulated human T cells (by 5μg/mL plate-coated anti-CD3/ 1μg/mL soluble anti-CD28 antibodies) treated with increasing concentrations (0 nM, 8 nM, 40 nM, 200 nM) of dasatinib, HLCX antibody, HLCX-dasatinib ADC and HLCX-ss-dasatinib ADC.

[042] **FIG. 13A** shows inhibition of TCR-triggered activation of human T cells, particularly inhibition of cytokine expression (Interleukin-2 and TNFα) of αCD3/αCD28-activated T cells by dasatinib (positive control, ~ 0.5 nM EC_{50}'s), unconjugated HLCX (negative control), HLCX-dasatinib non-cleavable ADC (32.1 ± 11.3 nM EC_{50} for IL-2, 66.1 ± 30.5 nM EC_{50} for TNFα) and HLCX-ss-dasatinib cleavable ADC (12.7 ± 5.8 nM EC_{50} for IL-2, 26.3 ± 9.0 nM EC_{50} for TNFα).

[043] **FIG. 13B** shows a Western blot for TCR complex signal transduction. T cells were stimulated in the presence of dasatinib (lane 2), HLCX (lane 3), HLCX-ss-dasatinib ADC (lane 4), or trastuzumab-ss-dasatinib (lane 5) at 37° C, then immediately lysed. Proteins were separated on SDS-PAGE, transferred to PVDF membrane and probed with pan anti-phosphotyrosine antibody. eIF4E served as the loading control.

[044] **FIG. 14A** shows suppression of T cell activation by dasatinib and related ADCs, particularly interferon gamma (IFNγ) secretion levels of T cells treated by dasatinib (~ 0.5 nM EC_{50}), HLCX (negative control), HLCX-dasatinib (~ 123.5 nM ± 43.3 nM EC_{50}), and HLCX-ss-dasatinib (58.7 nM ± 28.6 nM EC_{50})

[045] **FIG. 14B** shows viability of T cells activated by αCD3/αCD28 in the presence of dasatinib, HLCX antibody, and two dasatinib ADCs. Viability was checked 36 h after stimulation and normalized against that of untreated T cells.

[046] **FIG. 15** Cytokine secretion analysis of αCD3/αCD28-activated human T cells that were either treated with Herceptin conjugated with the dasatinib cleavable linker or dasatinib small molecule (positive control).

DETAILED DESCRIPTION OF THE INVENTION

[047] Kinase inhibitors have shown considerable success for the treatment of cancers, autoimmune diseases and inflammatory diseases. Unfortunately, many kinase inhibitors, including those currently in clinical use, suffer from a lack of selectivity for related kinase family members, leading to off-target toxicity. This issue has largely limited kinase inhibitors to the treatment of cancer, despite their considerable potential in other disease settings. For example, dasatinib has potent activity in

inhibiting T cell activation, but leads to severe side effects including nausea, neutropenia, and pleural effusions, which undermine its development as an immunosuppressive agent. Disclosed herein are methods and compositions for selectively targeting kinase inhibitors to immune cells/lymphocytes by incorporating kinase inhibitors into antibody-drug conjugates, wherein the antibody portion of the antibody-drug conjugate binds a lymphocyte antigen, thereby improving the kinase inhibitor therapeutic index.

[048] To selectively deliver dasatinib to T lymphocytes, antibody-drug conjugates may comprise an antibody that bind one or more of several T cell antigens, including CD3, CD4, CD71, and CD184 (CXCR4). Among these, CXCR4 is highly expressed on the surface of human T cells (**FIG. 1**), but has minimal to no expression on non-hematopoietic cells as well as resting neutrophils. Although CXCR4 is also expressed on hematopoietic stem cells (HSCs), B-cells, and monocytes, delivery of dasatinib to these cells is not likely to cause serious adverse effects. Moreover, it has been demonstrated that antibodies that bind CXCR4 are efficiently internalized, and their antagonism of CXCR4-signalling is not associated with significant adverse clinical effects. A bovine anti-CXCR4 antibody was recently developed that specifically binds to CXCR4 with high affinity by grafting a CXCR4 peptide antagonist into the extended complementarity determining region (CDR) of the bovine antibody (BLV1H12) scaffold. However, to use this antibody as a carrier of a dasatinib ADC, a human version with trastuzumab (HCLX) was generated in order to avoid a neutralizing immune response upon chronic administration. It is known that internalization efficiency depends on binding epitopes. The long CDR3H of HCLX could target the ligand binding pocket of CXCR4 deeply, which likely contributes to its high internalization efficiency.

Antibody-Drug Conjugates

[049] Disclosed herein are antibody-drug conjugates, comprising an antibody or antibody fragment attached to or fused to a drug. Generally, the drug is a kinase inhibitor. The antibody-drug conjugate may comprise more than one antibody or antibody fragment. The antibody-drug conjugate may comprise a first antibody or antibody fragment and a second antibody or antibody fragment. The first antibody or antibody fragment may bind a first cell surface molecule and the second antibody or antibody fragment may bind a second cell surface molecule. The first cell surface molecule and the second cell surface molecule may be the same. The first cell surface molecule and the second cell surface molecule may be different.

[050] The antibody-drug conjugate may comprise more than one kinase inhibitor. The antibody-drug conjugate may comprise a first kinase inhibitor and a second kinase inhibitor. The first kinase inhibitor may inhibit a first kinase and the second kinase inhibitor may inhibit a second kinase. The

first kinase and the second kinase may be the same. The first kinase and the second kinase may be different.

[051] The antibody-drug conjugate may have a high selectivity. The high selectivity may be due to the affinity of the kinase inhibitor for the kinase. The high selectivity may be due to the affinity of the antibody or antibody fragment for the cell surface molecule. The high selectivity may be due to both the affinity of the kinase inhibitor for the kinase and the affinity of the antibody or antibody fragment for the cell surface molecule. The high selectivity may be a high selectivity for a cell surface molecule. The high selectivity may be a high selectivity for a kinase. The high selectivity may be a high selectivity for a cell that expresses the cell surface molecule and the kinase.

[052] The antibody-drug conjugate may comprise one or more linkers that links the antibody or antibody fragment to the drug. The linker may be a non-peptide. The linker may contain one or more polyethylene glycol units. The linker may be a peptide.

[053] The antibody kinase inhibitor conjugate of claims 1, wherein the antibody kinase inhibitor conjugate can inhibit a target activity with an EC50 of less than about 15 nM, less than about 20 nM, less than about 25 nM, less than about 30 nM, less than about 35 nM, less than about 40 nM, less than about 45 nM, less than about 50 nM, less than about 55 nM, less than about 60 nM, less than about 65 nM, less than about 70 nM, less than about 75 nM, less than about 80 nM, less than about 85 nM, less than about 90 nM, less than about 95 nM, less than about 100 nM, , less than about 105 nM, less than about 110 nM, less than about 115 nM, less than about 120 nM, less than about 125 nM, less than about 130 nM, less than about 135 nM, less than about 140 nM, less than about 145 nM, or less than about 150nM.

Antibodies and Antibody Targets

[054] Disclosed herein are compositions and methods for treating a condition in a subject in need thereof comprising administering an antibody-drug conjugate, wherein the antibody-drug conjugate comprises an antibody or antibody fragment. The antibody or antibody fragment may bind a cell surface molecule on an immunomodulatory cell. The term “immunomodulatory cell,” as used herein, may refer to a cell capable of generating, producing and/or displaying an immune activity and/or inflammatory activity, which includes, but is not limited to cytokine production, chemokine production, chemotaxis, cellular migration, phagocytosis and antibody or pathogen recognition. Thus, immunomodulatory cells may include, but are not limited to, an adipose tissue macrophage, an alveolar macrophage, a B cell, a basophil, a dendritic cell, an eosinophil, an epithelioid cell, a gamma/delta T cell, a granulocyte, a histiocyte, a Hofbauer cell, an intraglomerular mesangial cell, a Kupffer cell, a Langerhans cell, a lymphocyte precursor cell, a macrophage, a myeloid precursor cell,

mast cell, a memory B cell, a memory T cell, a microglial cell, a monocyte, a natural killer cell, a natural killer T cell, a neutrophil, an osteoclast, a plasma cell, a regulatory T cell, a sinus histiocyte, and a T cell. The T cell may be CD4-positive. The T cell may be CD8-positive. The T cell may be both $CD4^+$ and $CD8^+$.

[055] Although immunomodulatory cells may be malignant (e.g. cancer cells) under various conditions that exist in nature, the term "immunomodulatory cell," also referred to as "benign immunomodulatory cell," as used herein, refers to a non-malignant (e.g. non-cancer) cell. Compositions and methods disclosed herein provide for antibody drug conjugates and antibody kinase inhibitor conjugates comprising antibodies and or antibody fragments that bind antigens on immunomodulatory cells (e.g. non-cancer cells). Compositions and methods disclosed herein also provide for antibody drug conjugates and antibody kinase inhibitor conjugates comprising antibodies and or antibody fragments that bind antigens on cancer cells (e.g. tumor associated antigens), thus targeting the drugs or kinase inhibitor to malignant/cancer cells.

[056] The antibody or antibody fragment may be a polyclonal antibody or polyclonal antibody fragment. The antibody or antibody fragment may be a monoclonal antibody or monoclonal antibody fragment.

[057] The antibody or antibody fragment of may bind a cell surface molecule selected from CD3, CD4, CD8, CD19, CD20, CD21, CD22, CD24, CD 25, CD27, CD28, CD34, CD44, CD45, CD62, CD62L, CD69, CD71, CD80, CD86, CD117, CD122, CD 152, CD184, CD195, CD197, and CD274.

[058] The antibody or antibody fragment may be selected from an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD8 antibody, an anti-CD19 antibody, an anti-CD20 antibody, an anti-CD21 antibody, an anti-CD22 antibody, an anti-CD24 antibody, an anti-CD25 antibody, an anti-CD27 antibody, an anti-CD28 antibody, an anti-CD34 antibody, an anti-CD44 antibody, an anti-CD45 antibody, an anti-CD62 antibody, an anti-CD62L antibody, an anti-CD69 antibody, an anti-CD71 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD117 antibody, an anti-CD122 antibody, an anti-CD 152 antibody, an anti-CD184 antibody, an anti-CD195 antibody, an anti-CD197 antibody, and an anti-CD274 antibody.

[059] The antibody or antibody fragment may be an anti-CXCR4 antibody or anti-CXCR4 antibody fragment. The anti-CXCR4 antibody may have a heavy chain sequence encoded by a sequence selected from: SEQ ID NOS. 1-3. The anti-CXCR4 antibody may have a light chain sequence encoded by SEQ ID NO. 4. The anti-CXCR4 antibody heavy chain may have a sequence wherein at least 90% of the nucleotides are identical to a sequence selected from SEQ ID NOS. 1-3. The anti-

CXCR4 antibody light chain may have a light chain sequence wherein at least 90% of the nucleotides are identical to SEQ ID NO. 4.

[060] As used herein, the antibody or antibody fragment, may also refer to a fusion antibody, wherein the fusion antibody comprises a non-targeting antibody or non-targeting antibody fragment and a targeting peptide, wherein the targeting peptide binds the cell surface molecule. The targeting peptide may be a non-antibody peptide, or in other words, a peptide that is not found or expressed within an antibody, wherein the antibody is produced by an animal immune system. In some instances, the non-targeting antibody or non-targeting antibody fragment may not bind the cell surface molecule. The non-targeting antibody or non-targeting antibody fragment may serve the purpose of extending the *in vivo* half life of the fusion antibody and/or increasing the stability of the fusion antibody, while the targeting peptide binds the cell surface molecule. As exemplified in the present application, the non-targeting antibody may be an anti-Her2 (trastuzumab), and the targeting peptide may be a CXCR4 binding peptide. The non-targeting antibody may bind an epitope that has low expression in the subject. The epitope may be expressed by cells that do not express or only express low levels of the drug target (e.g., the kinase), such that the antibody-drug conjugate has minimal off-target effects. In the present example of trastuzumab with the CXCR4 binding peptide, CXCR4-expressing cells are the predominant target, as Her2 (the target epitope of trastuzumab) has low expression in most subjects (i.e. subjects without breast cancer). Another ideal non-targeting antibody, for example, may be palivizumab, which binds an epitope expressed on respiratory syncytial virus, and therefore has low to no affinity for animal cell surface molecules.

[061] The targeting peptide may be an agonist. The targeting peptide may be an antagonist. The targeting peptide may be a cyclic peptide. The cyclic peptide may comprise a polypeptide chain wherein the amino termini and carboxyl termini, amino termini and side chain, carboxyl termini and side chain, or side chain and side chain are linked with a covalent bond that generates a ring, when the targeting peptide is not attached to the non-targeting antibody or non-targeting antibody fragment. The cyclic peptide may comprise a 2 or more amino acids. The cyclic peptide may be selected from a cyclic isopeptide, a cyclic depsipeptide, a bicyclic peptide and a homodetic cyclic peptide. The cyclic peptide may comprise a naturally occurring cyclic peptide. The cyclic peptide may comprise a synthetic cyclic peptide. The cyclic peptide may comprise a modified naturally occurring peptide. The targeting peptide may comprise a conformationally constrained peptide. The targeting peptide may comprise a peptide modified to comprise a conformationally constrained peptide. The conformationally constrained peptide may have a reduced conformational entropy relative to a respective peptide that is not conformationally constrained. The conformationally constrained peptide may comprise a rigid feature and/or a rigid region and/or a rigid domain. The

conformationally constrained peptide may be locked into a conformation by one or more bonds between non-consecutive amino acids. The one or more bonds may be a disulfide bond. A conformationally constrained peptide may have a greatly improved binding affinity and/or specificity to a target relative to endogenous or naturally-occurring binding partners of the target. By non-limiting example, the conformationally constrained peptide may be a peptide comprising a β-hairpin structure. The conformationally constrained peptide may comprise a region that is U-shaped, rigid, stalk-like, knob-like, pointed, angular or shaped to fit into a specific region of a target or binding partner. The targeting peptide may further comprise one or more turn sequences. The turn sequence may comprise one or more amino acids. The turn sequence comprise about 1, about 2, about 3, about 4 or about 5 amino acids. The turn sequence may comprise one or more amino acids selected from glycine, asparagine and proline. The turn sequence may provide the therapeutic agent with a target binding conformation. The targeting peptide may be a non-cyclic peptide. The targeting peptide may be less than about 30 peptides, less than about 25 peptides, less than 20 peptides, less than about 15 peptides or less than about 10 peptides. The targeting peptide may be synthesized. The targeting peptide may be genetically encoded. The targeting peptide may be naturally occurring. The targeting peptide may be synthetic. The targeting peptide may be a naturally occurring peptide comprising a modification. The modification can be an addition of one or more amino acids. The modification can be a deletion of one or more amino acids. The modification may be a re-arrangement of two or more amino acids.

[062] The targeting peptide may be fused to a terminus of the non-targeting antibody or non-targeting antibody fragment. The targeting peptide may be grafted within the non-targeting antibody or non-targeting antibody fragment. The targeting peptide may be fused to a region of the non-targeting antibody selected from: a light chain N-terminus, a heavy chain N-terminus, a light chain C-terminus, and a heavy chain C-terminus. The targeting peptide may be grafted into a region of the non-targeting antibody selected from: a light chain variable region, a light chain constant region, a heavy chain variable region, a heavy chain CDR1, a heavy chain CDR2, a heavy chain CDR3, and a hinge region. The targeting peptide may be grafted or fused to the non-targeting antibody or non-targeting antibody fragment via a targeting peptide linker. The targeting peptide linker may comprise a bovine ultralong CDR or portion thereof. The portion of the bovine ultralong CDR may comprise at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the naturally occurring bovine ultralong CDR. The targeting peptide linker may have a sequence homologous to a sequence of a bovine ultralong CDR3. Bovine ultralong CDR3s are described in the art, (see, *e.g.*, Zhang et al., Angew Chem Int Ed Engl. 2014 vol. 3: pp. 132-5, Zhang et al., Angew Chem Int Ed

Engl. 2013 vol. 5: pp. 8295-8, and Saini et al. Eur J Immunol. 1999 vol. 29: pp. 2420-6, all incorporated herein by reference). The bovine ultralong CDR3 may be a CDR3 of a BLV1H12 antibody. The targeting peptide linker may comprise a protein secondary structure. The protein secondary structure may comprise a beta strand. The protein secondary structure may comprise an alpha helix. The targeting peptide linker may comprise a protein tertiary structure. The protein tertiary structure may comprise a beta sheet. The protein tertiary structure may comprise a coiled coil. The targeting peptide linker may be flexible. The targeting peptide linker may be rigid, causing the targeting peptide to be less sterically hindered by the non-targeting antibody or non-targeting antibody fragment, and thus more available to bind the cell surface molecule, as opposed to a targeting peptide attached to the non-targeting antibody or non-targeting antibody fragment by a flexible linker. The targeting peptide linker may have a length of 1 or more amino acids. The targeting peptide linker may have a length of about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids or about 50 amino acids. The targeting peptide linker may provide for less steric hindrance between the targeting peptide and the cell surface molecule, thereby increasing binding and the therapeutic efficacy of the antibody-drug conjugate.

[063] The targeting peptide may comprise a secondary structure (e.g., beta strand, alpha helix), tertiary structure (e.g., coiled coil, beta strand), or a sequence that gives the targeting peptide a rigid or flexible structure.

Kinase Inhibitors

[064] Disclosed herein are compositions and methods for treating a condition in a subject in need thereof comprising administering an antibody-drug conjugate, wherein the antibody-drug conjugate comprises a drug. The drug may be a small molecule. The drug may be a peptide. The drug may be a kinase inhibitor.

[065] The kinase inhibitor may inhibit a kinase that promotes or suppresses a cellular activity selected from, but not limited to: growth, proliferation, cell signaling, apoptosis, and production of enzymes (e.g., growth factors, cytokines, extracellular matrix proteins/peptides, etc.).

[066] The kinase inhibitor may be a modulator of immune activity. The kinase inhibitor may suppress immune activity. As used herein, immune activity may also be referred to as "inflammatory activity" or "inflammation." The kinase inhibitor may modulate and/or suppress a T cell activity. The kinase inhibitor may modulate and/or suppress a T cell receptor activity. A T cell receptor activity may be selected from: phosphorylation of downstream effectors and complexing with co-receptors. The kinase inhibitor may modulate and/or suppress one or more T cell activities. The T

cell activity may be selected from: cellular proliferation, cellular migration, antibody production, target cell binding, cytokine production, and cell killing.

[067] The kinase inhibitor may inhibit a kinase selected from AATK, ABL, ABL2, ALK, AXL, BCR-ABL, BLK, BMX, BRAF, BTK, CSF1R, CSK, DDR1, DDR2, EGFR, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB6, ERBB1, ERBB2, ERBB3, ERBB4, FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRK, FYN, GSG2, HCK, IGF1R, ILK, INSR, INSRR, IRAK4, ITK, JAK1, JAK2, JAK3, KDR, KIT, KSR1, LCK, LMTK2, LMTK3, LTK, LYN, MATK, MERTK, MET, MLTK, MST1R, MUSK, NPR1, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PLK4, PTK2, PTK2B, PTK6, PTK7, PTPRC, RET, ROR1, ROR2, ROS1, RYK, SGK493, SRC, SRMS, STYK1, SYK, TEC, TEK, TEX14, TIE1, TNK1, TNK2, TNNI3K, TXK, TYK2, TYRO3, VEGFR1,VEGFR2, VEGFR3, YES1 and ZAP70.

[068] The kinase inhibitor may be selected from Afatinib, Axitinib, Bevacizumab, Bosutinib, Cetuximab, Crizotinib, Dasatinib, Erlotinib, Fostamatinib, Gefitinib, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Panitumumab, Pazopanib, Pegaptanib, Ranibizumab, Ruxolitinib, Sorafenib, Sunitinib, SU6656, Trastuzumab, Tofacitinib, Vandetanib, and Vemurafenib.

Therapeutic Uses

[069] Disclosed herein are compositions and methods for treating a condition in a subject in need thereof comprising administering an antibody-drug conjugate disclosed herein. The term "condition," as used herein, may refer to a disease, an infection, a disorder or a defect. The condition may be acute. The condition may be long-standing. The condition may be temporary. The condition may be permanent. The condition may be an immune condition. The condition may be an inflammatory condition. The condition may be an infection. The condition may be an autoimmune disease. The autoimmune disease may be selected from Acute disseminated encephalomyelitis (ADEM), Addison's disease, Agammaglobulinemia, Alopecia areata, Amyotrophic lateral sclerosis , Ankylosing Spondylitis, Antiphospholipid syndrome, Antisynthetase syndrome, Atopic allergy, Atopic dermatitis, Autoimmune aplastic anemia, Autoimmune cardiomyopathy, Autoimmune enteropathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune pancreatitis, Autoimmune polyendocrine syndrome, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune urticaria, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Behçet's disease, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous pemphigoid, Castleman's disease, Celiac disease, Chagas disease, Chronic

inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal osteomyelitis, Chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial pemphigoid, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Contact dermatitis, Cranial arteritis, CREST syndrome, Crohn's disease, Cushing's Syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Drug-induced lupus, Discoid lupus erythematosus, Eczema, Endometriosis, Enthesitis-related arthritis[32], Eosinophilic fasciitis, Eosinophilic gastroenteritis, Eosinophilic pneumonia, Epidermolysis bullosa acquisita, Erythema nodosum, Erythroblastosis fetalis, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibrosing alveolitis (or Idiopathic pulmonary fibrosis), Gastritis, Gastrointestinal pemphigoid, Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's encephalopathy, Hashimoto's thyroiditis, Henoch-Schonlein purpura, Herpes gestationis aka Gestational Pemphigoid, Hidradenitis suppurativa, Hughes-Stovin syndrome, Hypogammaglobulinemia, Idiopathic inflammatory demyelinating diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura , IgA nephropathy, Inclusion body myositis, Chronic inflammatory demyelinating polyneuropathy, Interstitial cystitis, Juvenile idiopathic arthritis aka Juvenile rheumatoid arthritis, Kawasaki's disease, Lambert-Eaton myasthenic syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease (LAD), Lupoid hepatitis, Lupus erythematosus, Majeed syndrome, Ménière's disease, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed connective tissue disease, Morphea, Mucha-Habermann disease (Pityriasis lichenoides et varioliformis acuta), Multiple sclerosis, Myasthenia gravis, Microscopic colitis, Myositis, Narcolepsy, Neuromyelitis optica (also Devic's disease), Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord's thyroiditis, Palindromic rheumatism, PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonage-Turner syndrome, Pars planitis, Pemphigus vulgaris, Pernicious anemia, Perivenous encephalomyelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progressive inflammatory neuropathy, Psoriasis, Psoriatic arthritis, Pyoderma gangrenosum, Pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syndrome, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatic fever, Sarcoidosis, Schmidt syndrome another form of APS, Schnitzler syndrome, Scleritis, Scleroderma, Serum Sickness, Sjögren's syndrome, Spondyloarthropathy Still's disease (Juvenile Rheumatoid Arthritis), Stiff person syndrome, Subacute bacterial endocarditis

(SBE), Susac's syndrome, Sweet's syndrome, Sydenham chorea see PANDAS, Sympathetic ophthalmia, Systemic lupus erythematosus see Lupus erythematosus, Takayasu's arteritis, Temporal arteritis (also known as "giant cell arteritis"), Thrombocytopenia, Tolosa-Hunt syndrome, Transverse myelitis, Ulcerative colitis , Undifferentiated connective tissue disease , Undifferentiated spondyloarthropathy, Urticarial vasculitis, Vasculitis, Vitiligo, and Wegener's granulomatosis.

[070] Disclosed herein are compositions that have an immunosuppressive activity. The immunosuppressive activity may reduce the efficacy of the immune system, inhibit an immune system activity and/or weaken the immune system. The immunosuppressive activity may inhibit inflammation. The immunosuppressive activity may inhibit an immune and/or inflammatory activity selected from: cytokine production, chemokine production, chemotaxis, cellular migration, cellular proliferation, cellular apoptosis, phagocytosis, antibody recognition, or pathogen recognition, and any combination thereof.

EXAMPLES

[071] The following illustrative examples are representative of embodiments of the invention, and methods described herein and are not meant to be limiting in any way.

Example 1 – Production of chimeric antibody specific for CXCR4

[072] CXCR4 is highly expressed on the surface of human T cells (**FIG. 1**). To engineer a humanized CXCR4 antibody, the long CDR3H of the bovine anti-CXCR4 antibody was grafted into CDR3H of trastuzumab, an antibody with minimal immunogenicity in humans (**FIG. 2A**). The long CDR3H of the bovine anti-CXCR4 consists of a disulfide cross-linked β-hairpin peptide that specifically binds the membrane-buried ligand binding pocket of CXCR4. The CXCR4 targeting hairpin peptide was inserted into the CDR3H between Arg98 and Asp108, replacing the original Trp99–Met107 loop in CDR3H of trastuzumab to afford the humanized antibody HLCX (**FIG. 2A and 2B**). HLCX was transiently expressed in HEK 293F cells, purified by Protein G chromatography with a final yield of ~5-10 mg/L. Denaturing SDS/PAGE gel electrophoresis demonstrated that the antibody was > 90% pure and resolved into bands of ~150 kDa (non-reducing conditions, full length IgG) and ~50 and ~25 kDa (reducing conditions, heavy and light chains, respectively) (**FIG. 3A**). Further analysis of HLCX by electrospray-ionization mass spectrometry (ESI-MS) indicated the expected molecular weight (**FIG. 3B**). The binding of HCLX to cell-surface CXCR4 was examined by flow cytometry. Incubation of 10 nM HLCX with Jurkat T cells (CXCRhigh) resulted in a peak shift of 96.2% by flow cytometry analysis (**FIG. 2C**). On the contrary, incubation of HLCX with MDA-MB435 cells (CXCRneg) did not result in any shift (**FIG. 2D**), suggesting that HLCX binds human CXCR4 specifically. Given that HLCX was derived from

trastuzumab scaffold, the binding of HLCX to Her2-transfected MDA-MB435 cells was also tested (**FIG. 4**). A minimal peak shift (**FIG. 2E**) demonstrated that the fusion into the CDR3H of trastuzumab has abrogated its recognition of cognate antigen (Her2). To further characterize the binding profile of HLCX, a series of flow cytometry analyses were performed on additional cell lines expressing different levels of CXCR4 (**FIG. 5**), which further confirmed that HLCX is a selective antibody towards CXCR4.HLCX's binding affinity with human CXCR4 was measured using Tag-lite homogeneous time-resolved fluorescence (HTRF), a highly specific method to measure CXCR4 binding. The K_d value for HLCX was determined to be 2.1 ± 0.2 nM (**FIG. 2F**).

Antibody expression purification

[073] Genes containing the engineered antibody were assembled by overlapping PCR and inserted into the pFuse backbone vector (InvivoGen, CA). The antibody was expressed by transient transfection of FreeStyle 293F cells (Life Technologies, CA). For maintenance, 293-F cells were cultured between 0.2×10^6 and 2×10^6 cells/mL in FreeStyle medium (Life Technologies, CA) in Minitron shakers at 37 °C. On the day of transfection, 293-F cells at a density of 10^6 cells/mL were transfected with heavy chain plasmid, light chain plasmid and 293fectin at a ratio of 2:1:6 as suggested by Life Technologies. Expression medium containing secreted proteins was harvested every 48 h twice after transfection. The antibody was purified by standard Protein A/G chromatography (Thermo Fisher Scientific, IL) and was analyzed by coomassie brilliant blue staining of SDS-PAGE, followed by ESI-mass spectrometric analysis.

Flow Cytometry Analysis

[074] For anti-CXCR4 binding assays with cell lines, cells were first blocked with blocking buffer (PBS / 3% BSA) at 4° C for 10 min and then incubated with 10 nM anti-CXCR4 antibody HLCX in the blocking buffer for 1 h. Cells were then washed with PBS and incubated with Alexa Fluor 647 or FITC conjugated goat anti-human IgG (Life Technologies, CA) in the blocking buffer following manufacturer's instruction. After incubation, cells were washed twice with the same medium and analyzed on a LSR II flow cytometer (Becton Dickinson). For antigen-expression analysis on T cells, the following fluorophore-conjugated anti-human antibodies were used: αCD3-PB (UCHT1, Biolegend, CA), αCXCR4-PE (12G5, Biolegend, CA), αCD69-PE (FN50, Biolegend, CA), αCD25-APC/Cy7 (M-A251, Biolegend, CA). Cells were stained with these antibodies in Hanks' balanced salt solution (HBSS, Mediatech, VA)/2% FBS for 45 min at 4° C, after which they were washed twice with HBSS/2% FBS and analyzed on LSR II flow cytometer equipped with a high throughput sampler (BD Bioscience, CA). All the results were processed via FlowJo software (Tree Star Inc.).

Tag-lite HTRF Binding Assay

[075] The Tag-lite HTRF binding assay was performed by following manufacturer's suggested procedure. Briefly, 10^6 Tag-lite labeled CXCR4 cells were thawed at 37 °C, centrifuged for 5 min at 1200g, and re-suspended in 2.7 mL 1X Tag-lite buffer after removal of supernatant. The cells were incubated with increasing concentrations of antibodies and 50 nM of fluorescent ligand (Chemokine CXCR4 receptor red agonist) for 3 h at room temperature. The signal was recorded by an EnVision multilabel plate reader (PerkinElmer) at 620 nm and 665 nm with 340 nm excitation. The binding between CXCR4 and SDF-1 was represented by ratio of signal 665/620 ×10000. The K_d between antibodies and CXCR4 were calculated based on the Cheng-Prusoff equation: $K_d=IC_{50}/([A]/EC_{50}+1)$, where [A] is the fixed concentration of SDF-1 and EC_{50} is the concentration of SDF-1 that results in half maximal activation of the CXCR4 receptor.

Example 2- Internalization of anti-CXCR4 antibodies

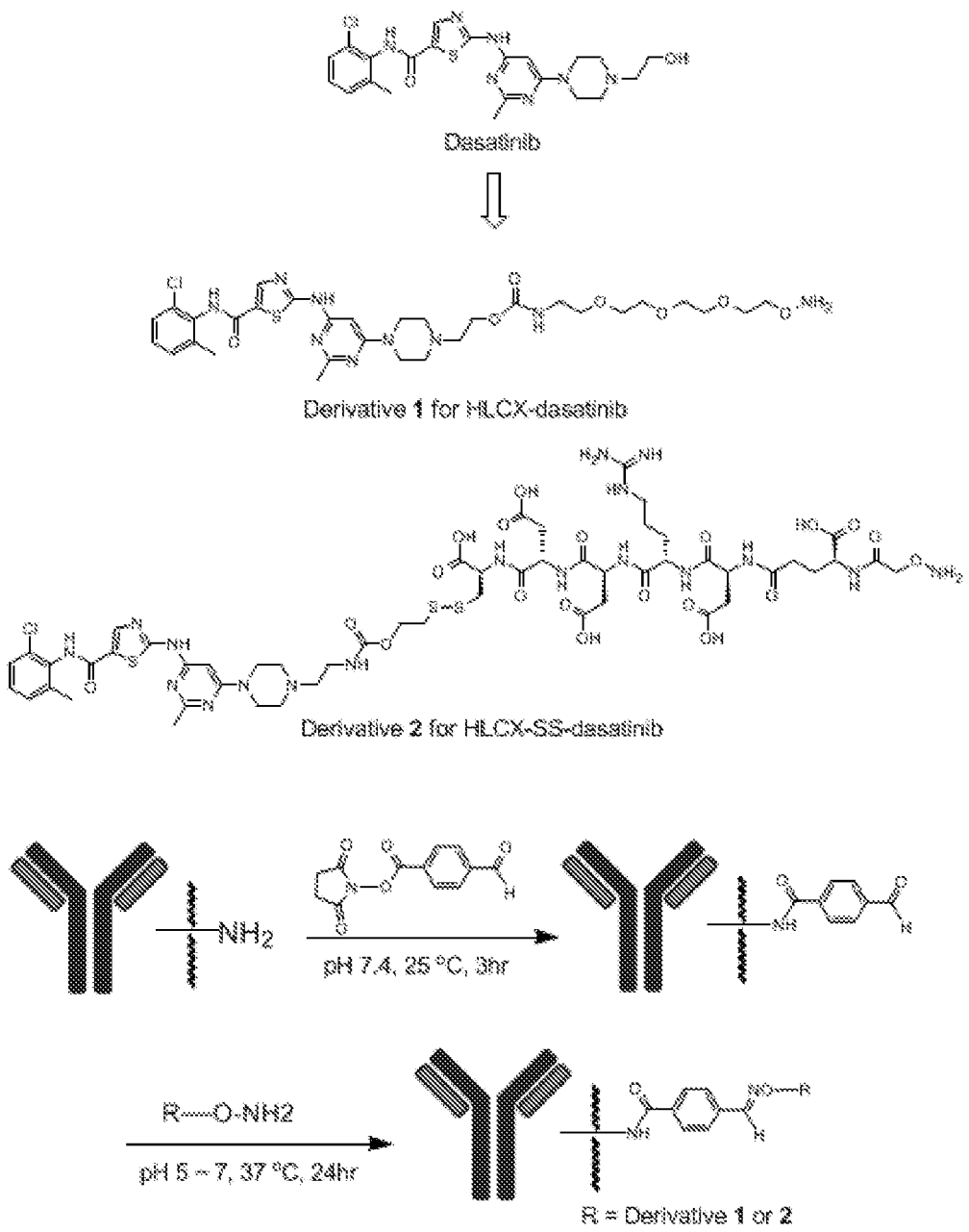
[076] Given its selectivity and high affinity, the internalization of HLCX was evaluated. The antibody was conjugated to Alexa Fluor 488 (AF488) (15x AF488-NHS, pH 7.4, 25° C, 3 hr.) at a drug-to-antibody ratio (DAR) of ~ 4 (**FIG. 6**), followed by confocal microscopic analysis of its endocytosis into human T cells (**FIG. 7**). For comparison, a common anti-CXCR4 commercial clone (12G5) was also conjugated with AF488 at the similar DAR (**FIG. 8**), and analyzed for internalization. As shown in **FIG. 2A**, HLCX-AF488 (green spots) was observed in the cytoplasm of T cells within 30 min at 37° C (**FIG. 7A**), thus indicating efficient endocytosis. This internalization could be inhibited by 4° C treatment (**FIG. 7B**) or a 20-fold excess of unconjugated HLCX antibody (**FIG. 7D**), suggesting it is CXCR4-mediated. In contrast, less 12G5-AF488 was observed inside the cytoplasm, suggesting that 12G5 internalized less efficiently than HLCX (**FIG. 7C**).

Confocal Microscopic Analysis

[077] Two million human T cells were suspended in an eppendorf tube containing 600 µL RPMI media supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were treated with 50 nM (final concentration) anti-CXCR4 IgG-AF488 in the presence or absence of 20-fold excess of unlabeled anti-CXCR4 IgG, at 37° C or 4° C for 30 min. Samples were then spun at 4° C, washed with PBS (3× 1mL), and fixed with 4% paraformaldehyde for 15 min. Cells were then washed with PBS/3% BSA (2× 1mL), followed by staining with Hoechst dye and Alexa Fluor 594-conjugated wheat germ agglutinin under manufacturer instructions (Life Tech, CA). Finally-prepared samples were imaged using Zeiss LSM 710 laser scanning confocal microscope.

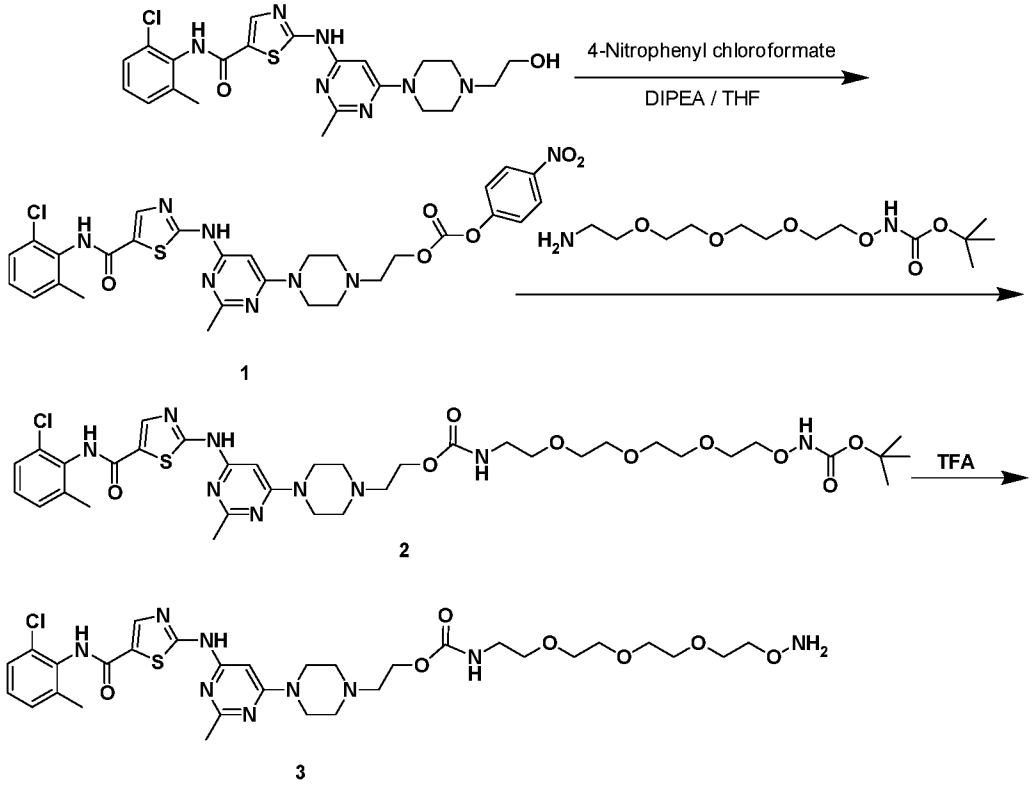
Example 3- Design and synthesis of dasatinib linker derivatives

[078] Exemplary Scheme 1 – synthetic scheme of dasatinib linker derivatives for conjugation with HLCX.

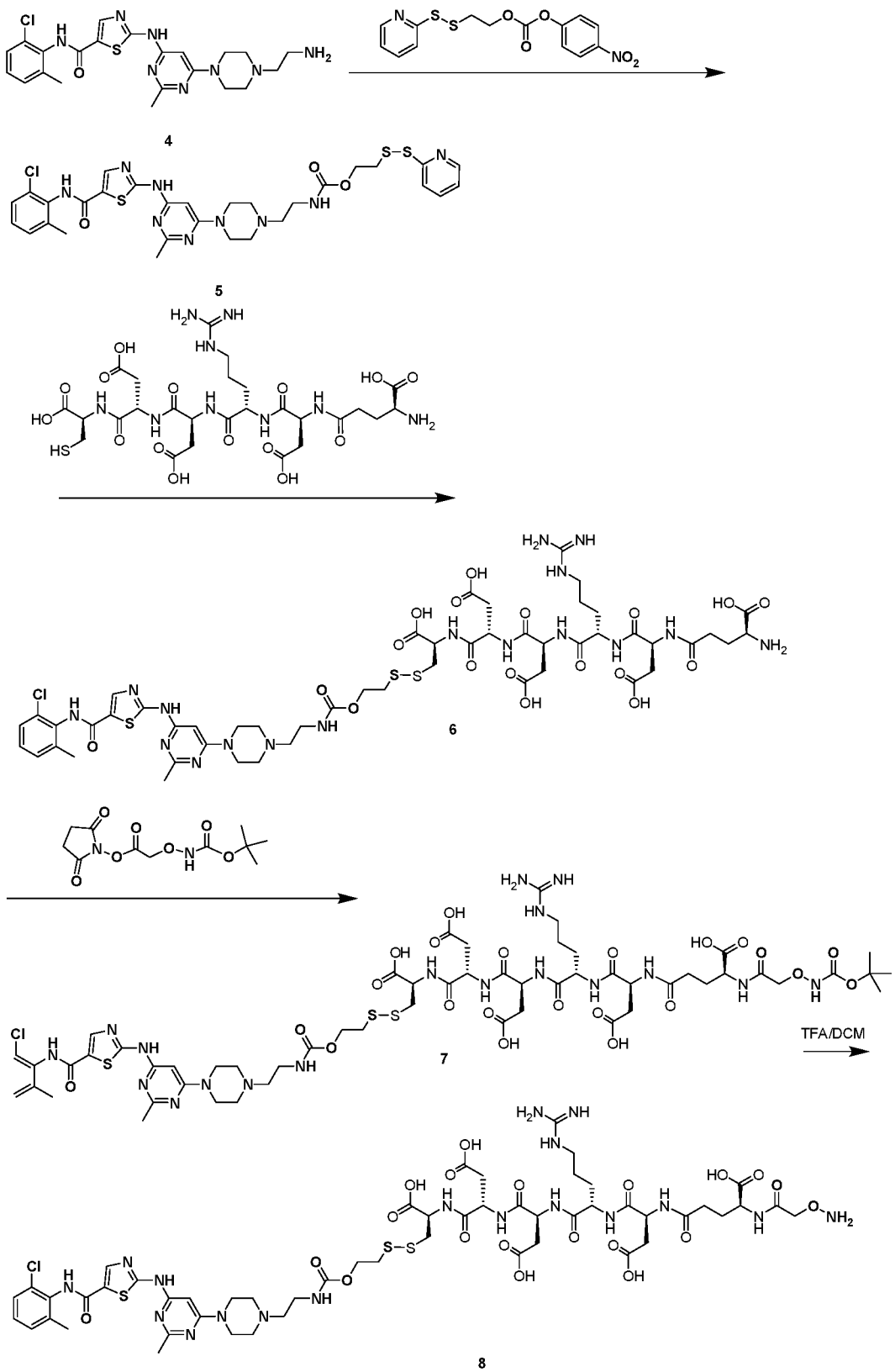


[079] Exemplary Scheme 1 shows synthesis of HLCX-based antibody drug conjugates (ADCs). Lysines of the antibody were reacted with N-succinimidyl-4-formylbenzamide to convert the amino group to benzaldehyde at a defined ratio that could then be conjugated with dasatinib derivatives (non-cleavable or cleavable with disulfide bond) bearing a terminal alkoxy amine.

[080] Exemplary Scheme 2 -- synthetic scheme of dasatinib derivative with a non-cleavable linker



[081] **Exemplary Scheme 3** -- synthetic scheme of dasatinib derivative with a disulfide-cleavable linker



[082] The hydroxyl group of dasatinib was modified through a mild acylation with p-nitrophenyl chloroformate, followed by carbamation via the tetra-polyethylene glycol (PEG) linker bearing a

protected aminooxy moiety (**FIG. 3**). The resulting intermediate **2** was readily deprotected to afford the desired derivative **3** with a non-cleavable linker. An in vitro kinase inhibition assay with Lck (**FIG. 9B**) confirmed that the derivative had a similar potency as the parent compound (EC_{50}'s of 64 pM vs. 22 pM, respectively). Dasatinib was also prepared with a disulfide-cleavable linker, which consisted of a peptide spacer unit to increase solubility, and a disulfide bond to be selectively cleaved inside cells to release the parent dasatinib compound (**FIG. 9A**). For this purpose, dasatinib was derivatized to have an –amino end, which maintained its activity while facilitated multi-step modifications to attach the linker.

[083] To facilitate comparison of activities between HLCX-dasatinib conjugates of different linkers, a two-step coupling procedure was developed that would allow for synthesis of ADCs with a defined conjugation ratio. As illustrated, the first step involved a large scale preparation of antibody with lysines converted to benzaldehydes (Solulink) (30x linker, pH 7.4, 25° C, 3 hr.), followed by removal of the small molecules through buffer exchange on Amicon filter or desalting columns. The purified antibody tagged with a fixed ratio of benzaldehydes was then reacted with different aminooxy-derived dasatinib linker compounds. The second step (oxime ligation with benzaldehyde) took place at 37° C, pH 6.5, 30x linker and was >95% complete after 24 hours by ESI-MS. The resulting conjugates with dasatinib non-cleavable linker and with disulfide-cleavable linker were designated as HLCX-dasatinib and HLCX-ss-dasatinib, respectively. Both ADCs were then purified by size exclusion chromatography (Superdex-200) to remove excess small molecules. As demonstrated by SDS-PAGE (**FIG. 10**) and ESI-MS (**FIG. 11**), the dasatinib ADCs were > 90% pure with DAR of ~3, and have expected molecular weights.

Detailed Synthesis of dasatinib linker derivatives

[084] Dasatinib powder (100mg, 0.20mmol) was mixed with 4-nitrophenyl chloroformate (45mg, 0.22 mmol) and 100 μL DIPEA in 50mL anhydrous THF. The solution was stirred at room temperature for eight hours, after which another 45 mg 4-nitrophenyl chloroformate was added. After overnight stirring, the solution turned clear and the solvent was evaporated under reduced pressure. The residue was neutralized by acetic acid and purified by flash column chromatography on silica gel (5% methanol in ethyl acetate) to afford **1** in yellowish color (108mg, yield 83%). 1HNMR (600MHz, DMSO-d6): δ11.06 (br s, 1H), 9.91(br s, 1H), 8.24(s, 1H), 8.12(d, J=8.4Hz, 2H), 7.40(dd, J=7.5Hz, 1.8Hz, 1H), 7.28(dd, J=7.6Hz, 7.5Hz, 1H), 7.26(dd, J=7.5Hz, 1.8Hz, 1H), 6.94(d, J=8.4Hz, 2H), 6.14(s, 1H), 4.36(br, 2H), 3.75-3.13(m, 10H), 2.45(s, 3H), 2.24(s, 3H); 13CNMR (151MHz, DMSO-d6): 166.2, 165.4, 163.9, 162.4, 161.9, 159.8, 157.3, 157.2, 140.8, 139.6, 138.8, 133.4, 132.4, 129.0, 128.2, 127.0, 126.2(2), 115.8(2), 83.4, 73.0, 55.3, 55.0, 53.5, 41.8, 40.7, 25.6, 18.3; HRMS calculated for $C_{29}H_{29}ClN_8O_6S$ [MH]+ 653.1698, observed 653.1694.

[085] A mixture of 2-[2-(2-(N-(tert-butyloxycarbonyl)aminooxyethoxy)ethoxy)-ethoxy]ethylamine (114 mg, 0.37 mmol, synthesized as reported1) and compound **1** (200 mg, 0.31 mmol) were dissolved in 30 mL anhydrous tetrahydrofuran, together with DIPEA (90 uL, 0.5 mmol). The solution was stirred under nitrogen until compound **1** was fully reacted as monitored by LC-MS. The solvent was then evaporated under vacuum and the mixture was purified by prep-HPLC to finally yield the desired product (158mg, yield 62%) (see Exemplary Scheme 1). 1HNMR (600MHz, CD3OD): δ 8.18 (s, 1H), 7.36(dd, J=7.5Hz, 1.8Hz, 1H), 7.26(dd, J=7.6Hz, 7.5Hz, 1H), 7.24 (dd, J=7.5Hz, 1.8Hz, 1H), 6.23 (s, 1H), 4.46(t, J=4.8Hz, 2H), 3.93-3.31(m, 26H), 2.53(s, 3H), 2.32(s, 3H), 1.46(s, 9H); 13CNMR (151MHz, CD3OD): 165.2, 163.6, 162.8, 161.3, 159.0, 158.4, 157.8, 140.3, 136.2, 135.4, 134.2, 130.9,130.2, 129.6, 128.4, 84.7, 82.2, 76.5, 71.6, 71.5, 71.4, 71.2, 70.8, 69.8, 59.7, 57.5, 55.8, 53.1, 52.9, 42.4, 41.9, 28.5 (3), 25.1, 18.7; ESI-MS calculated for $C_{36}H_{52}ClN_9O_9S$ [MNa]+ 844.3 observed 844.3.

[086] Compound **2** (100 mg, 0.12 mmol) in 10 mL of 20% trifluoroacetic acid (in dichloromethane) was stirred at room temperature for one hour, after which the solution was diluted with toluene and dried under vacuum. The residue was re-suspended in water and purified by prep-HPLC as described above to yield compound **3** (**Exemplary Scheme 2**) as white-colored powder (67mg, yield 67%). 1HNMR (600MHz, CD3OD): δ 8.17 (s, 1H), 7.36(dd, J=7.8Hz, 1.8Hz, 1H), 7.26(dd, J=7.8Hz, 7.7Hz, 1H), 7.24 (dd, J=7.8Hz, 1.8Hz, 1H), 6.15(s, 1H), 4.46(t, J=5.0Hz, 2H), 4.27-3.24(m, 26H), 2.52(s, 3H), 2.33(s, 3H); 13CNMR (151MHz, CD3OD): 167.7, 165.0, 163.8, 163.2, 159.0, 158.0, 142.0, 140.4, 134.3, 134.2, 130.2, 129.6, 128.4, 127.2, 84.5, 75.0, 71.6, 71.4, 71.1(2), 71.0(2),59.8, 57.4, 55.8, 53.1, 47.9, 42.4, 41.8, 25.5, 17.3 HRMS calculated for $C_{31}H_{44}ClN_9O_7S$ [MH]+ 722.2851, observed 722.2847; [MNa]+ 744.2671, observed 744.2665.

[087] Compound **4** (**Exemplary Scheme 3**) was synthesized based on a published procedure (Fischer, J. J.; Dalhoff, C.; Schrey, A. K.; Graebner, O. Y.; Michaelis, S.; Andrich, K.; Glinski, M.; Kroll, F.; Sefkow, M.; Dreger, M.; Koester, H. *Journal of proteomics* 2011, 75, 160.) and purified by HPLC to yield as a white solid. ^{1}HNMR(600MHz, CD3OD): δ 8.19 (s, 1H), 7.36(dd, J=7.5Hz, 1.8Hz, 1H), 7.27(dd, J=7.6Hz, 7.5Hz, 1H), 7.25 (dd, J=7.5Hz, 1.8Hz, 1H), 6.30 (s, 1H), 3.91-3.04 (m, 12H), 2.55(s, 3H), 2.32(s, 3H); ^{13}CNMR (151MHz, CD3OD): 165.2, 162.6, 162.5, 162.3, 157.2, 140.3, 136.7, 135.0, 134.1, 131.7, 130.2, 129.7, 128.3, 84.5, 55.8, 54.9, 53.2, 44.4 (2), 36.2, 24.1, 18.6; HRMS calculated for $C_{22}H_{27}ClN_8OS$ $[MH]^+$ 487.1795 , observed 487.1788.

[088] (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate was prepared according to a reported procedure [3], and 175 mg (0.50 mmol) of it was stirred with compound **4** (200mg, 0.41 mmol) and 150 μL DIPEA in 50 mL anhydrous tetrahydrofuran. After overnight reaction, the solvent was evaporated under reduced pressure. The mixture was purified by flash column chromatography with

10% methanol in ethyl acetate to yield the desired product **5 (Exemplary Scheme 3)** as a colorless solid (258mg, yield 90%). ^{1}HNMR (600MHz, DMSO-d_6): δ 9.91(br s, 1H), 9.73(br, 1H), 8.47(d, J=4.8Hz, 1H), 8.24(s, 1H), 7.84(td, J=7.2Hz, 1.8Hz, 1H), 7.78(dd, J=7.8Hz, 1.8Hz, 1H), 7.40(d, J=7.2Hz, 1H), 7.32-7.25(m, 3H), 6.14(s, 1H), 4.37-3.10(m, 16H), 2.45(s, 3H), 2.24(s, 3H); ^{13}CNMR (151MHz, DMSO-d_6): 165.4, 162.4, 161.9, 159.8, 158.8, 157.2, 156.0, 149.7, 140.8, 138.8, 137.8, 133.4, 132.4, 129.0, 128.2, 127.0, 126.0, 121.3, 119.3, 83.4, 62.0, 54.9, 50.6(2), 40.7(2), 37.2, 35.0, 25.5, 18.3; HRMS calculated for $C_{30}H_{34}ClN_9O_3S_3$ $[MH]^+$ 700.1714, observed 700.1708.

[089] The cysteine-ended spacer unit γGlu-Asp-Arg-Asp-Asp-Cys-OH was synthesized on solid phase, cleaved and purified based on reported protocol[4]. The linker (30 mg, 0.04 mmol) was mixed with compound **5** (**Exemplary Scheme 3**) (28 mg, 0.04 mmol) in 20 mL of THF/water (1:1), and stirred overnight under argon. After removal of THF by vacuum, the mixture was washed by extraction with ethyl acetate (three times). The water phase was concentrated and further purified by prep-HPLC to afford compound **6 (**see **FIG. 5)** (24mg, yield 45%). ^{1}HNMR (600MHz, DMSO-d_6): δ 10.11(br s, 3H), 9.91(br s, 1H), 8.74(br, 1H), 8.24(s, 1H), 8.21-8.06(br m, 6H), 7.95(br s, 2H), 7.48(br, 2H), 7.40(d, J=7.4 Hz, 1.8Hz, 1H), 7.30-7.25(m, 3H), 6.15(s, 1H), 4.54-3.02(m, 24H), 2.89-2.45(m, 12H), 2.38(s, 3H), 2.24(s, 3H), 1.94-1.50(m, 4H); ^{13}CNMR (151MHz, DMSO-d_6): 174.0, 173.9, 172.8, 171.9, 171.6, 171.1, 170.6, 170.4, 170.0, 168.2, 165.4, 162.4, 161.9, 159.8, 156.1, 140.7, 138.8, 133.4, 132.4, 129.0, 128.2, 127.0, 126.0, 83.4, 62.2, 59.9, 55.0, 52.3, 52.1, 51.7, 51.6, 51.4, 50.6, 49.6(2), 49.4, 41.8, 40.5, 38.3, 36.9, 36.0, 35.7, 35.0, 33.1, 27.8, 27.6, 25.6, 24.7, 18.3 ESI-MS calculated for $C_{51}H_{70}ClN_{17}O_{18}S_3$ $[MH]^+$ 1340.4, observed 1340.4; HRMS calculated for $[M+2H]^{2+}$ 670.7046, observed 670.7041.

[090] Previously prepared compound **6** (**Exemplary Scheme 3**) (20 mg, 0.015 mmol) was mixed with t-Boc-aminooxyacetic acid N-hydroxysuccinimide ester (8.6 mg, 0.03 mmol) in 10 mL anhydrous DMF, along with 50 uL DIPEA. The solution was stirred for eight hours at room temperature to allow for a complete conversion of compound **6** (**Exemplary Scheme 3**). The mixture was then vacuum-concentrated and purified via HPLC. The eluted fraction with the correct UV absorption and mass spectrometry was dried to yield the desired compound **7** (12mg, yield 53%). ^{1}HNMR (600MHz, D_2O): δ 8.21(s, 1H), 7.44(m, 1H), 7.35-7.33(m, 2H), 6.44(s, 1H), 4.48-3.02(m, 26H), 2.87-2.79(m, 6H), 2.64(s, 3H), 2.49-2.44(m, 2H), 2.30(s, 3H), 2.18-1.98(m, 4H), 1.83-1.60(m, 4H), 1.47(s, 9H); ^{13}CNMR (151MHz, D_2O): 176.2, 173.6, 173.4, 172.8, 172.4, 171.9, 171.4, 171.3, 171.0, 164.4, 163.9, 161.7, 159.9, 158.4, 157.9, 157.8, 156.2, 138.9, 138.4, 131.8, 130.7(2), 129.0, 128.9, 126.9, 83.6, 77.4, 74.1, 61.1, 58.1, 55.8, 53.9, 53.1, 52.7, 52.4, 51.7, 50.6, 49.8, 49.7, 49.6, 42.1, 41.4, 39.9, 38.1, 36.4, 34.7, 34.4, 33.2, 27.2, 27.0, 26.9(3),25.9, 24.7, 17.3 HRMS calculated

for $C_{58}H_{81}ClN_{18}O_{22}S_3$ $[MH]^+$ 1513.4702, observed 1513.4694; $[M+2H]^{2+}$ 757.2390, observed 757.2372

[091] Compound **7** (**Exemplary Scheme 3**) (10mg, 0.007mmol) was suspended in 5 mL solution of 20% TFA in DCM under argon. After stirring for 30 minutes at room temperature, the reaction was quenched by addition of 10 mL toluene. The solvent was removed under vacuum and the residue was purified by prep-HPLC following the aforementioned conditions. The desired fraction was eventually achieved as a white-colored solid as compound **8** (4mg, yield 40%) (**Exemplary Scheme 3**). ^{1}HNMR (600MHz, D_2O): δ 8.21(s, 1H), 7.44(m, 1H), 7.35-7.33(m, 2H), 6.36(s,1H), 4.54-3.00(m, 26H),2.94-2.79(m, 6H), 2.61(s, 3H), 2.49-2.44(m, 2H), 2.30(s, 3H), 2.12-1.88(m, 4H), 1.75-1.58(m, 4H); ^{13}CNMR (151MHz, D_2O): 176.4, 173.7, 173.4, 172.8, 172.4, 171.9, 171.4, 171.3,171.2, 163.8, 163.1, 161.9, 160.3, 157.9, 157.8, 156.2,138.5, 138.3, 131.9, 130.8, 130.6, 129.0, 128.9, 126.9, 84.0, 72.0, 61.1, 56.7, 55.8, 53.9, 53.1, 52.6, 52.4, 51.7, 50.7, 49.8(2), 49.7, 42.0, 41.3, 39.9, 37.1, 36.9, 34.9, 34.7, 33.2, 27.3, 27.0, 25.4, 24.7, 17.2; ESI-MS calculated for $C_{53}H_{73}ClN_{18}O_{20}S_3$ $[MH]^+$ 1413.4, observed 1413.4, HRMS for $[M+2H]^{2+}$ 707.2128, observed 707.2118.

Example 4. Inhibition of TCR triggered activation of human T cells

[092] To evaluate their *in vitro* potency, the two dasatinib ADCs were incubated with freshly isolated human T cells that were stimulated with anti-CD3/anti-CD28 antibodies. Analysis by flow cytometry (**FIG. 12**) and ELISA (**FIG. 13A**) showed that dasatinib itself significantly inhibited the expression of CD69 and CD25, and blocked the secretion of IL-2, TNFα, and IFNγ with EC_{50}'s ~ 0.2 - 1 nM. Both ADCs showed potent inhibition of CD69/CD25 expression and suppressed cytokine secretion, while the unconjugated HLCX antibody displayed minimal effects (EC_{50} > 200 nM), (**FIGS. 12, 13A, and 14**). The HLCX-ss-dasatinib was ~2-fold more potent than HLCX-dasatinib in suppressing IL-2 (EC_{50} = 12.7 ± 5.8 nM vs. 32.1 ± 11.3 nM), TNFα (EC_{50} = 26.3 ± 9.0 nM vs. 66.1 ± 30.5 nM), and IFNγ (EC_{50} = 58.7 ± 28.6 nM vs. 123.5 ± 43.3 nM). Although the suppression of T cell activation by dasatinib ADC is CXCR4-dependent that the underlying activity was due to inhibition of Lck by dasatinib was confirmed. To this end, western blot analysis was carried out to examine the phorsphorylation of down-stream kinases during TCR-induced T cell activation (**FIG. 13B**). As shown in lane 1, TCR activation occurs after crosslinking T cells with OKT3 at 37° C. Dasatinib, as a positive control, efficiently blocked Lck-mediated phosphorylation of tyrosine on multiple kinases including ZAP70 (lane 2). As indicated in lane 4, the HLCX-ss-dasatinib ADC also blocked Lck signaling similarly to dasatinib, while the CXCR4 antibody (lane 3) had no effect on phosphorylation. In addition, trastuzumab-ss-dasatinib (negative control) displayed a negligible effect on phosphorylation (lane 5), which demonstrated both the linker stability and the antibody-dependence in this assay. Taken together, suppression of T cell activation by HLCX-ss-dasatinib

ADC is both antibody and small-molecule dependent, suggesting that a promiscuous kinase inhibitor can be successfully delivered to T cells by the anti-CXCR4 antibody HLCX.

[093] While dasatinib derivatives in both ADCs may escape from the endosome-lysosome cycles, the cleavable linker derivative could be further reduced to release the parent dasatinib compound. The released compound is more potent than the dasatinib non-cleavable linker, which could lead to the potency difference between the ADC constructs. To ensure this effect was not due to any prematurely released dasatinib, the disulfide cleavable dasatinib was conjugated to an irrelevant antibody, trastuzumab, and its activity in cytokine secretion assays was evaluated. Trastuzumab-ss-dasatinib showed negligible effects at concentrations up to 200 nM, whereas dasatinib completely suppressed cytokine secretions (**FIG. 15**). Additionally, to rule out the possibility that the observed immunosuppression was due to cytotoxicity, viability of T cells was also measured using CellTiter Glo (**FIG. 14B**). Compared to the untreated T cells, the activated T cells did not manifest any significant decrease of viability after incubation with either dasatinib or ADCs at concentrations up to 200 nM.

Conjugation and purification of Antibody Drug Conjugate

[094] A solution of anti-CXCR4 IgG HLCX (10mg, 67 nmol) in PBS buffer (pH 7.4, 1mL) was added N-succinimidyl-4-formylbenzamide (495 μg, 2 μmol) (Solulink, CA), and the reaction mixture was incubated at room temperature for three hours with shaking (50 rpm). The modified antibody was purified by excess buffer exchange using an Amicon filter (30kDa MWCO, EMD Millipore,Ireland), or Zeba spin desalting columns (7k MWCO, Thermo Scientific, IL) as reported before 5-7. The purified antibody could either proceed to a second step conjugation or be stored in aliquots at -80C until future use. For the second step conjugation, the antibody tagged with benzaldehyde (1mg, 6.7 nmol) was buffer exchanged into 50 mM phosphate buffer (pH6.5, 150 mM NaCl), and then added the synthesized dasatinib derivative 8 (0.28mg, 200 nmol) for HLCX-ss-dasatinib ADC. For synthesis of HLCX-dasatinib ADC, the antibody was buffer exchanged to 50 mM sodium acetate buffer (pH 5.0) to increase the solubility of dasatinib derivative 3 (0.14 mg, 200 nmol). The mixture was then incubated at 37C for 24 h, with the reaction progress monitored by electrospray ionization (ESI) protein mass spectrometry (Scripps center for metabolomics and mass spectrometry). Usually after 24 h of incubation, the benzaldehyde tags on protein were >95% conjugated with aminooxy-linker derivatives. The final ADC was purified by FPLC using size exclusion column (Superdex 200 10/300 GL, GE Healthcare, PA), or alternatively by buffer exchange on amicon or Zeba columns as described above.

T cell isolation and activation

[095] Whole blood were collected from healthy human donor by normal blood donor service (The Scripps Research Institute, CA), and were purified with conventional Ficoll-Hypaque gradient centrifugation (GE Healthcare, CA) to afford peripheral blood mononuclear cells (PBMC). T cells were then isolated from PBMC with a human T cell enrichment kit (Stem cell technologies, Canada), to yield > 95% CD3-positive cells as monitored by flow cytometry. For activation studies, freshly isolated T cells were suspended in RPMI media supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin, and plated at 200 K cells per well. Small molecule dasatinib, HLCX, and ADCs were prepared by serial dilutions in PBS as 10× stock, and then added in triplicate to T cells, respectively. The samples were incubated at 37° C, 5% CO2 for 24 hours, after which point they were activated by 5 μg/mL plate-coated OKT3 (eBioscience, CA) and 1 μg/mL soluble anti-CD28 (eBioscience, CA). Cellular supernatants were collected approximately 18 h after stimulation for IL-2 analysis, and 36 h from another set of separate treatments for TNFα and IFNγ analysis. All three cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA) using DuoSet development kits (R&D systems, MN), with the results processed by GraphPad Prism (GraphPad Software, CA). Flow cytometry analysis of T cell antigen markers were performed with another separate set of samples, around 24 hours after stimulation for anti-CD69, or 72 hours after for anti-CD25.

Western blotting for T cell receptor signaling studies

[096] Four million freshly isolated human T cells were suspended in 500 μL RPMI media supplemented with penicillin/streptomycin, and incubated at 37° C with 100 nM dasatinib, HLCX, HLCX-ss-dasatinib ADC, or Herceptpin-ss-dasatinib ADC, respectively. After overnight incubation, all samples were transferred to 1.5 mL Eppendorf tubes and cooled down on ice for 15 min, after which pre-chilled OKT3 (10 μg/mL final concentration) and goat anti-mouse IgG F(ab')2 (5 μg/mL) were added. The mixture was incubated on ice for another 15 min, and then transferred to 37° C water bath for 5 min to allow for TCR-mediated phosphorylation. T cells treated with PBS blank buffer were activated in the same way as a control. All the samples after 37° C incubation were immediately chilled on ice and centrifuged at 1800 rpm at cold room to pellet cells. After removal of residual solution, each cell pellet was lysed by 30 μL of cellLytic buffer (Sigma Aldrich, MO) mixed with protease/phosphatase inhibitors, followed by separation of proteins on 10% SDS-PAGE. Magic markers (Life technologies, CA) were used for labeling the migration. The separated proteins were then transferred to PVDF membrane (Life Technologies, CA) that was later incubated for 2 hours at RT in blocking buffer consisting of 5% BSA, 10 mM Tris, 100 mM NaCl, and 1% Tween-20. The blocked membrane was probed with horseradish peroxidase (HRP)-conjugated anti-phosphotyrosine

mAb (P-Tyr-100, Cell signaling, MA) at a dilution of 1/2500 for 1 hr. at RT, followed by multiple washings with PBST. Detection of HRP was performed using SuperSignal West Pico (Thermo Scientific, IL), with chemiluminescence recorded on HyBlot CL films (Denville Scientific, NJ). For detection of loading controls, the membrane was stripped, re-blocked with blocking buffer, and probed with anti-eukaryotic initiation factor 4E (eIF4E) primary antibody (cell signaling, MA) (1/1000 dilution), followed by HRP-conjugated anti-rabbit secondary antibody.

In vitro Cytotoxicity Studies

[097] Freshly purified human T cells were incubated with increasing concentrations of dasatinib, HLCX, HLCX-dasatinib ADC, or HLCX-ss-dasatinib ADC and then activated under the same conditions as described above. After 36 hours of T cell stimulation, 50 µL out of 150 µL cell culture was pipetted out from each sample well, and transferred into a new plate. Cell viabilities were evaluated by measuring the ATP content with CellTiter Glo (Promega, WI) for which the luminescence was detected on Gemini EM microplate reader (Molecular Devices, CA). Viability of unstimulated T cells was used as 100% viability control.

[098] Further evaluations of the conjugate in a more advanced surrogate model (e.g., cynomolgus monkeys) could be suitable to determine its therapeutic relevance.

Table 1. Immunoglobulin Light Chain (LC) and Heavy Chain (HC)–Nucleotide Sequence

NAME	SEQ ID NO	SEQUENCE
HLCX heavy chain	1	CAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAGCCT GGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGGTTCAAT ATTAAGGACACTTACATCCACTGGGTCCGCCAGGCTCCAGGG AAGGGGCTGGAGTGGGTCGCACGTATTTATCCTACCAATGGT TACACACGCTACGCAGACTCCGTGAAGGGCCGATTCACCATC TCCGCAGACACTTCCAAGAACACGGCGTATCTTCAAATGAAC AGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTTCGAGA GAAACTAAGAAATACCAGAGC*TATCGCAAATGTAGAGGAGGCC* *GAAGGTGGTGCTACCAAAAG*TCTTATACCTACAATTATGAAGA CTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAGCCTC CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGG CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCC CTCTAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAAC CCAAATCTTGCGACAAAACTCACACATGCCCACCGTGCCCAG CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGT CACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC

Table 1. Immunoglobulin Light Chain (LC) and Heavy Chain (HC)–Nucleotide Sequence		
NAME	**SEQ ID NO**	**SEQUENCE**
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC CAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAT GAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC CTCTCCCTGTCTCCGGGTAAATGATAA
HMCX heavy chain	2	CAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAGCCT GGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGGTTCAAT ATTAAGGACACTTACATCCACTGGGTCCGCCAGGCTCCAGGG AAGGGGCTGGAGTGGGTCGCACGTATTTATCCTACCAATGGT TACACACGCTACGCAGACTCCGTGAAGGGCCGATTCACCATC TCCGCAGACACTTCCAAGAACACGGCGTATCTTCAAATGAAC AGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTTCGAGA GAAACTAAGAAA*TATCGCAAATGTAGAGGAGGCCGAAGGTGGT GCTACCAAAAG*TACAATTATGAAGACTACTGGGGCCAAGGAA CCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGG TCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCA CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC CGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACTGTGCCCTCTAGCAGCTTGGGCAC CCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACAC CAAGGTGGACAAGAAAGTTGAACCCAAATCTTGCGACAAAA CTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGG ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAA AACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGG TGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCG ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG GGTAAATGATAA

Table 1. Immunoglobulin Light Chain (LC) and Heavy Chain (HC)–Nucleotide Sequence		
NAME	**SEQ ID NO**	**SEQUENCE**
HSCX heavy chain	3	CAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAGCCT GGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGGTTCAAT ATTAAGGACACTTACATCCACTGGGTCCGCCAGGCTCCAGGG AAGGGGCTGGAGTGGGTCGCACGTATTTATCCTACCAATGGT TACACACGCTACGCAGACTCCGTGAAGGGCCGATTCACCATC TCCGCAGACACTTCCAAGAACACGGCGTATCTTCAAATGAAC AGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTTCGAGA *TATCGCAAATGTAGAGGAGGCCGAAGGTGGTGCTACCAAAAG*GA CTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAGCCTC CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGG CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCC CTCTAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAAC CCAAATCTTGCGACAAAACTCACACATGCCCACCGTGCCCAG CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGT CACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC CAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAT GAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC CTCTCCCTGTCTCCGGGTAAATGATAA
HLCX light chain	4	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTG TAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGATG TGAATACCGCGGTCGCATGGTATCAGCAGAAACCAGGGAAA GCCCCTAAGCTCCTGATCTATTCTGCATCCTTCTTGTATAGTG GGGTCCCATCAAGGTTCAGTGGCAGTAGATCTGGGACAGATT TCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA CTTACTACTGTCAACAGCATTACACTACCCCTCCGACGTTCGG CCAAGGTACCAAGCTTGAGATCAAACGAACTGTGGCTGCACC ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT GGAACTGCCTCTGTCGTGTGCCTGCTGAATAACTTCTATCCCA GAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAA TCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAA GGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA

Table 1. Immunoglobulin Light Chain (LC) and Heavy Chain (HC)–Nucleotide Sequence		
NAME	**SEQ ID NO**	**SEQUENCE**
		CCCATCAGGGCCTGTCCTCGCCCGTCACAAAGAGCTTCAACA GGGGAGAGTGT

For heavy chain SEQ ID NOs, the targeting peptide is *italicized.*

CLAIMS

What is claimed is:

1. An antibody kinase inhibitor conjugate that interacts with a cell surface molecule on a target cell comprising:
   a. an antibody or antibody fragment; and
   b. a kinase inhibitor,
   wherein the antibody or antibody fragment is attached to the kinase inhibitor.
2. The antibody kinase inhibitor conjugate of claim 1, wherein the antibody or antibody fragment binds the cell surface molecule on the target cell.
3. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a kinase expressed by a hematopoietic cell.
4. The antibody kinase inhibitor conjugate of claim 3, wherein the hematopoietic cell is selected from a lymphocyte, a B cell, a T cell, a monocyte and a macrophage.
5. The antibody kinase inhibitor conjugate of claim 3, wherein the hematopoietic cell is a T cell.
6. The antibody kinase inhibitor conjugate of claim 5, wherein the kinase inhibitor is a modulator of a T cell activity.
7. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a kinase that modulates an immune and/or inflammatory activity.
8. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a kinase downstream of a protein selected from: an epidermal growth factor receptor (EGFR), a vascular endothelial growth factor receptor (VEGFR), a platelet derived growth factor receptor (PDGFR), a hepatocyte growth factor receptor (HGFR), and a mast/stem cell growth factor receptor (SCFR).
9. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a Src kinase.
10. The antibody kinase inhibitor conjugate of claim 9, wherein the Src kinase is selected from Src, Yes, Fyn, Fgr, Lck, Hck, Blk, Lyn, and Frk.
11. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a tyrosine kinase.
12. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a kinase selected from: an Abelson murine leukemia viral oncogene homolog 1 (Abl), a breakpoint cluster region protein- Abelson murine leukemia viral oncogene homolog 1 fusion (Bcr-Abl), a Src kinase, an anaplastic lymphoma kinase (ALK), a spleen tyrosine kinase (Syk), a Bruton's tyrosine kinase (BTK), a janus kinase (JAK), and a RET tyrosine kinase.

13. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor inhibits a kinase selected from Abl and BCR-Abl.
14. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor is a non-peptide small molecule.
15. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor is selected from afatinib, axitinib, bosutinib, cetuximab, crizotinib, dasatinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, mubritinib, nilotinib, pazopanib, pegaptanib, ruxolitinib, sorafenib, sunitinib, SU6656, tofacitinib, vandetanib, and vemurafenib.
16. The antibody kinase inhibitor conjugate of claim 1, wherein the kinase inhibitor is dasatinib.
17. The antibody kinase inhibitor conjugate of claim 1, wherein the antibody or antibody fragment binds a cell surface molecule on a target cell.
18. The antibody kinase inhibitor conjugate of claim 1, comprising a targeting peptide, wherein the targeting peptide interacts with the cell surface molecule on the target cell.
19. The antibody kinase inhibitor conjugate of claim 18, wherein the targeting peptide is attached to the antibody or antibody fragment.
20. The antibody kinase inhibitor conjugate of claim 18, wherein the targeting peptide is selected from a peptide that is conformationally constrained, a peptide comprising a beta strand, a peptide comprising a beta-hairpin, a peptide that can bind a deep ligand binding pocket of the cell surface molecule, and combinations thereof.
21. The antibody kinase inhibitor conjugate of claim 1, wherein the target cell is a hematopoietic cell.
22. The antibody kinase inhibitor conjugate of claim 21, wherein the hematopoietic cell is selected from a lymphocyte, a B cell, a T cell, a monocyte, and a macrophage.
23. The antibody kinase inhibitor conjugate of claim 21, wherein the hematopoietic cell is a T cell.
24. The antibody kinase inhibitor conjugate of claim 1, wherein the cell surface molecule is a T cell antigen.
25. The antibody kinase inhibitor conjugate of claim 24, wherein the T cell antigen is selected from CD3, CD4, CD71, CD69, CD25, CD8, and CXCR4.
26. The antibody kinase inhibitor conjugate of claim 1, wherein the cell surface molecule is CXCR4.
27. The antibody kinase inhibitor conjugate of claim 1, wherein the antibody or antibody fragment is selected from a rabbit antibody, a rodent antibody, an avian antibody, a simian

antibody, a human antibody, a humanized antibody, a chimeric antibody, a bovine antibody, and fragments thereof, and combinations thereof.

28. The antibody kinase inhibitor conjugate of claim 1, wherein the antibody or antibody fragment comprises a bovine ultralong complementary determining region or portion thereof.
29. The antibody kinase inhibitor conjugate of claim 1, wherein the antibody or antibody fragment binds the cell surface molecule with a K_d value equal to or less than about 2 nM.
30. The antibody kinase inhibitor conjugate of claim 17, wherein the targeting peptide binds the cell surface molecule with a K_d value equal to or less than about 2 nM.
31. The antibody kinase inhibitor conjugate of claim 1, further comprising a linker, wherein the linker attaches the kinase inhibitor to the antibody or antibody fragment.
32. The antibody kinase inhibitor conjugate of claim 31, wherein the linker is uncleavable.
33. The antibody kinase inhibitor conjugate of claim 31, wherein the linker is cleavable.
34. The antibody kinase inhibitor conjugate of claim 31, wherein the linker comprises a disulfide bond.
35. The antibody kinase inhibitor conjugate of claim 31, wherein the linker is selectively cleaved inside the cell.
36. The antibody kinase inhibitor conjugate of claim 31, wherein the linker comprises a peptide that increases solubility.
37. An antibody drug conjugate that interacts with a cell surface molecule on a benign immunomodulatory cell comprising:
    a. an antibody or antibody fragment that interacts with the cell surface molecule; and
    b. a drug that modulates an immune activity of the benign immunomodulatory cell.
38. The antibody drug conjugate of claim 37, wherein the benign immunomodulatory cell is selected from a lymphocyte, a B cell, a T cell, a monocyte and a macrophage.
39. The antibody drug conjugate of claim 37, wherein the benign immunomodulatory cell is a T cell.
40. The antibody drug conjugate of claim 37, wherein the drug is a non-peptide small molecule.
41. The antibody drug conjugate of claim 37, wherein the drug is a kinase inhibitor.
42. The antibody drug conjugate of claim 37, wherein the drug is dasatinib.
43. The antibody drug conjugate of claim 37, further comprising a targeting peptide, wherein the targeting peptide interacts with the cell surface molecule on the target cell.
44. The antibody drug conjugate of claim 43, wherein the targeting peptide is selected from a peptide that is conformationally constrained, a peptide comprising a beta strand, a peptide

comprising a beta-hairpin, and a peptide that can bind a deep ligand binding pocket of the cell surface molecule.

45. The antibody drug conjugate of claim 37, wherein the cell surface molecule is a T cell antigen.
46. The antibody drug conjugate of claim 45, wherein the T cell antigen is selected from CD3, CD4, CD71, CD69, CD25, CD8, and CXCR4.
47. The antibody drug conjugate of claim 37, wherein the cell surface molecule is CXCR4.
48. The antibody drug conjugate of claims 37, wherein the antibody or antibody fragment is selected from a rabbit antibody, a rodent antibody, an avian antibody, a simian antibody, a human antibody, a humanized antibody, a chimeric antibody, a bovine antibody, and fragments thereof, and combinations thereof.
49. The antibody drug conjugate of claim 37, wherein the antibody or antibody fragment comprises a bovine ultralong complementary determining region or portion thereof.
50. The antibody drug conjugate claim 37, wherein the antibody or antibody fragment binds the cell surface molecule with a K_d value equal to or less than about 2 nM.
51. The antibody drug conjugate of claim 43, wherein the targeting peptide binds the cell surface molecule with a K_d value equal to or less than about 2 nM.
52. The antibody drug conjugate of claim 37, further comprising a linker, wherein the linker attaches the drug to the antibody or fragment thereof.
53. The antibody drug conjugate of claim 52, wherein the linker is uncleavable.
54. The antibody drug conjugate of claim 52, wherein the linker is cleavable.
55. The antibody drug conjugate of claim 52, wherein the linker comprises a disulfide bond.
56. The antibody drug conjugate of claim 52, wherein the linker is selectively cleaved inside the cell.
57. The antibody drug conjugate of claim 52, wherein the linker comprises a peptide that increases solubility.
58. The antibody drug conjugate of claim 37, wherein the antibody-drug conjugate has an immunosuppressive activity.
59. A method of treating a condition in a subject in need thereof comprising administering the antibody kinase inhibitor conjugate or the antibody drug conjugate of any one of claims 1-58.
60. The method of claim 59, wherein the condition is an immune condition.
61. The method of claim 59, wherein the condition is an autoimmune disease.
62. The method of claim 59, wherein the condition is an inflammatory condition.
63. The method of claim 59, wherein the condition is not a cancer.

64. Use of the antibody kinase inhibitor conjugate or the antibody drug conjugate of any one of claims 1-58 in the manufacture of a medicament for the treatment of an autoimmune disease.

FIG. 1

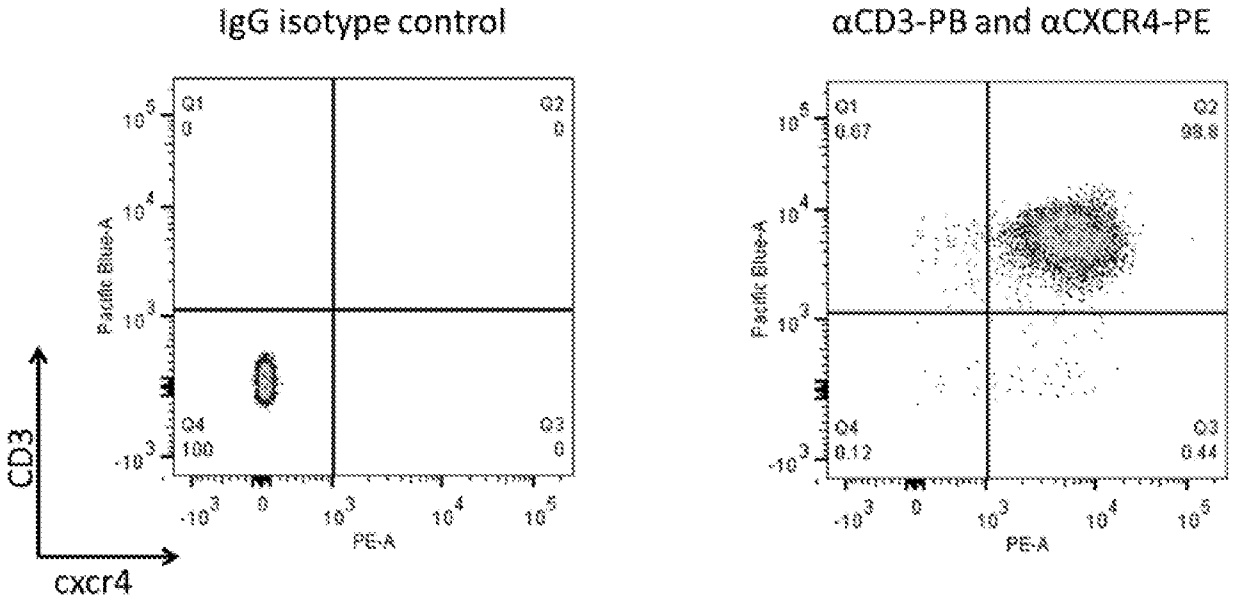

IgG isotype control
αCD3-PB and αCXCR4-PE
Q1
0
Q2
0
Q4
100
Q3
0
Q1
0.67
Q2
98.8
Q4
0.12
Q3
0.44
Pacific Blue-A
PE-A
CD3
cxcr4

FIG. 2A

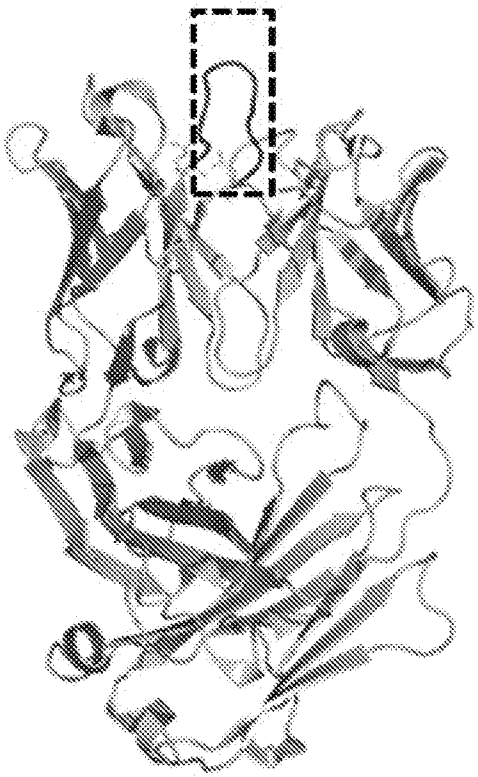


FIG. 2B

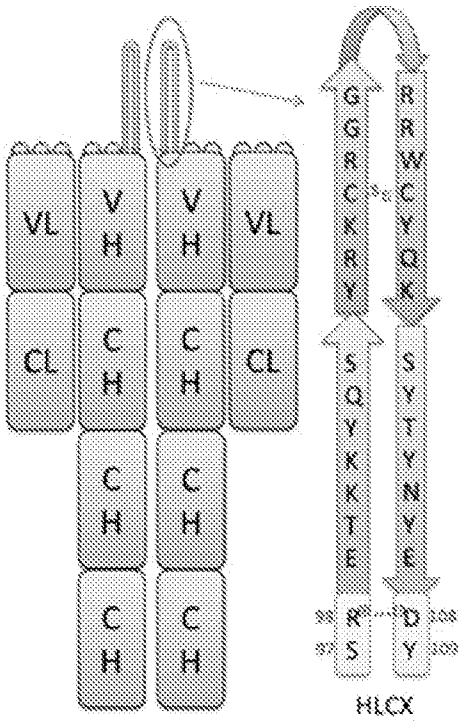

VL
VH
VH
VL
CL
CH
CH
CL
CH
CH
CH
CH
GGRCKRY
SQYKKTE
RRWCYQK
SYTTYNYE
R
S
D
Y
HLCX

FIG. 2C

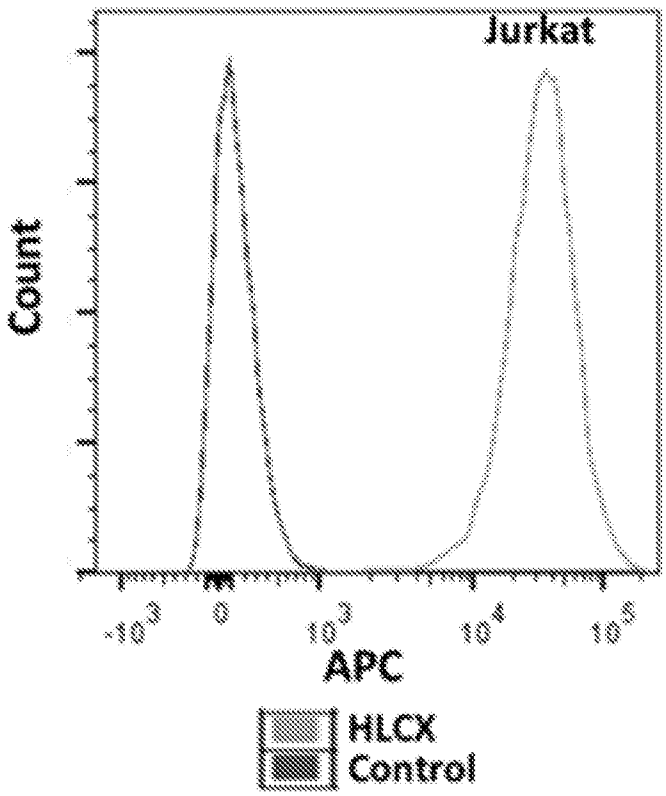

Jurkat
Count
-10^3
0
10^3
10^4
10^5
APC
HLCX
Control

FIG. 2D

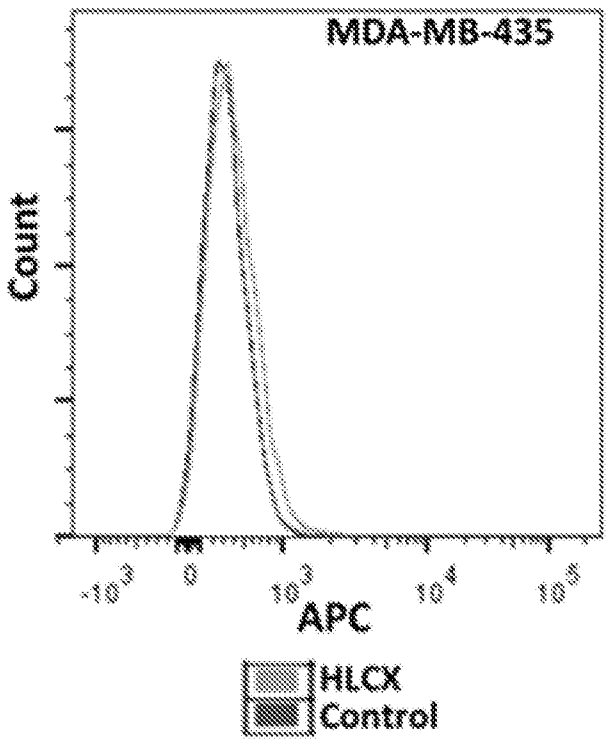

MDA-MB-435
Count
-10^3
0
10^3
10^4
10^5
APC
HLCX
Control

FIG. 2E

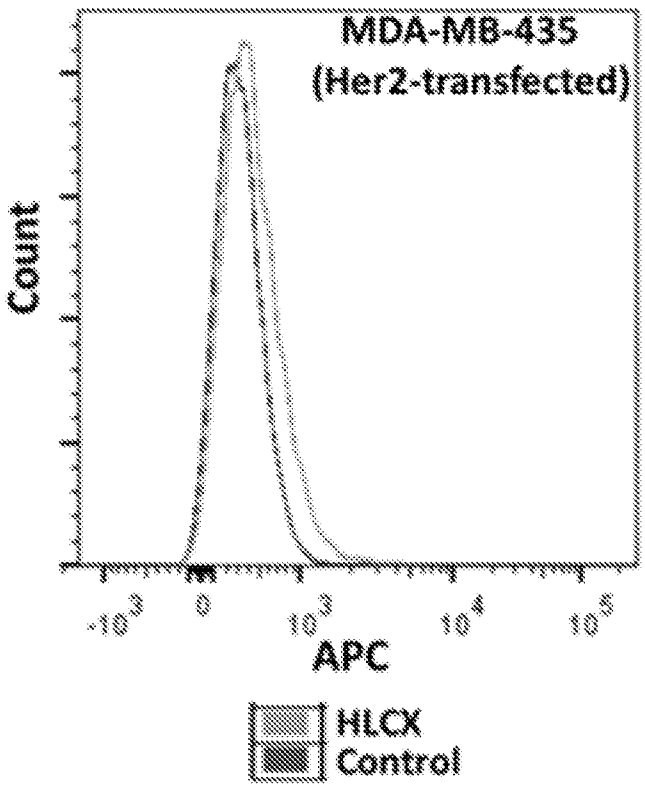

MDA-MB-435
(Her2-transfected)
Count
-10^3
0
10^3
10^4
10^5
APC
HLCX
Control

FIG. 2F

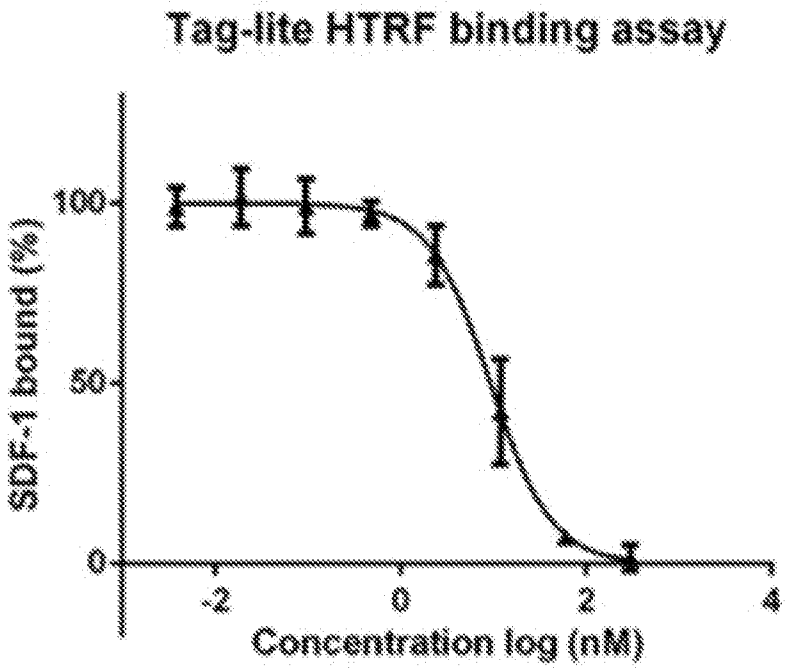

Tag-lite HTRF binding assay
SDF-1 bound (%)
100
50
0
-2
0
2
4
Concentration log (nM)

FIG. 3A

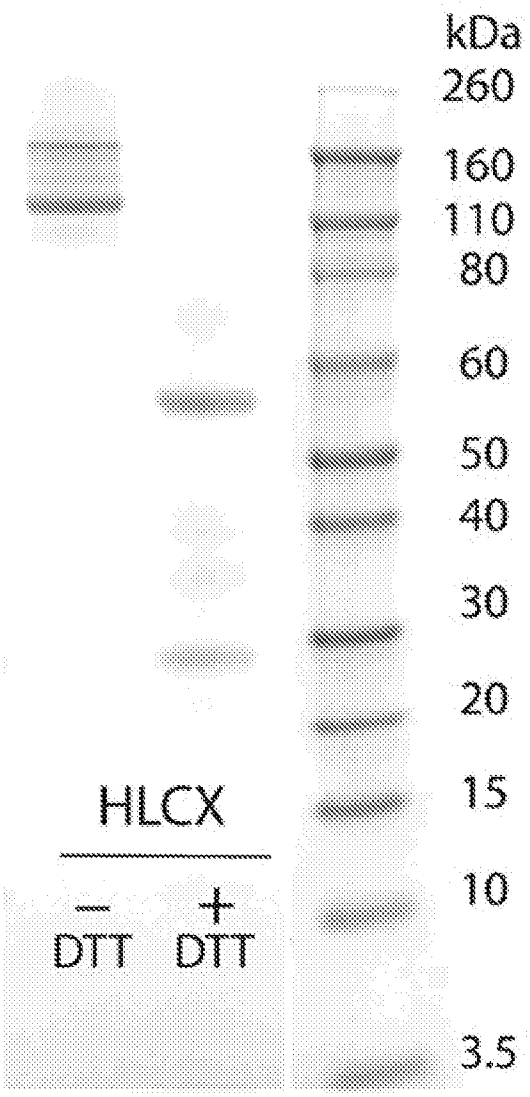


FIG. 3B

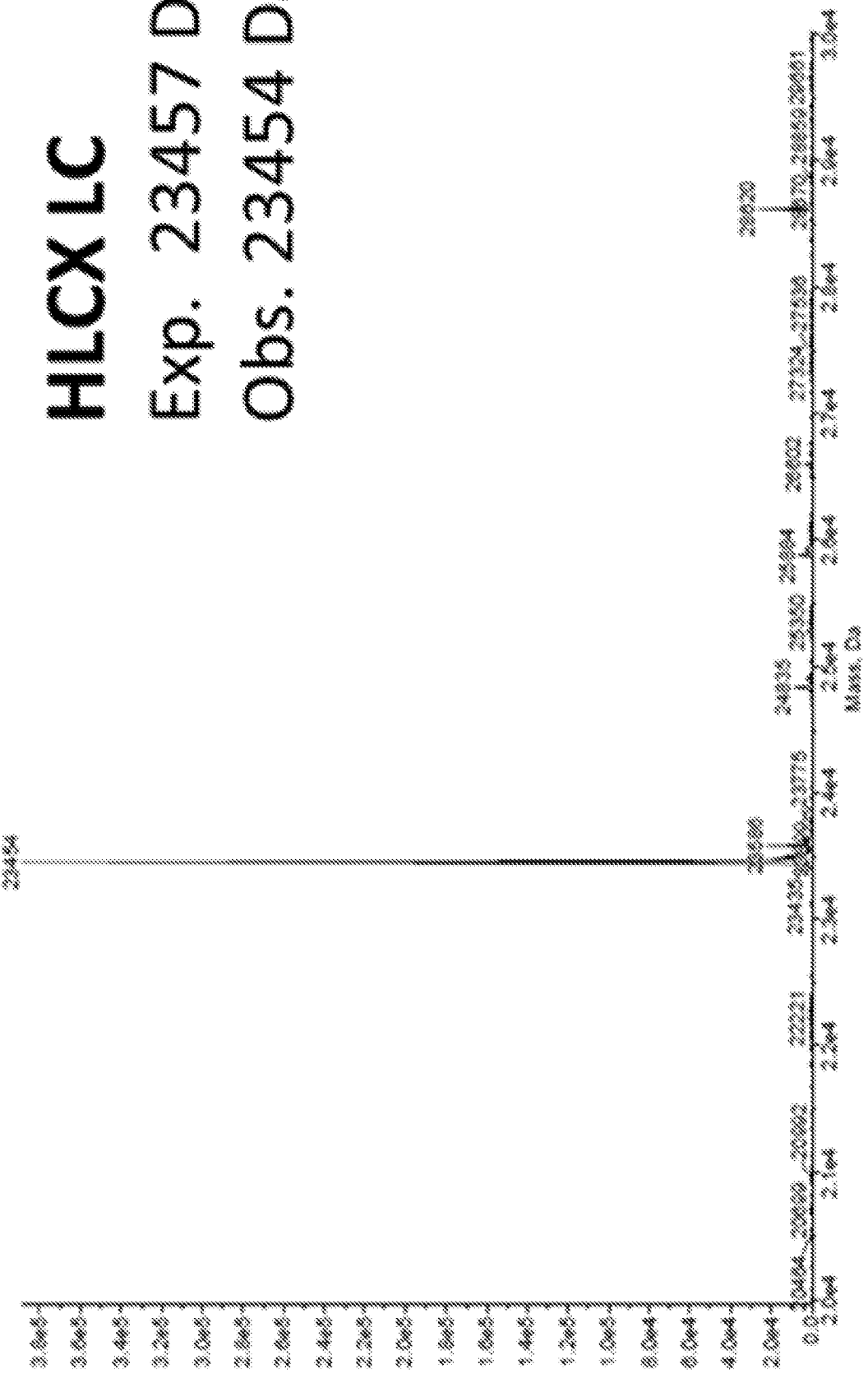


FIG. 3C

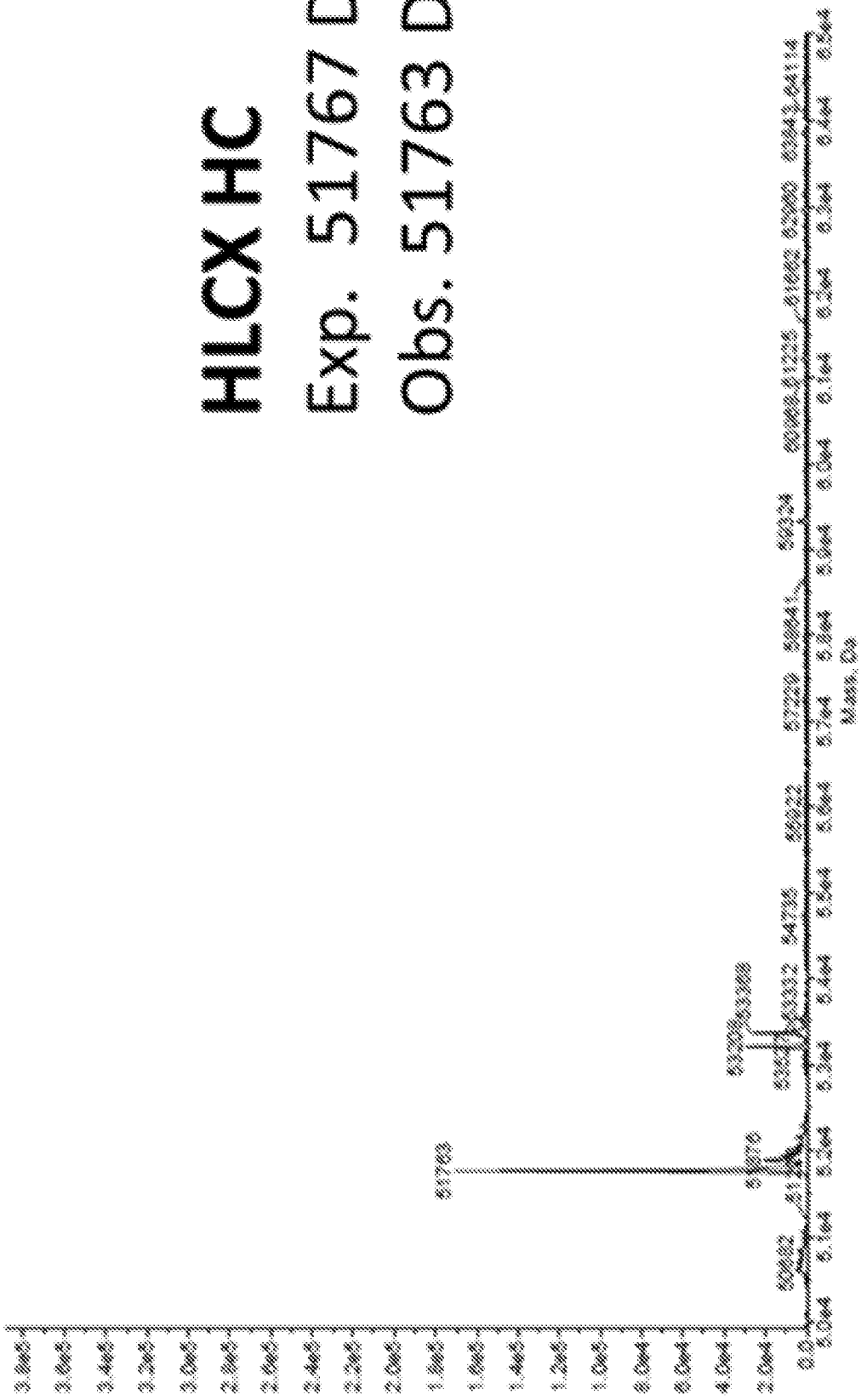


FIG. 4

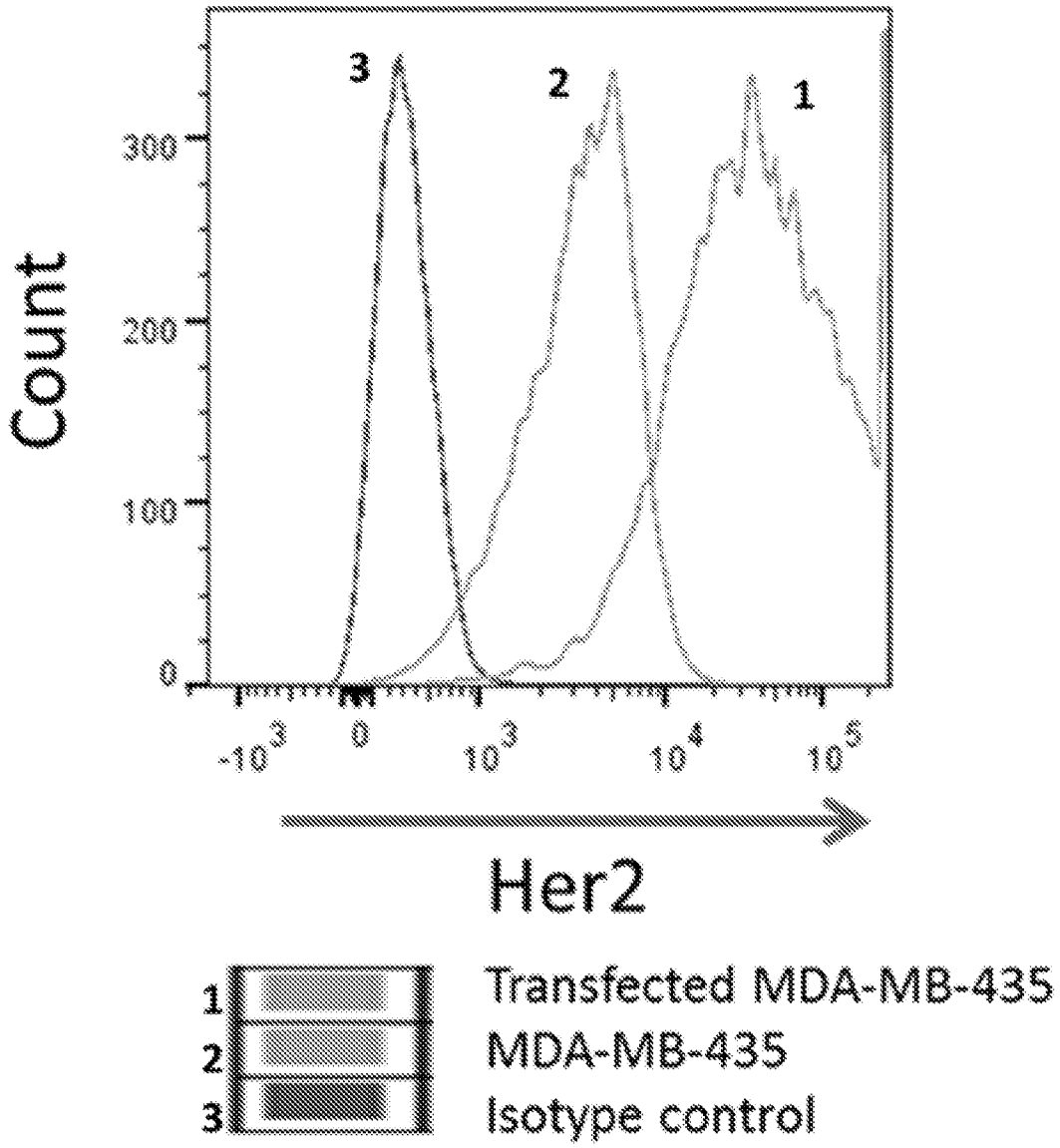

3
2
1
300
200
100
0
Count
-10^3
0
10^3
10^4
10^5
Her2
1
2
3
Transfected MDA-MB-435
MDA-MB-435
Isotype control

FIG. 5A

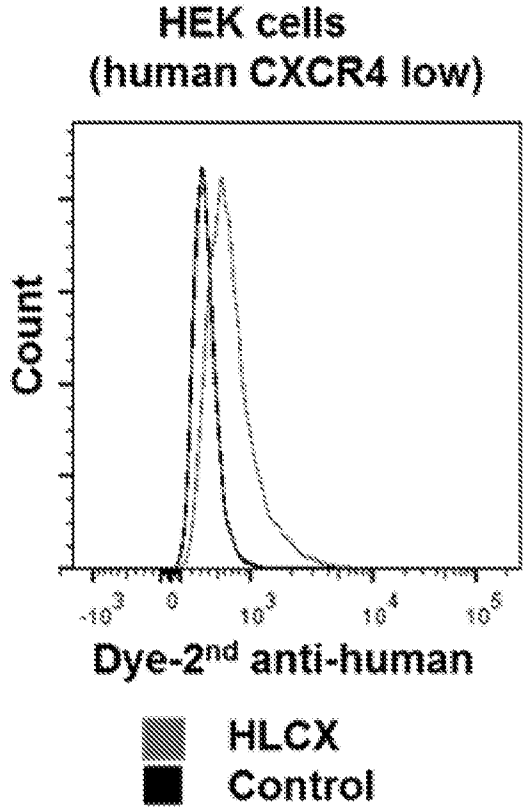

HEK cells
(human CXCR4 low)
Count
-10^3
0
10^3
10^4
10^5
Dye-2nd anti-human
HLCX
Control

FIG. 5B

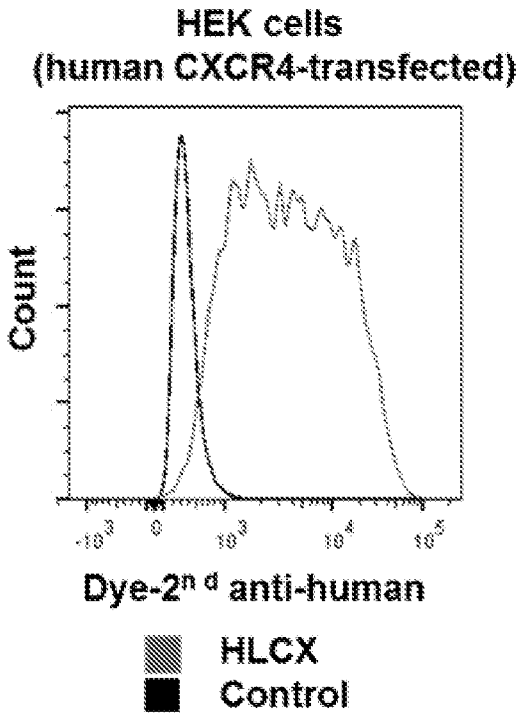

HEK cells
(human CXCR4-transfected)
Count
-10^3
0
10^3
10^4
10^5
Dye-2nd anti-human
HLCX
Control

FIG. 5C

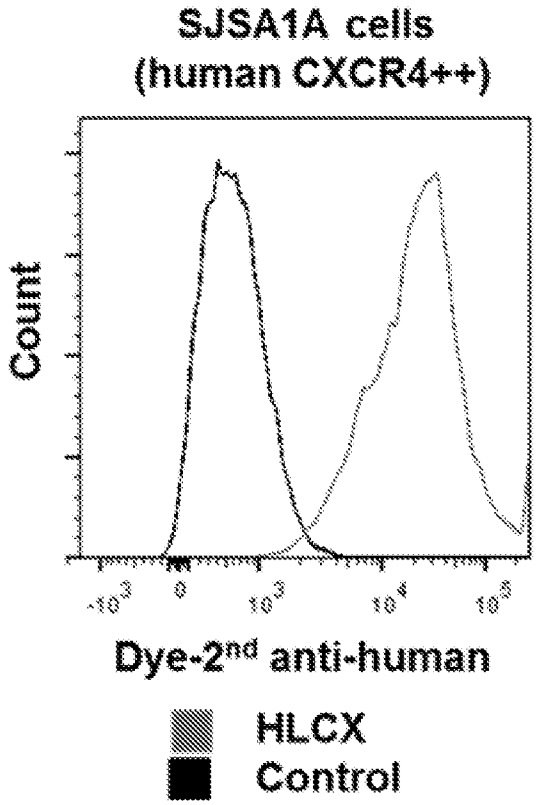

SJSA1A cells
(human CXCR4++)
Count
-10^3
0
10^3
10^4
10^5
Dye-2nd anti-human
HLCX
Control

FIG. 5D

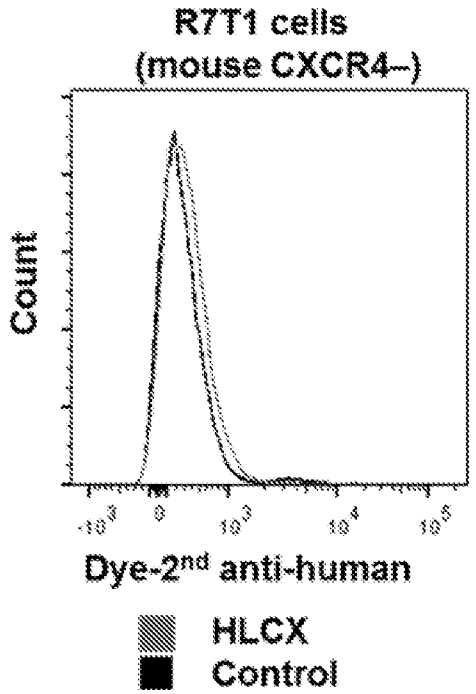

R7T1 cells
(mouse CXCR4–)
Count
-10^3
0
10^3
10^4
10^5
Dye-2nd anti-human
HLCX
Control

FIG. 5E

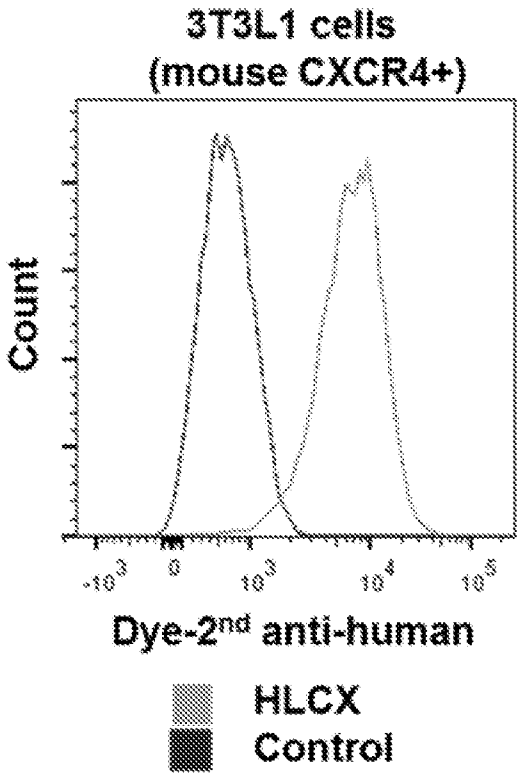

3T3L1 cells
(mouse CXCR4+)
Count
-10^3
0
10^3
10^4
10^5
Dye-2nd anti-human
HLCX
Control

FIG. 6A

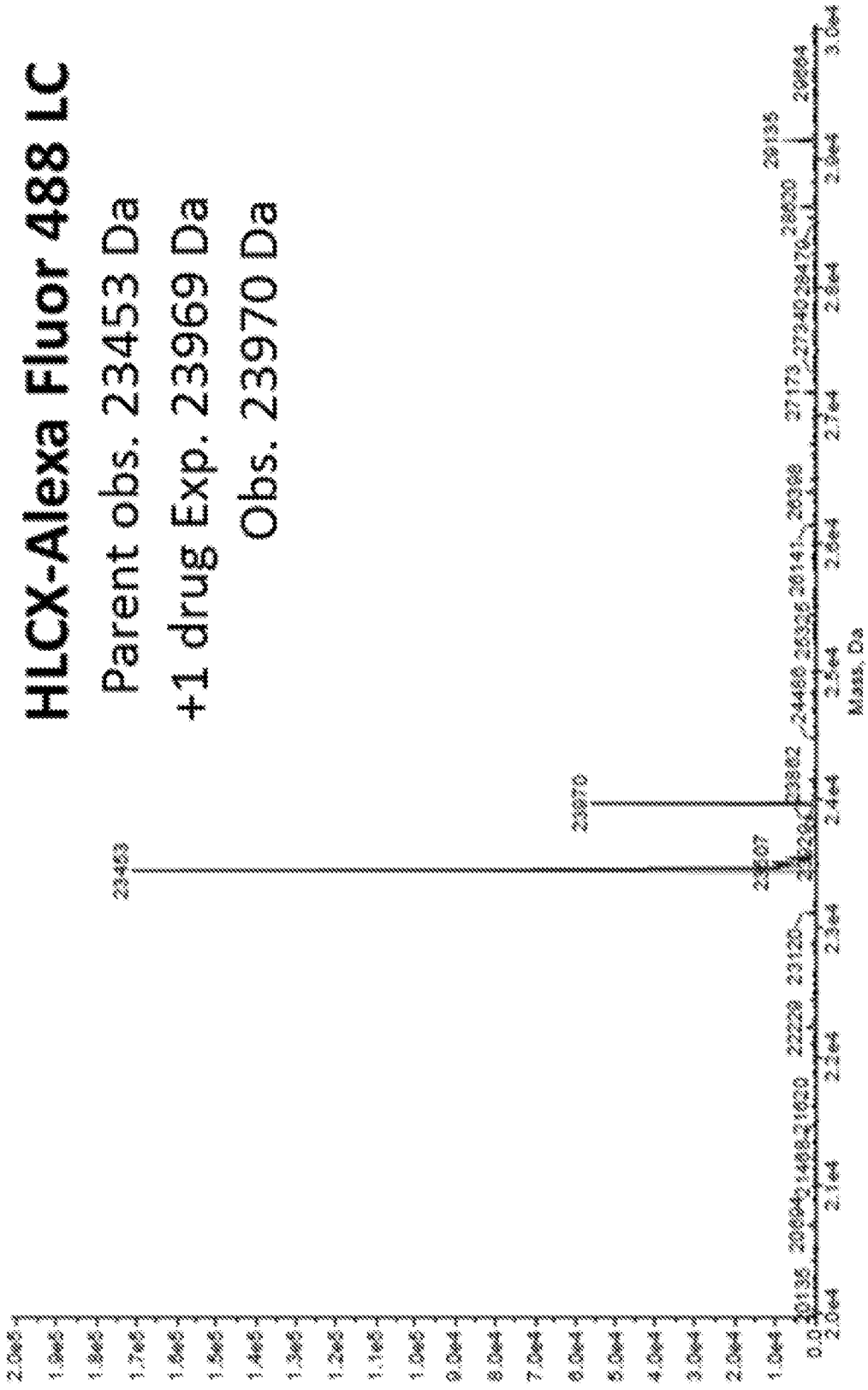

HLCX-Alexa Fluor 488 LC
Parent obs. 23453 Da
+1 drug Exp. 23969 Da
Obs. 23970 Da
23453
23970
Mass, Da

FIG. 6B

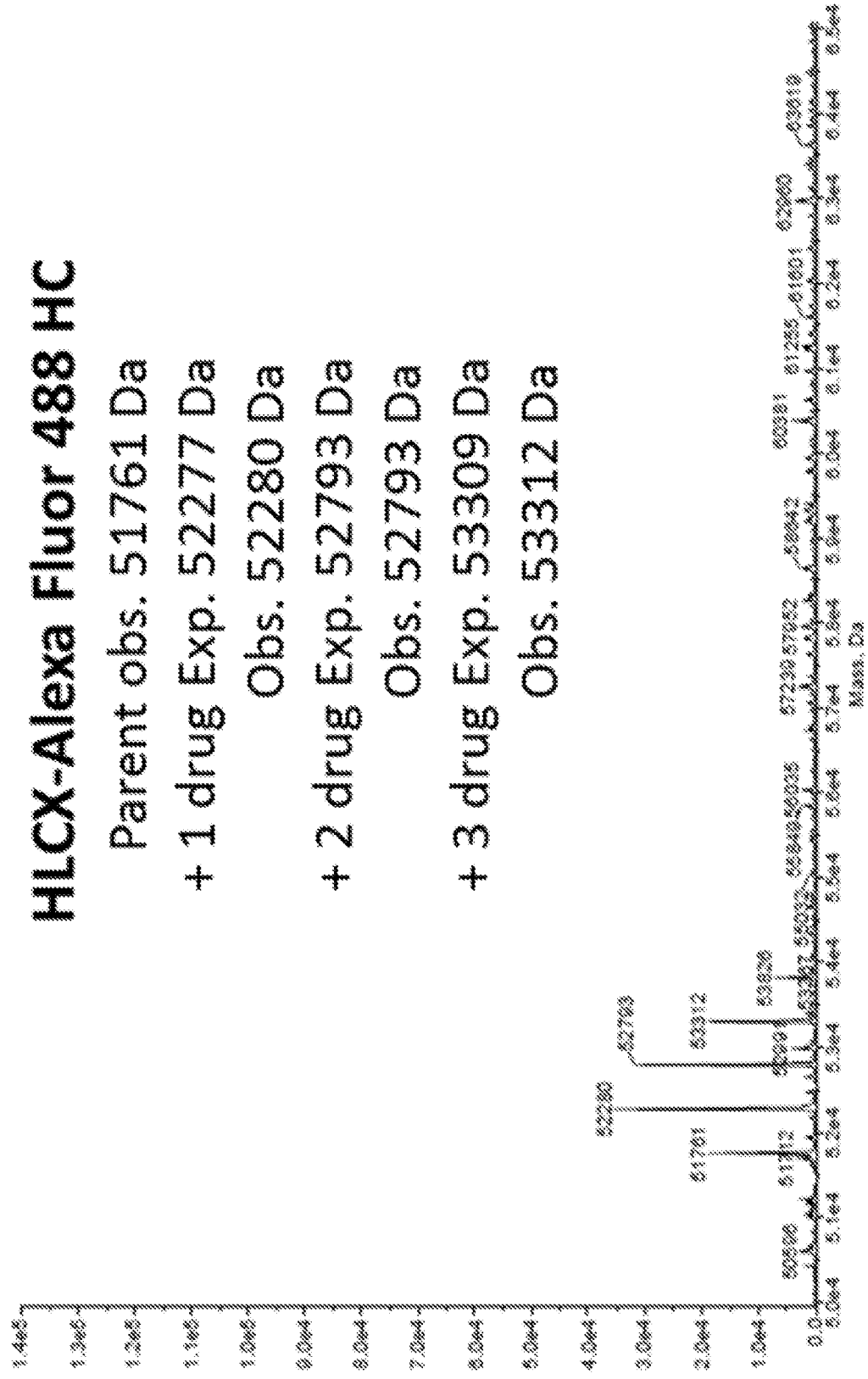

HLCX-Alexa Fluor 488 HC
Parent obs. 51761 Da
+ 1 drug Exp. 52277 Da
Obs. 52280 Da
+ 2 drug Exp. 52793 Da
Obs. 52793 Da
+ 3 drug Exp. 53309 Da
Obs. 53312 Da
Mass, Da

FIG. 7A

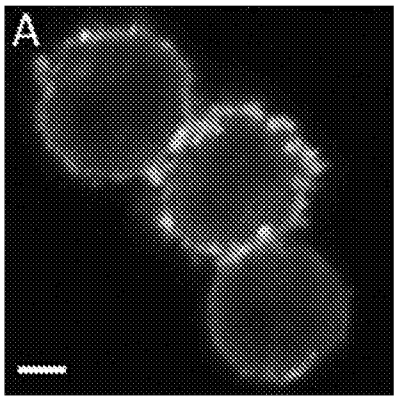


FIG. 7B

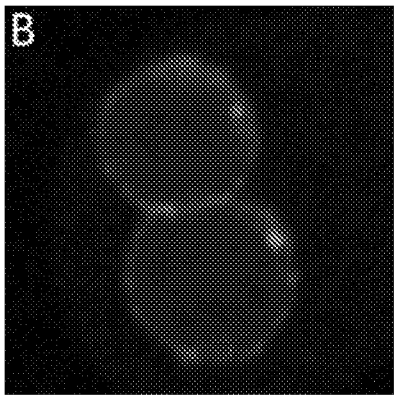


FIG. 7C

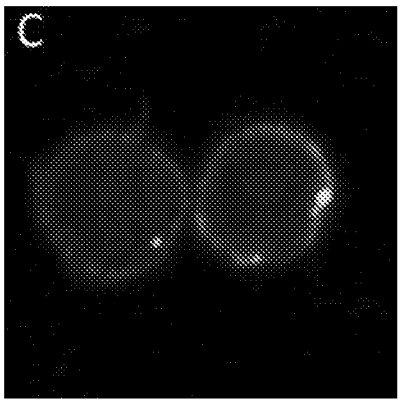


FIG. 7D

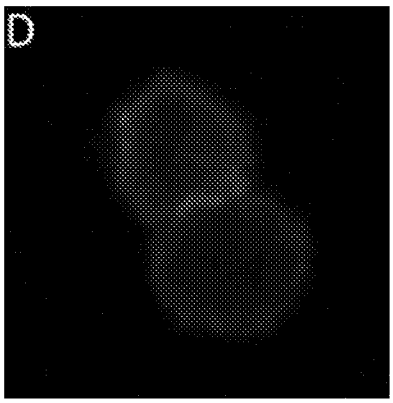


FIG. 8A

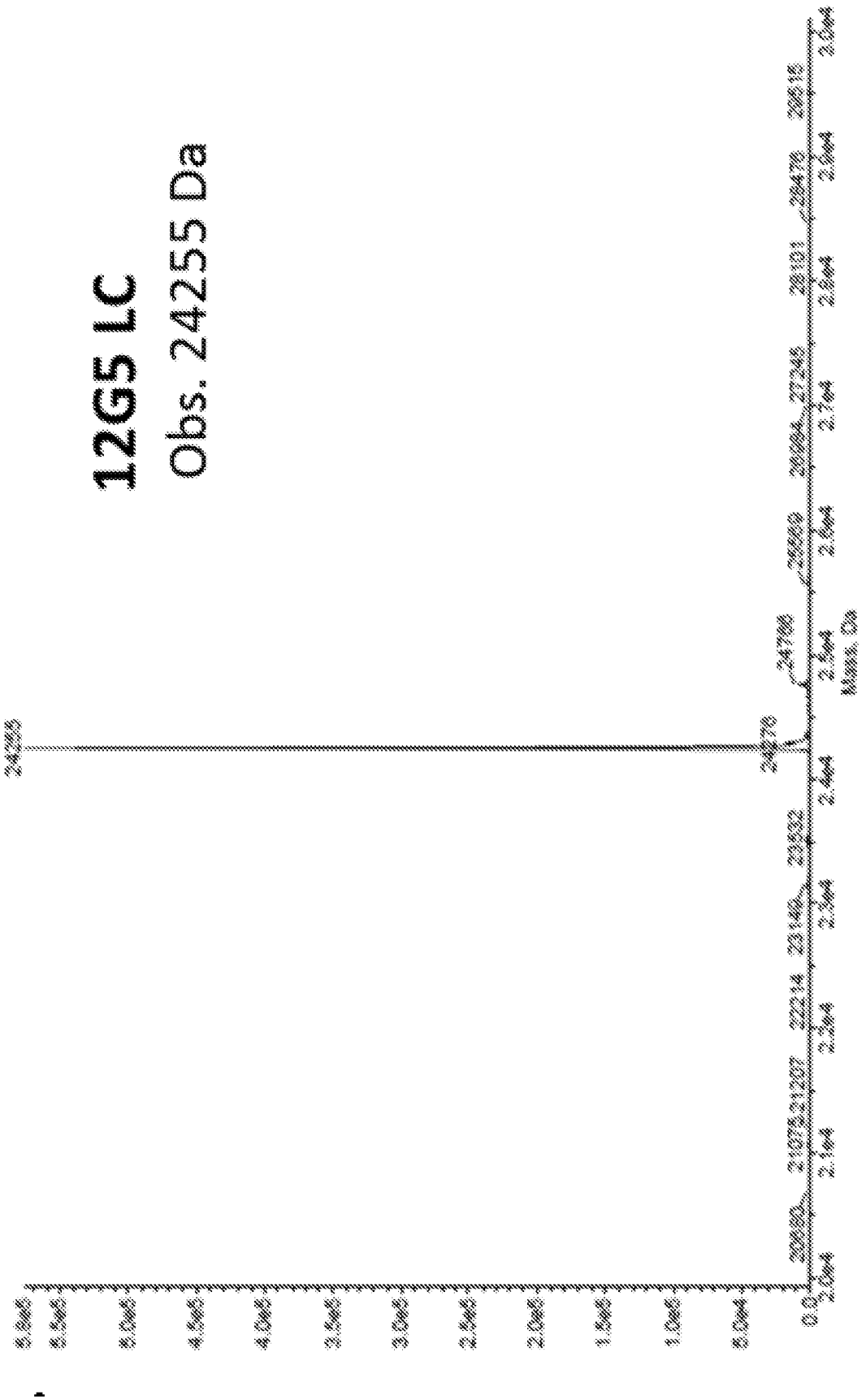


FIG. 8B

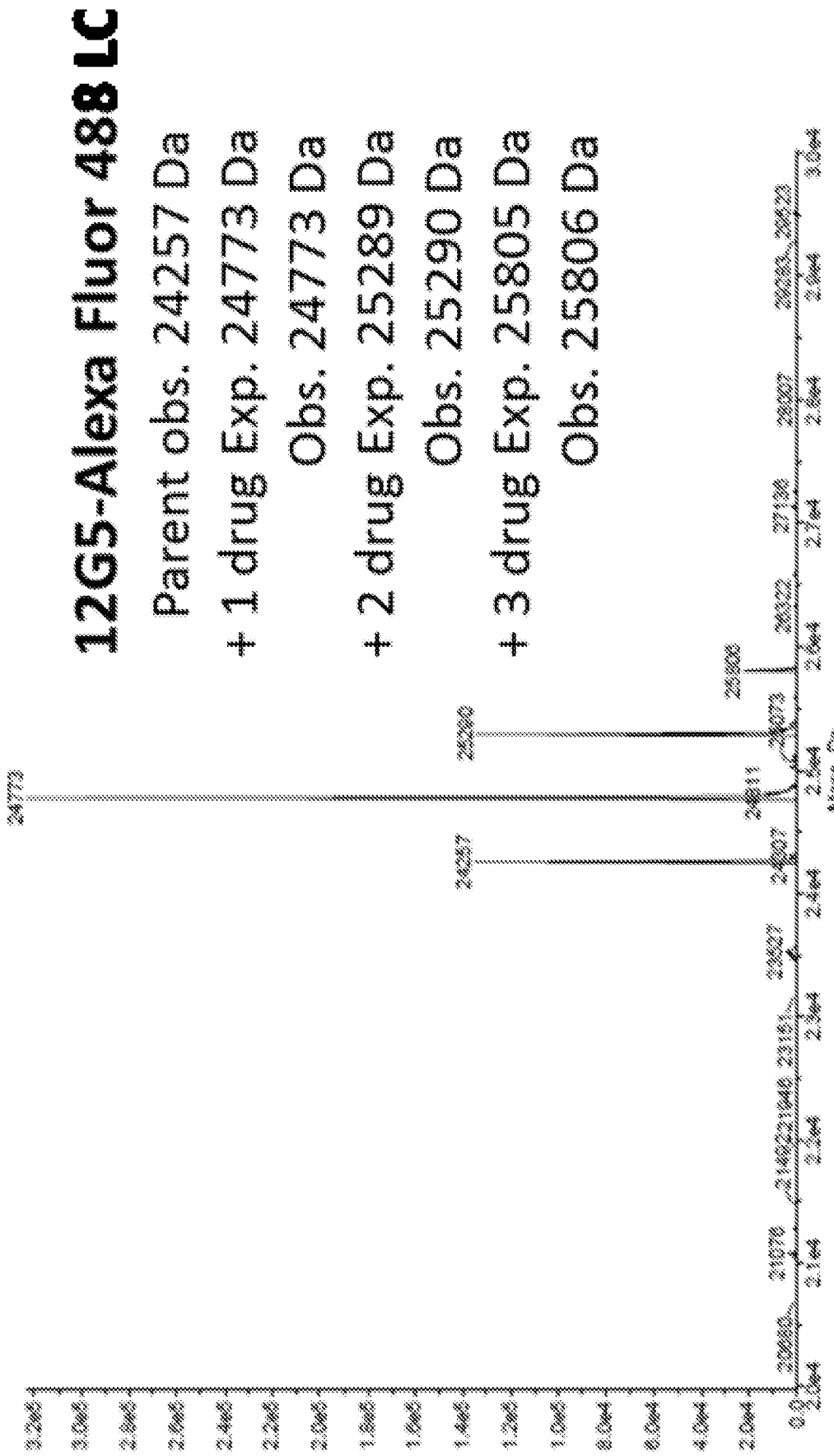

12G5-Alexa Fluor 488 LC
Parent obs. 24257 Da
+ 1 drug Exp. 24773 Da
Obs. 24773 Da
+ 2 drug Exp. 25289 Da
Obs. 25290 Da
+ 3 drug Exp. 25805 Da
Obs. 25806 Da
24773
25290
24257
25806
Mass, Da

FIG. 8C

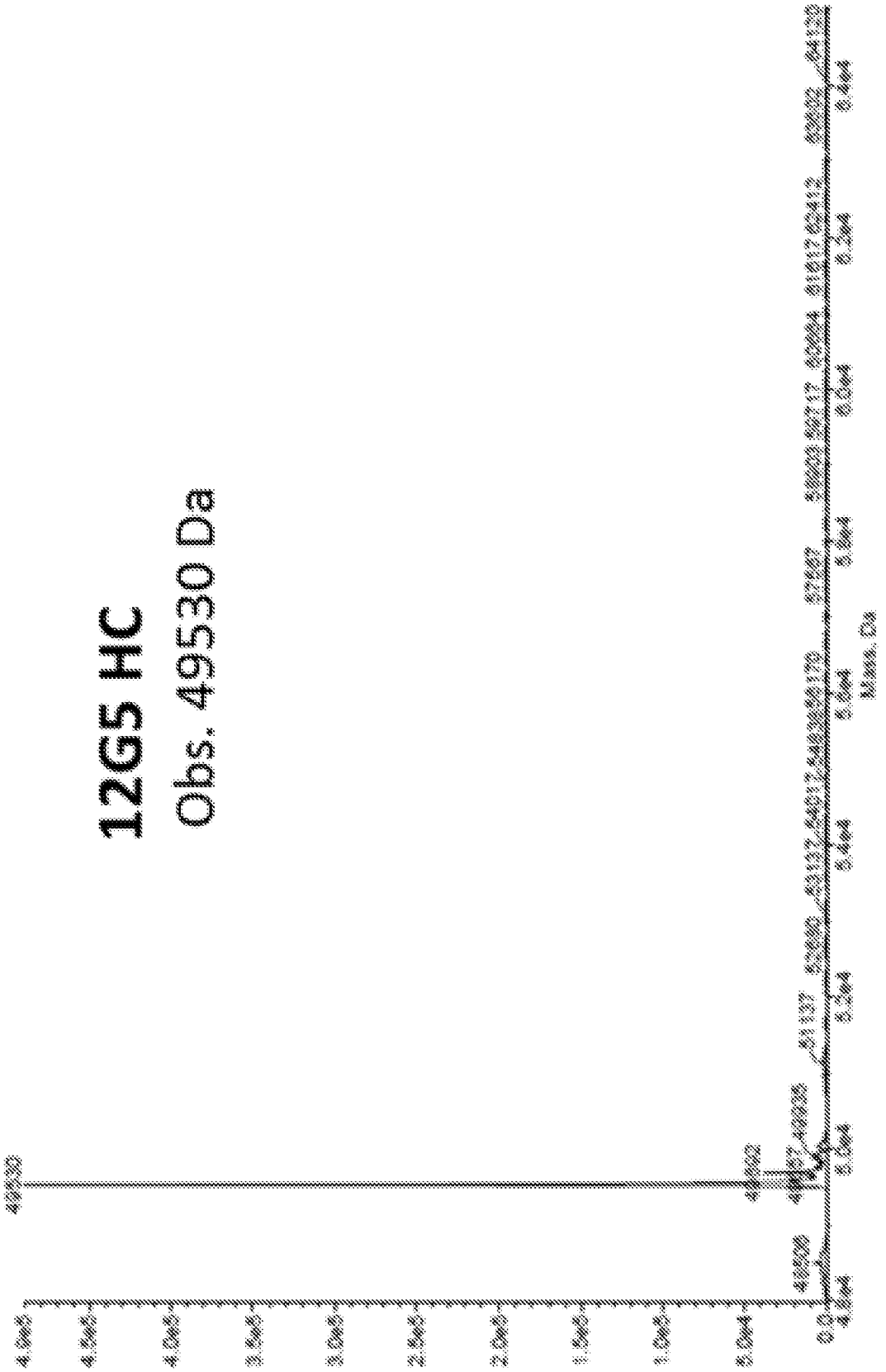


FIG. 8D

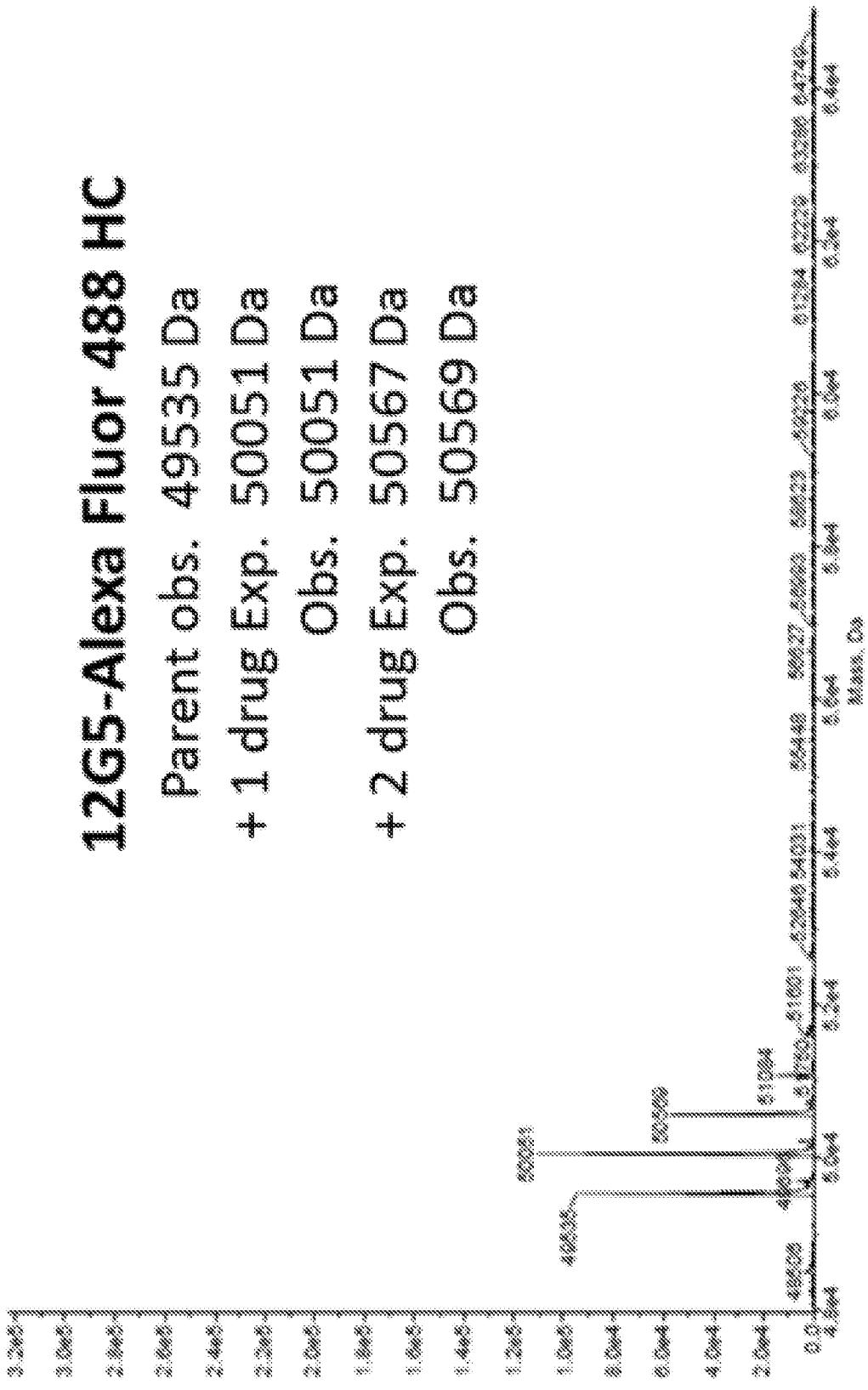

12G5-Alexa Fluor 488 HC
Parent obs. 49535 Da
+ 1 drug Exp. 50051 Da
Obs. 50051 Da
+ 2 drug Exp. 50567 Da
Obs. 50569 Da
Mass, Da

FIG. 9A

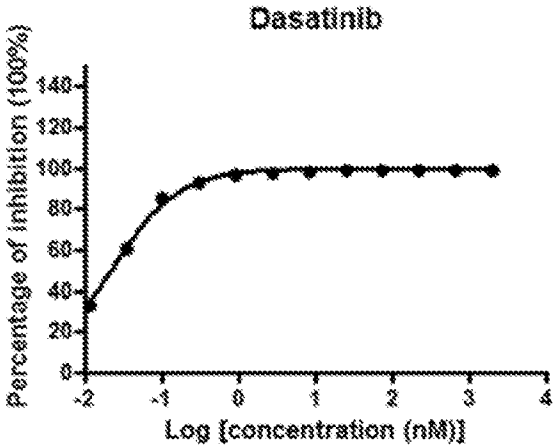


FIG. 9B

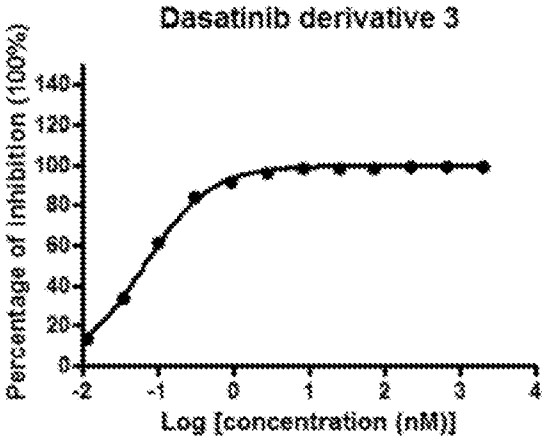


FIG. 9C

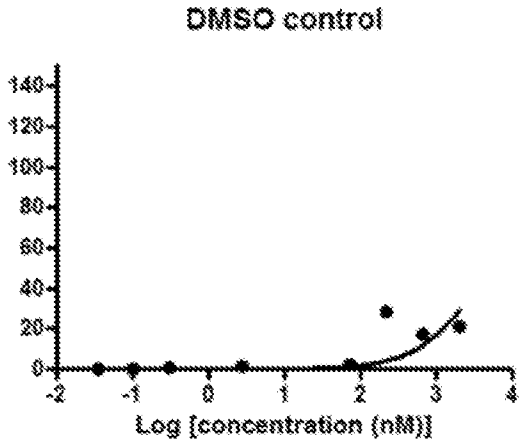


FIG. 10

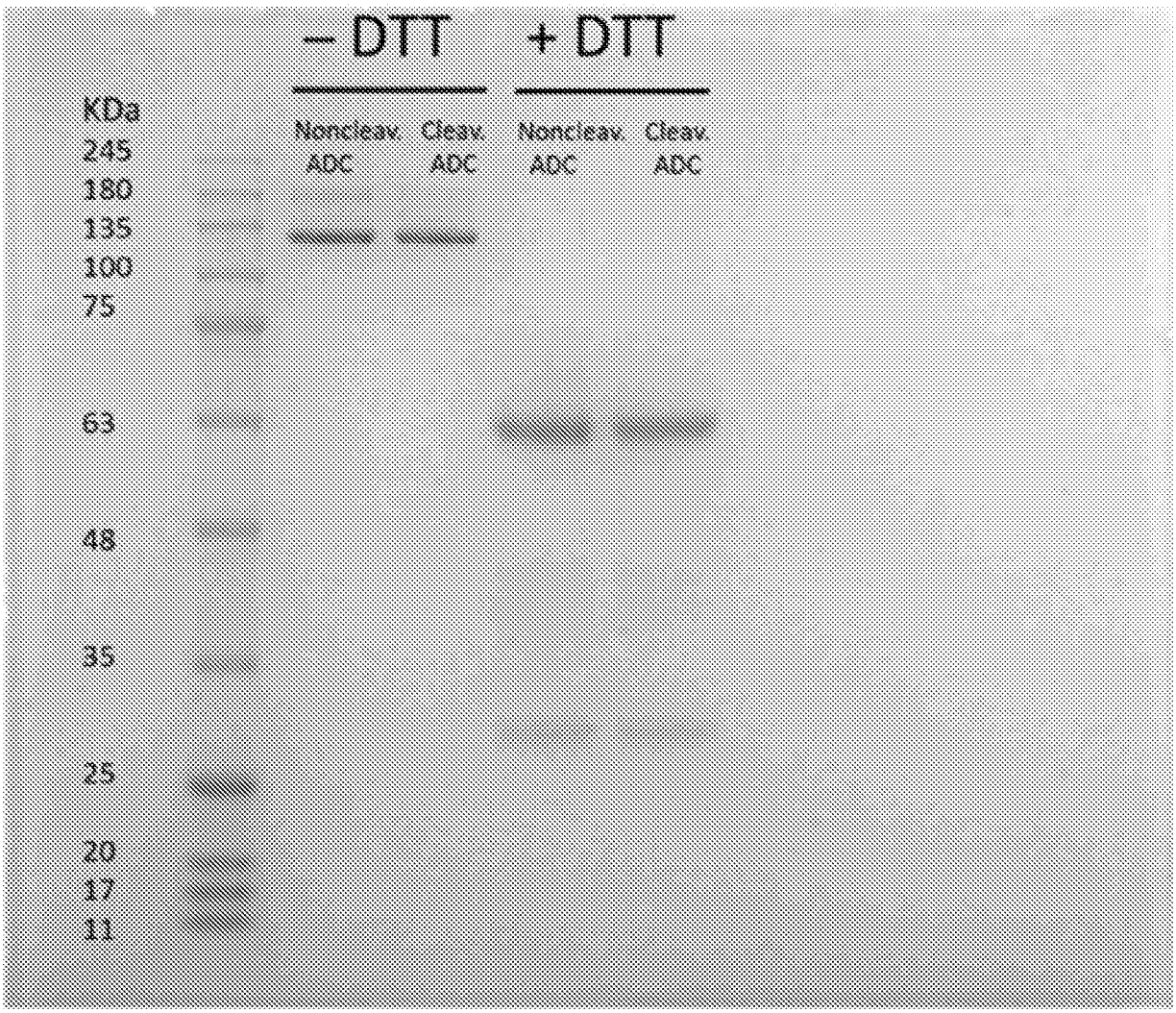

– DTT
+ DTT
KDa
Noncleav. ADC
Cleav. ADC
Noncleav. ADC
Cleav. ADC
245
180
135
100
75
63
48
35
25
20
17
11

FIG. 11A

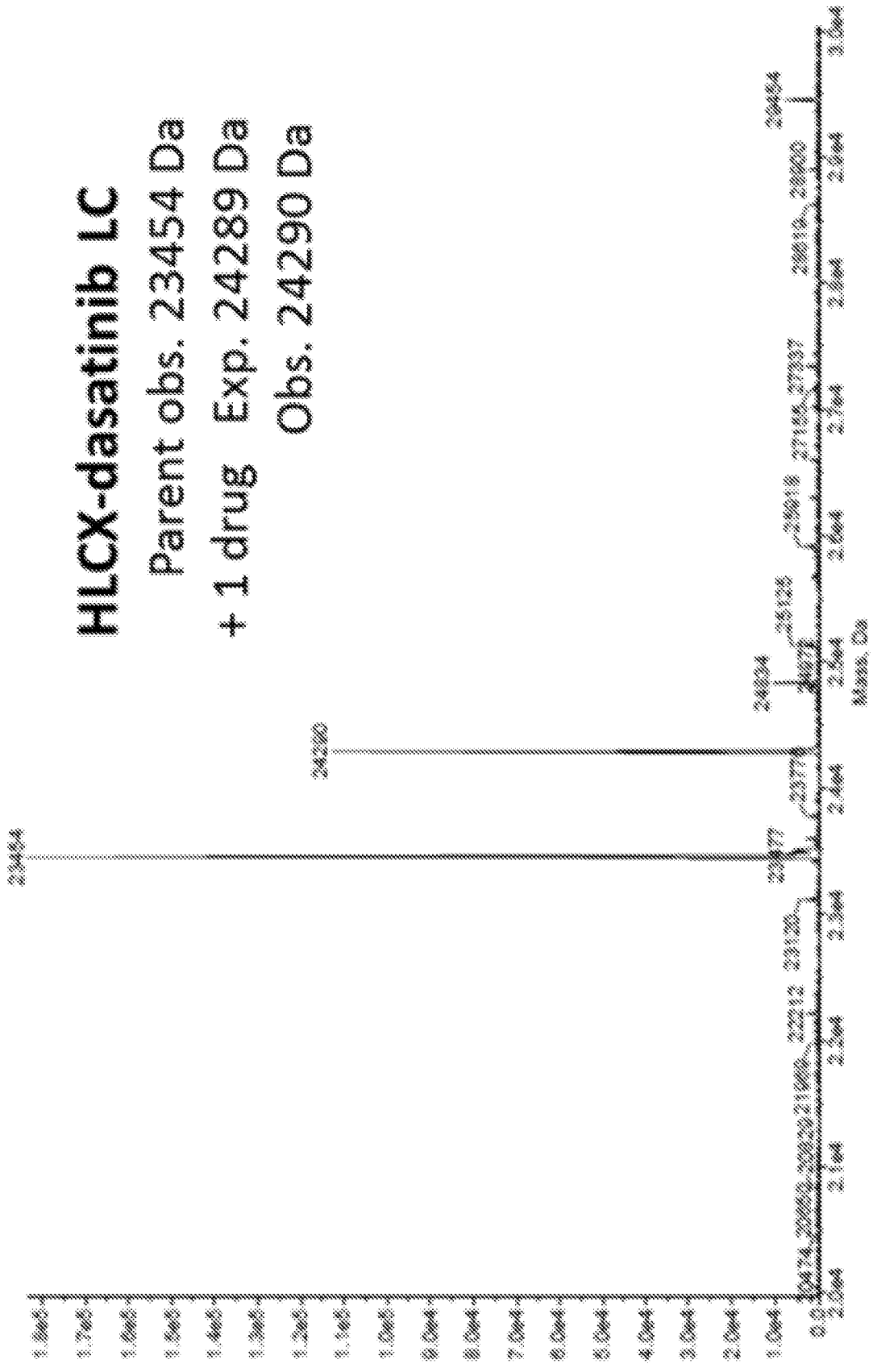

HLCX-dasatinib LC
Parent obs. 23454 Da
+ 1 drug Exp. 24289 Da
Obs. 24290 Da
23454
24290
Mass, Da

FIG. 11B

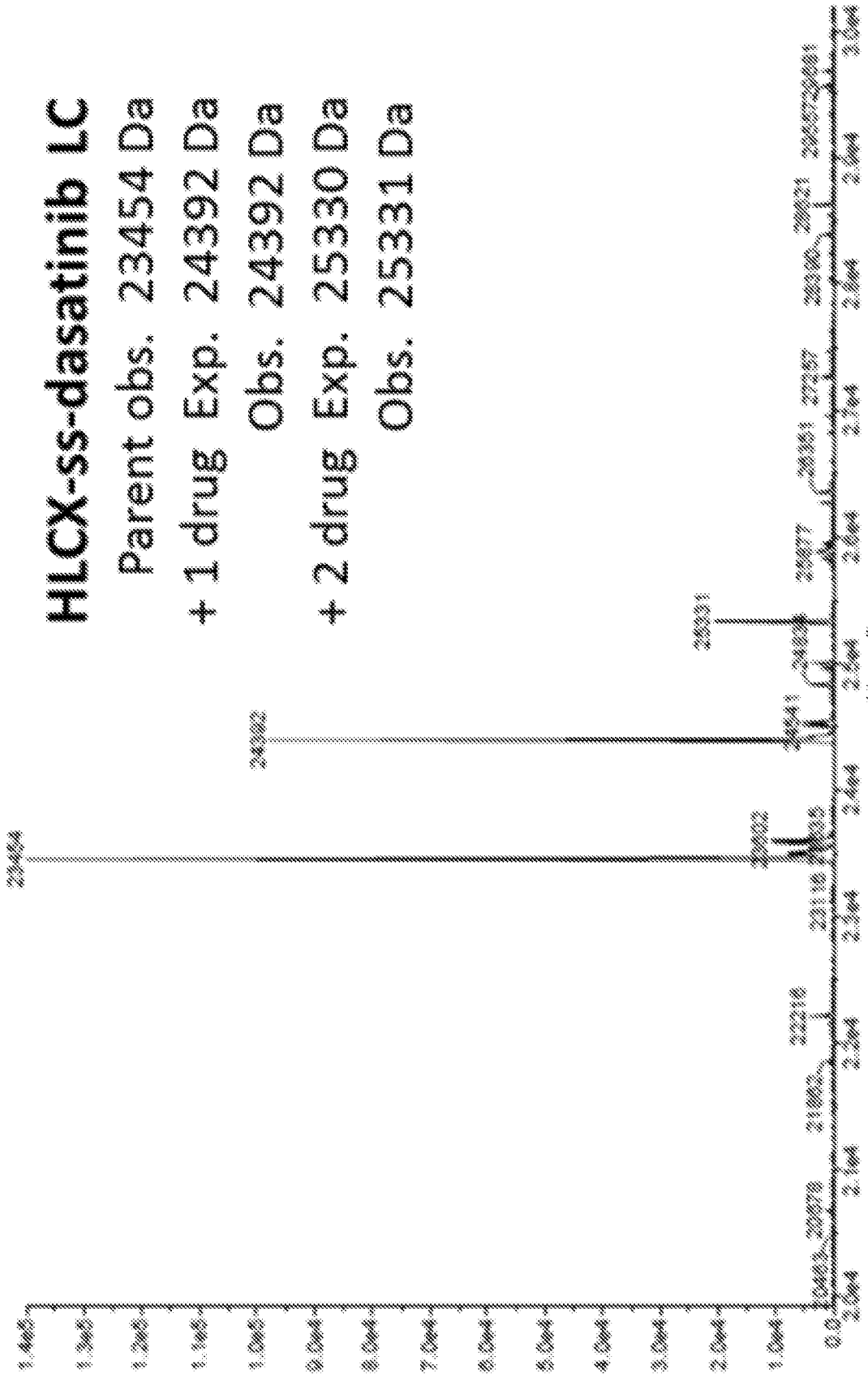

HLCX-ss-dasatinib LC
Parent obs. 23454 Da
+ 1 drug Exp. 24392 Da
Obs. 24392 Da
+ 2 drug Exp. 25330 Da
Obs. 25331 Da
23454
24392
25331
Mass, Da

FIG. 11C

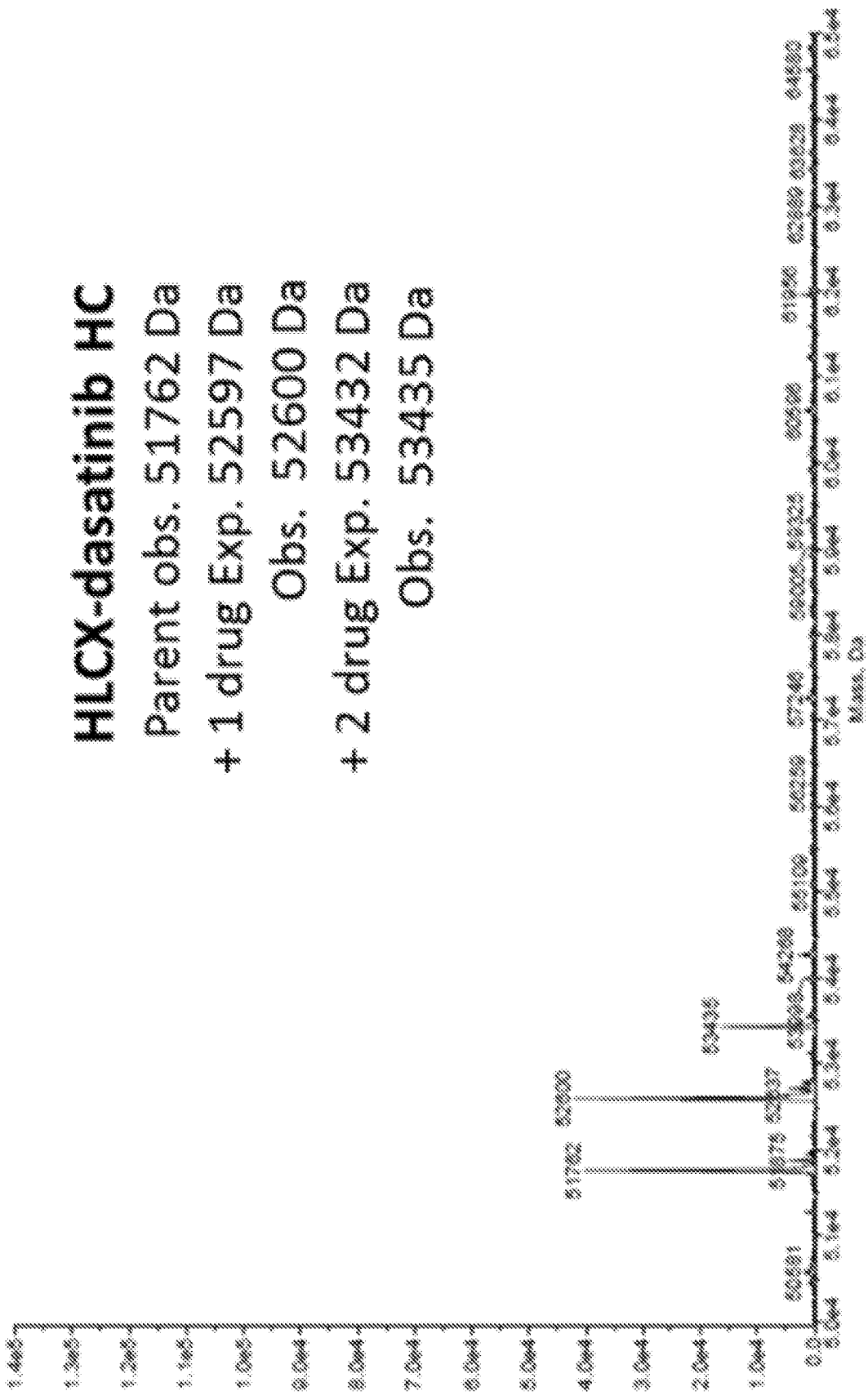

HLCX-dasatinib HC
Parent obs. 51762 Da
+ 1 drug Exp. 52597 Da
Obs. 52600 Da
+ 2 drug Exp. 53432 Da
Obs. 53435 Da
51762
52600
53435
Mass, Da

FIG. 11D

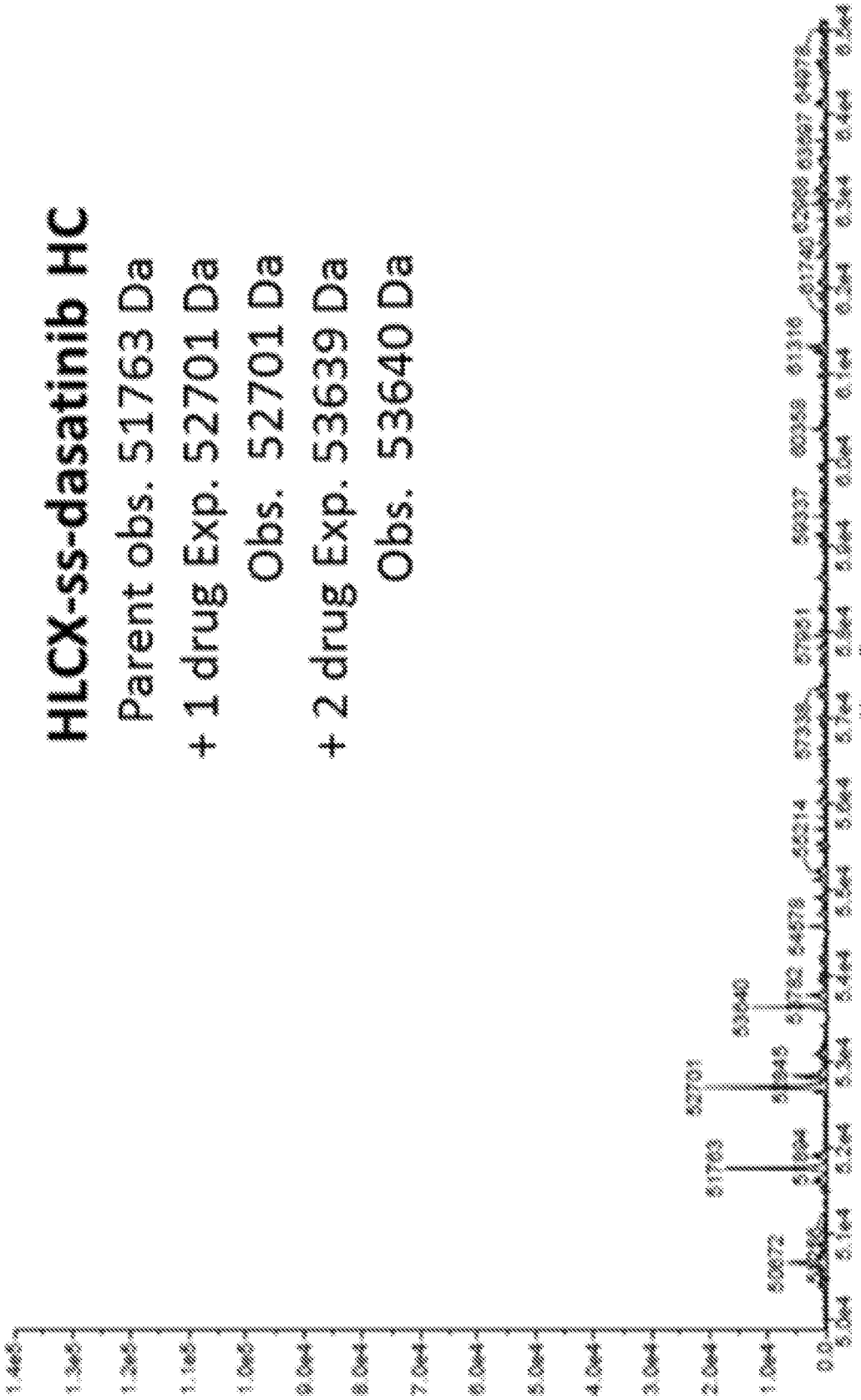

HLCX-ss-dasatinib HC
Parent obs. 51763 Da
+ 1 drug Exp. 52701 Da
Obs. 52701 Da
+ 2 drug Exp. 53639 Da
Obs. 53640 Da
Mass, Da

FIG. 12

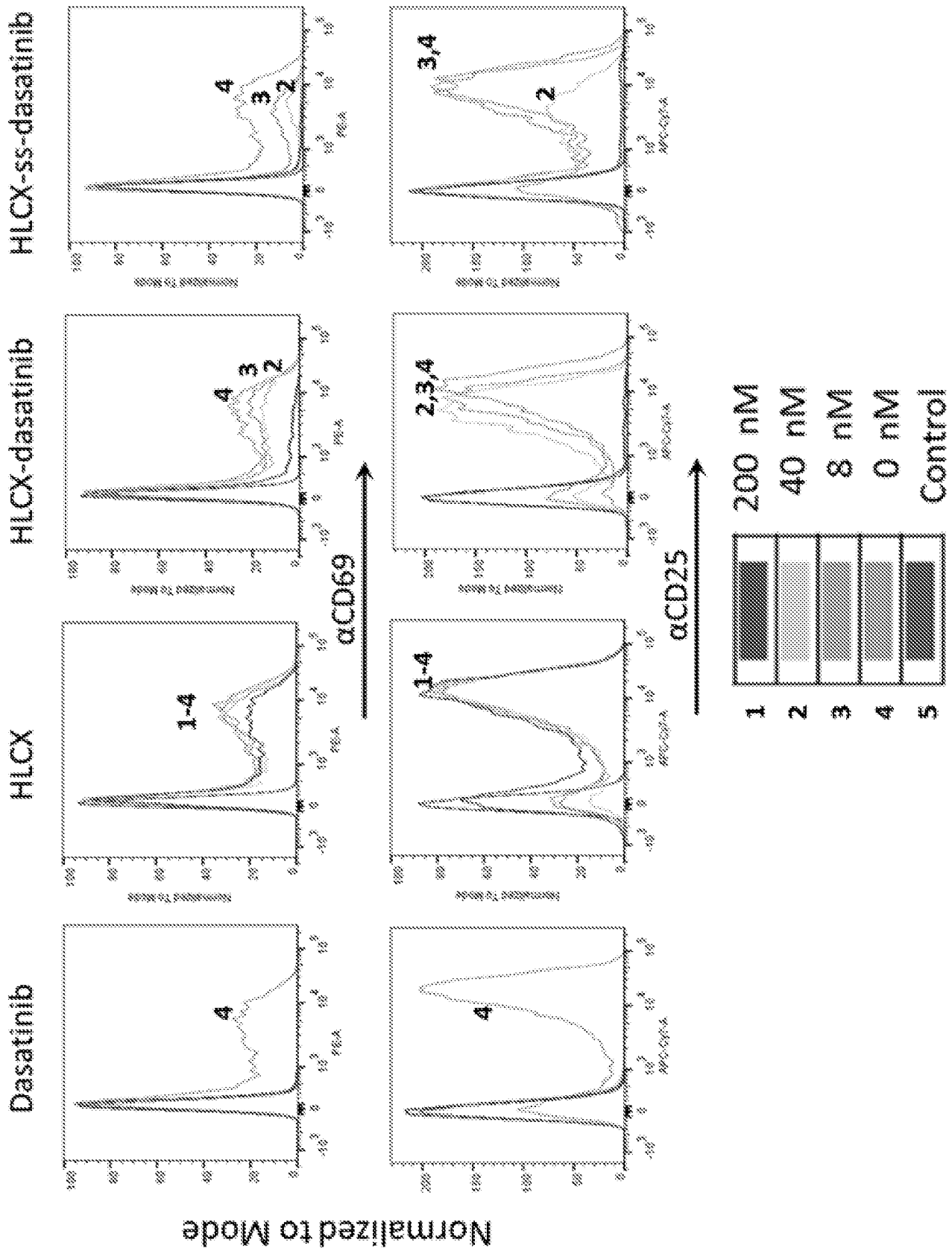

Dasatinib
HLCX
HLCX-dasatinib
HLCX-ss-dasatinib
αCD69
αCD25
Normalized to Mode
1-4
4
2,3,4
3,4
2
3
200 nM
40 nM
8 nM
0 nM
Control
1
2
3
4
5

FIG. 13A

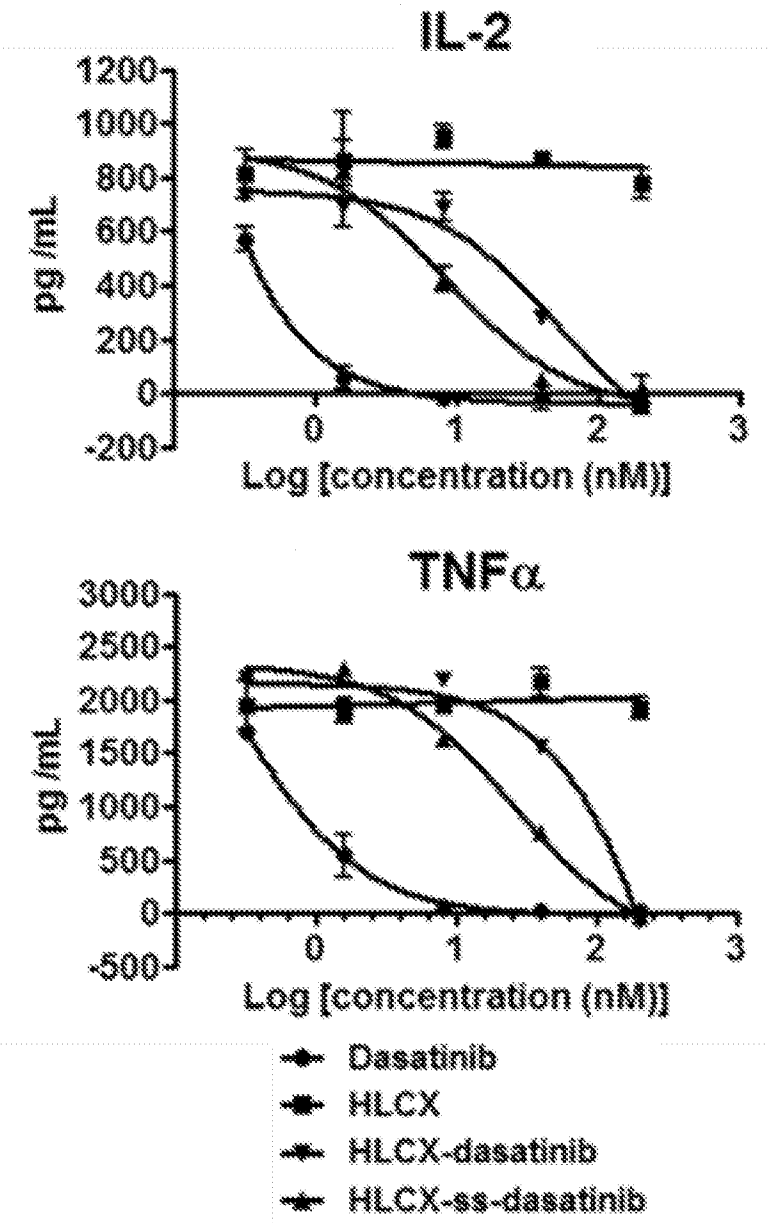

IL-2
1200
1000
800
600
400
200
0
-200
pg /mL
0
1
2
3
Log [concentration (nM)]
TNFα
3000
2500
2000
1500
1000
500
0
-500
pg /mL
0
1
2
3
Log [concentration (nM)]
Dasatinib
HLCX
HLCX-dasatinib
HLCX-ss-dasatinib

FIG. 13B

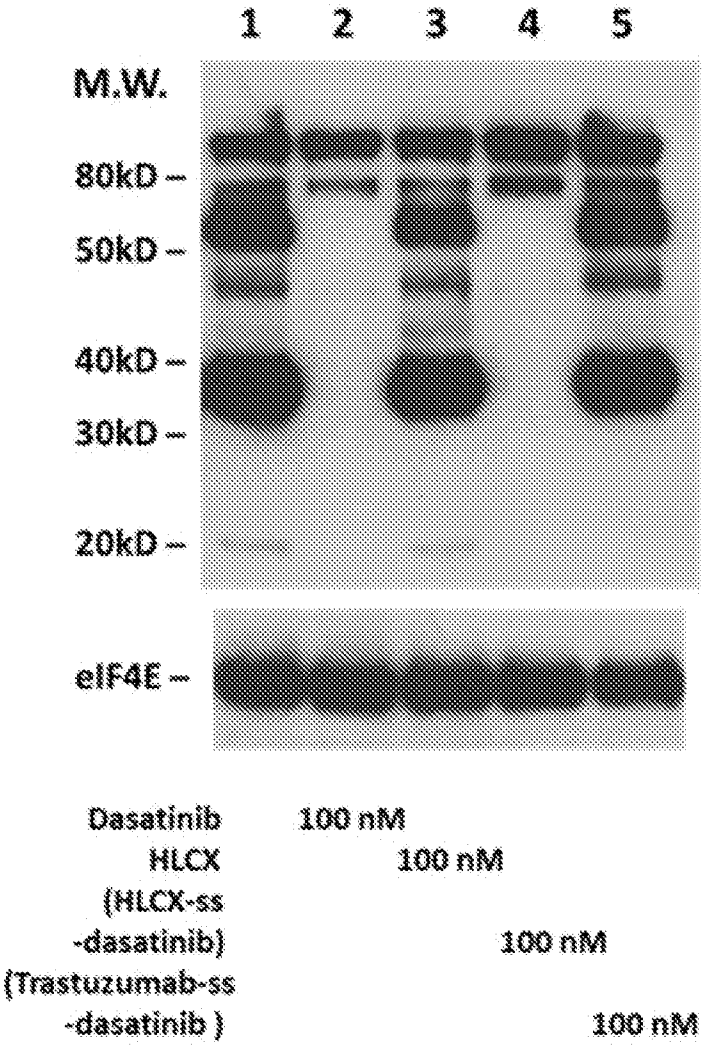

1
2
3
4
5
M.W.
80kD –
50kD –
40kD –
30kD –
20kD –
eIF4E –
Dasatinib 100 nM
HLCX 100 nM
(HLCX-ss -dasatinib) 100 nM
(Trastuzumab-ss -dasatinib) 100 nM

FIG. 14A

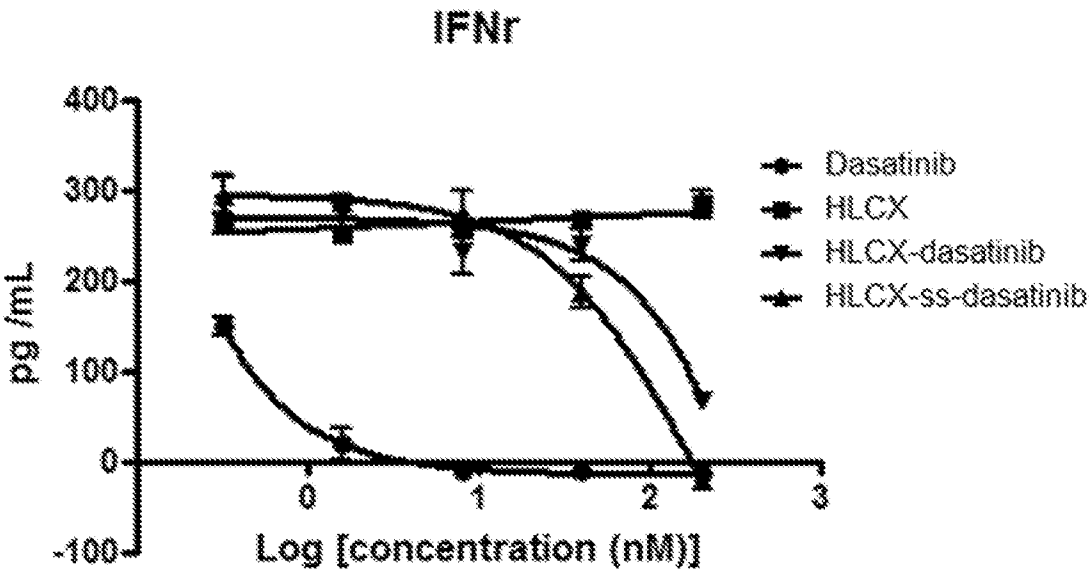


FIG. 14B

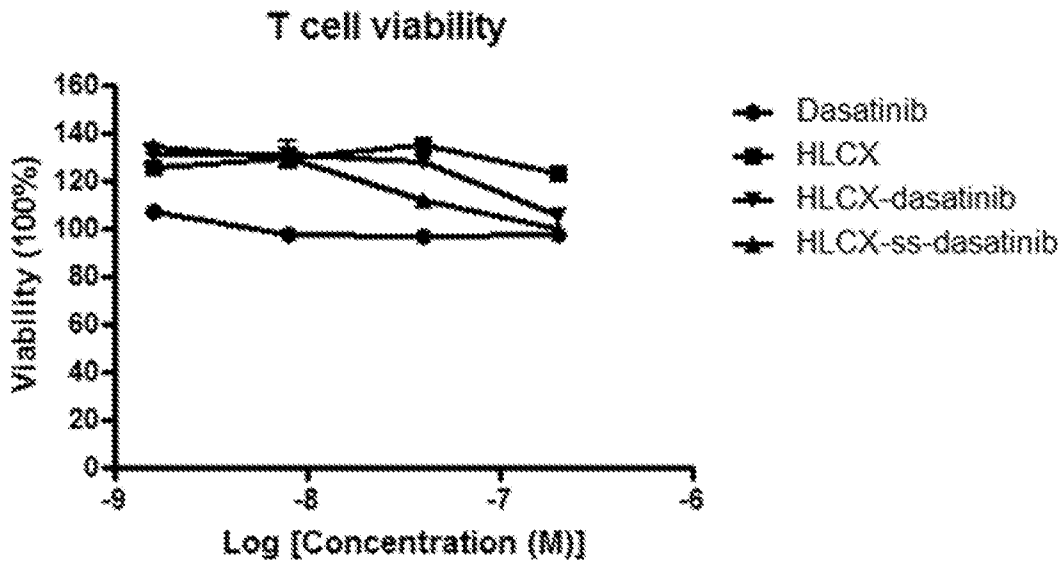
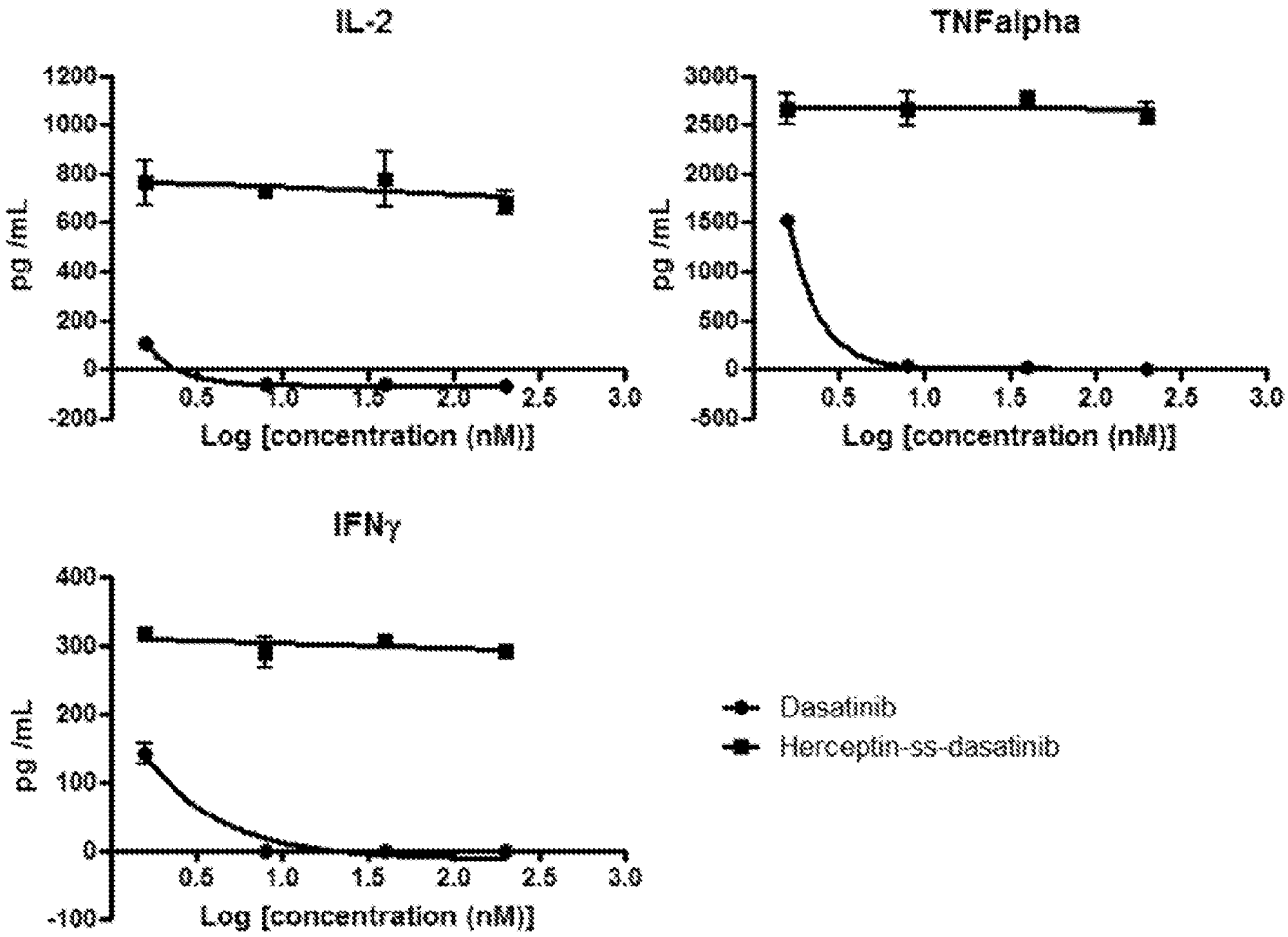


FIG. 15


IL-2
pg /mL
1200
1000
800
600
400
200
0
-200
0.5
1.0
1.5
2.0
2.5
3.0
Log [concentration (nM)]
TNFalpha
pg /mL
3000
2500
2000
1500
1000
500
0
-500
0.5
1.0
1.5
2.0
2.5
3.0
Log [concentration (nM)]
IFNγ
pg /mL
400
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
3.0
Log [concentration (nM)]
Dasatinib
Herceptin-ss-dasatinib

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/13189

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00, A61K 45/00, C07K 16/28 (2016.01)
CPC - C07K 16/28, C07K 16/3061, A61K 47/48384
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)
IPC(8): A61K 39/00, A61K 45/00, C07K 16/28 (2016.01)
CPC: C07K 16/28, C07K 16/3061, A61K 47/48384

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: A61K 47/48561, C07K 2317/21, C07K 2317/70

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: antibody kinase inhibitor conjugate, hematopoietic cell, cell surface, B cell, T cell modulator, monocyte, macrophage, Src, Yes, Fyn, Fgr, Lek, Hck, PDGF, VEGF, EGFR, CD3, CD4, CD71, CD69, CD25, CXCR4, linker, cleavable, increase solubility, autoimmune, inflammatory,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2014/080251 A1 (HANGZHOU DAC BIOTECH CO., LTD) 30 May 2014 (30.05.2014) abstract, claims 8,9, pg 2, ln 10-14, pg 3, ln 26-28, pg 4, ln 17-21, ln 25-29 - pg 5, ln 1-2, pg 19 ln 3, pg 20, ln 5-6, ln 19-21, pg 22, ln 5-7, pg 23, ln 1-24, pg 28, ln 5-8, ln21, pg 29, ln 27-29, pg 30, ln 15, ln 26, pg 32, ln 7-9, pg 38, ln 15-18, pg 39, ln 17-25, pg 40, ln 22-25, ln 30, pg 43, ln 8-9	1-2, 8,11-27,31-48,52-58, (59-64)/(1-2,8,11-27, 31-48,52-58) ---------- 3-7,9-10, 28-30, 49-51, (59-64)/(3-7,9-10, 28-30, 49-51)
Y	US 6,498,165 B1 (ARMSTRONG et al.) 24 December 2002 (24.12.2002) col 2, ln 40-52, col 22, ln 38- 50	3-7, 9-10, (59-64)/(3-7, 9-10)
Y	US 2014/0050720 A1 (SMIDER et al.) 20 February 2014 (20.02.2014) para [0004]	28 49, (59-64)/(28,49)
Y	US 2011/0027286 A1 (THURSTON et al.) 3 February 2011 (03.02.2011) para [0034], [0055], [0066], [0082]	29-30,50-51, (59-64)/(29-30,50-51)

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"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
21 April 2016 (21.04.2016)	16 MAY 2016

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/13189

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 filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: