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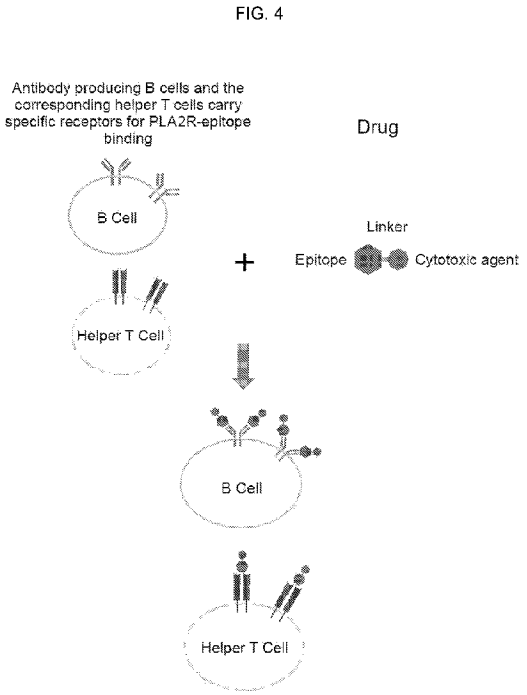

(57) Abstract: Compositions, methods, and kits are for the diagnosis, prevention and/or treatment of autoimmune diseases by detecting, targeting, and/or eliminating epitope-specific autoimmune cells. The compositions include a conjugate of an epitope and an agent that allows for detecting, targeting, and/or eliminating epitope-specific autoimmune cells.

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DIAGNOSIS, PREVENTION, AND/OR TREATMENT OF AUTOIMMUNE DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001[This application claims the benefit of U.S. Provisional Application No. 62/374,382, filed August 12, 2016, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING IN ELECTRONIC FORMAT

[0002[The present application is being filed along with an Electronic Sequence Listing as an ASCII text file via EFS-Web. The Electronic Sequence Listing is provided as a file entitled IMWO001WOSEQLIST.txt, created and last saved on August 11, 2017, which is 72,810 bytes in size. The information in the Electronic Sequence Listing is incorporated herein by reference in its entirety in accordance with 35 U.S.C. § 1.52(e).

BACKGROUND

Field

[0003[The present disclosure generally relates to compositions, methods, and/or kits for diagnosis, prevention and/or treatment of autoimmune system diseases by detecting, targeting, and/or eliminating epitope-specific autoimmune cells.

Description of the Related Art

[0004[Autoimmune diseases can be T cell mediated, B cell mediated, or both and can be associated with organ and/or tissue damage.

SUMMARY

[0005[In some embodiments, a method of treating a patient with membranous nephropathy (MN) is provided. In some embodiments, the method of treating a patient with MN comprises identifying a patient with MN, and administering to the patient a complex comprising a PLA2R epitope and a drug, wherein the epitope is comprised within a PLA2R fragment, thereby eliminating or reducing an anti-PLA2R autoantibody producing B cell population in the patient.

[0006[In some embodiments of the method of treating a patient with MN, the PLA2R epitope is as provided in SEQ ID NO: 13. In some embodiments of the method, the

sequence of the PLA2R fragment is as provided in SEQ ID NO: 1. In some embodiments of the method, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2. In some embodiments of the method, the PLA2R fragment is as provided in SEQ ID NO: 2 and at least about 5% of the sequence provided in SEQ ID NO: 3. In some embodiments of the method, the PLA2R fragment is as provided in SEQ ID NO: 4. In some embodiments of the method, the PLA2R fragment is as provided in SEQ ID NO: 5. In some embodiments of the method, the PLA2R fragment is as provided in SEQ ID NO: 5 and at least about 5% of the sequence provided in SEQ ID NO: 6. In some embodiments of the method, the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine. In some embodiments of the method, an efficacy of eliminating the anti-PLA2R autoantibody producing B cell population ranges from about 70% to about 100%. In some embodiments of the method, the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-PLA2R autoantibody producing B cell population.

[0007[In some embodiments, a complex comprising a PLA2R epitope and a drug is provided. In some embodiments of the complex, the epitope is comprised within a PLA2R fragment. In some embodiments of the complex, the PLA2R epitope is as provided in SEQ ID NO: 13. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 1. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2 and at least about 5% of the sequence provided in SEQ ID NO: 3. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 4. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5. In some embodiments of the complex, the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5 and at least about 5% of the sequence provided in SEQ ID NO: 6. In some embodiments of the complex, the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine. In some embodiments of the complex, the drug is linked to the PLA2R fragment via a valine-citrulline linker.

[0008[In some embodiments, a method of delivering a drug to a subject having autoimmune B cells or T cells is provided. In some embodiments, the method of delivering a

drug comprises providing the drug in an epitope-drug conjugate (EDC) comprising an epitope conjugated to the drug via a linker, the epitope being recognized by receptors on the autoimmune B cells or T cells in the subject, the drug having an activity that blocks the B cells from stimulating other cells, and intradermally or subcutaneously administering the EDC to the subject. In some embodiments of the method of delivering a drug, the autoimmune B cells or T cells are circulating in the blood of the subject. In some embodiments of the method of delivering a drug, the autoimmune B cells or T cells are in lymph nodes of the subject. In some embodiments of the method of delivering a drug, the drug kills the B cell or the T cell. In some embodiments of the method of delivering a drug, the drug is selected from the group consisting of Duocarmycin A, Duocarmycin B1, Duocarmycin B2, Duocarmycin C1, Duocarmycin C2, Duocarmycin D, Duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil or capecitabine, Monomethyl auristatin E (MMAE), anthracyclines, oxaliplatin, or bortezomib. In some embodiments of the method of delivering a drug, the drug blocks release of cytokines from the B cell or the T cell or block cytokine signaling in the B cell or the T cell. In some embodiments of the method of delivering a drug, the drug is selected from the group consisting of Rapamycin, Ciclosporin, Tacrolimus, Mycophenolate, Fingolimod, Imatinib, Temsirolimus, Sorafenib, Sunitinib, Pirfenidone, Src family tyrosine kinase inhibitors (Dasatinib, Saracatinib, Bosutinib, Bafetinib), MEK kinase inhibitors (Selumetinib, Trametinib, and Refametinib). In some embodiments of the method of delivering a drug, the subject is not in an acute phase of active autoimmune disease. In some embodiments of the method of delivering a drug, the subject has been treated with an immunosuppressant such that the patient is not in the acute phase of active autoimmune disease. In some embodiments of the method of delivering a drug, the EDC has a molecular weight of 14-70 kDa.

[0009[In some embodiments, a method of treating a patient with Pemphigus vulgaris (PV) is provided. In some embodiments, the method of treating a patient with PV comprises identifying a patient with PV, and administering to the patient a complex comprising a desmoglein 1 or a desmoglein 3 epitope and a drug, wherein the epitope is comprised within a desmoglein 1 or desmoglein 3 fragment, thereby eliminating or reducing an anti- desmoglein 1 or anti- desmoglein 3 autoantibody producing B cell population in the patient.

[0010[In some embodiments of the method of treating a patient with PV, the desmoglein 1 epitope is as provided in SEQ ID NO: 16. In some embodiments of the method of treating a patient with PV, the sequence of the desmoglein 1 fragment is as provided in SEQ ID NO: 16. In some embodiments of the method of treating a patient with PV, the desmoglein 3 epitope is as provided in SEQ ID NO: 17. In some embodiments of the method, the sequence of

the desmoglein 3 fragment is as provided in SEQ ID NO: 17. In some embodiments of the method, the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine. In some embodiments of the method, an efficacy of eliminating the anti-desmoglein 1 autoantibody producing B cell population ranges from about 70% to about 100%. In some embodiments of the method, an efficacy of eliminating the anti-desmoglein 3 autoantibody producing B cell population ranges from about 70% to about 100%. In some embodiments of the method, the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-desmoglein 1 autoantibody producing B cell population. In some embodiments of the method, the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-desmoglein 3 autoantibody producing B cell population.

[0011[In some embodiments, an epitope drug complex (EDC) is provided. In some embodiments, the EDC comprises a drug and a desmoglein 1 epitope or desmoglein 3 epitope, wherein the desmoglein 1 epitope is comprised within a desmoglein 1 fragment and wherein the desmoglein 3 epitope is comprised within a desmoglein 3 fragment.

[0012[In some embodiments of the EDC, the desmoglein 1 epitope is as provided in SEQ ID NO: 16. In some embodiments of the EDC, the sequence of the desmoglein 1 fragment is as provided in SEQ ID NO: 16. In some embodiments of the EDC, the desmoglein 3 epitope is as provided in SEQ ID NO: 17. In some embodiments of the EDC, the sequence of the desmoglein 3 fragment is as provided in SEQ ID NO: 17. In some embodiments of the EDC, the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine.

[0013[In some embodiments, a method for diagnosing an autoimmune disease in a subject is provided. In some embodiments, the method of diagnosing comprises detecting a presence of a population of autoimmune B and/or T cells in the subject by collecting a sample from the subject, incubating the sample with an EDC comprising an epitope and a fluorophore, wherein the epitope is specific for one or more B cell receptors expressed by the autoimmune B cells and/or one or more T cell receptors expressed by the autoimmune T cells, detecting a binding of the EDC to the B cells and/or T cells by microscopy, thereby diagnosing an autoimmune disease in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014[FIG. 1 shows an embodiment of the topological folding and structural domains of PLA2R in a cell membrane.

[0015[FIG. 2 shows an embodiment of topological folding of PLA2R with the dominant conformational epitope.

[0016[FIG. 3 shows a schematic of an embodiment of antigen processing and recruitment of T cell help by B cells.

[0017[FIG. 4 shows a schematic of the molecular basis of an embodiment of epitope-drug conjugate (EDC) design.

[0018[FIG. 5 shows a schematic illustration of an embodiment of a mechanism of EDC treatment.

[0019[FIG. 6 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 1).

[0020[FIG. 7 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 2).

[0021[FIG. 8 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 3).

[0022[FIG. 9 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 4).

[0023[FIG. 10 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 5).

[0024[FIG. 11 shows the amino acid sequence of an embodiment of PLA2R fragment (SEQ ID NO: 6).

[0025[FIG. 12 shows the amino acid sequence of an embodiment of CysR domain (SEQ ID NO: 7) of PLA2R.

[0026[FIG. 13 shows the amino acid sequence of an embodiment of CysR and FNII domains (SEQ ID NO: 8) of PLA2R.

[0027[FIG. 14 shows the amino acid sequence of an embodiment of CysR, FNII and CTLD1 domains (SEQ ID NO: 9) of PLA2R.

[0028[FIG. 15 shows the amino acid sequence of an embodiment of CysR, FNII, and CTLD1, 2 domains (SEQ ID NO: 10) of PLA2R.

[0029[FIG. 16 shows the amino acid sequence of an embodiment of CysR, FNII and CTLD1, 2, 3 domains (PLA2R-1-5 domains) (SEQ ID NO: 11) of PLA2R.

[0030[FIG. 17 shows the amino acid sequence of an embodiment of CysR, FNII and CTLD1, 2, 3, 4 domains (SEQ ID NO: 12) of PLA2R.

[0031[FIG. 18 shows the amino acid sequence of an embodiment of the epitope (SEQ ID NO: 13) of PLA2R against which autoantibodies are present in MN patients.

[0032[FIG. 19 shows the amino acid sequence of an embodiment of full-length PLA2R (SEQ ID NO: 14). Shaded region indicates an embodiment the epitope (SEQ ID NO: 13) of PLA2R against which autoantibodies are present in MN patients. Underlined region indicates an embodiment of CysR, FNII and CTLD1 domains (SEQ ID NO: 9) of PLA2R.

[0033[FIG. 20 shows potential N-Linked glycosylation sites in the N-terminal 272 amino acids of PLA2R (SEQ ID NO: 15).

[0034[FIG. 21 shows a schematic of a drug development platform for autoimmune disease treatment. Top panel shows design of epitope drug conjugate (EDC). Middle panel shows EDC binds to the antigen-specific receptors on the surface of the B cells and T cells. Bottom panel shows the effect of EDC on the targeted immune cells (e.g., cell death).

[0035[FIG. 22 shows a schematic of delivery of EDC to memory B cells residing in the peripheral lymph nodes.

[0036[FIG. 23 shows design of an embodiment of a PLA2R epitope drug conjugate (EDC).

[0037[FIG. 24 shows detection of PLA2R epitope specific memory B cells in total B cells isolated from PLA2R-Ab positive PMN patient blood samples.

[0038[FIG. 25 shows the effect of PLA2R-epitope-MMAE conjugate on B cells isolated from PLA2RAb positive PMN patients.

[0039[FIG. 26 shows topological folding of desmoglein in the cell membrane. The shorter vertical line indicates the immunodominant epitope region in Dsg1 for autoantibody binding (EC1-2), and the longer vertical line indicates the immunodominant region in Dsg3 for autoantibody binding (EC1-3).

[0040[FIG. 27 shows design of an embodiment of an epitope drug conjugate (EDC) for mucocutaneous PV treatment.

[0041[FIG. 28 Purification and autoantibody binding to the drug conjugated epitopes.

[0042[FIG. 29 shows detection of Dsg1 and Dsg3 epitope specific memory B cells in total B cells isolated from mucocutaneous PV patient blood samples.

[0043[FIG. 30 shows the effect of Dsg1-epitope-MMAE and Dsg3-epitope-MMAE conjugate on B cells isolated from mucocutaneous PV patients.

[0044[FIG. 31 shows the amino acid sequence of an embodiment of an epitope sequence of Dsg1 (SEQ ID NO: 16) for developing EDC.

[0045[FIG. 32 shows the amino acid sequence of an embodiment of an epitope sequence of Dsg3 (SEQ ID NO: 17) for developing EDC.

DETAILED DESCRIPTION

[0046[Currently, there are no effective treatments for autoimmune diseases except to use high doses of steroid hormones and immunosuppressive agents. These treatments are nonspecific, have significant side-effects and often cannot stop the disease from progressing. The major challenge of these treatments is the frequent disease relapse when the dose of the reagents is reduced (e.g., after the disease is in clinical remission), which severely impair the function of the affected organ(s).

A new drug development platform for autoimmune disease diagnosis, prevention and/or treatment

[0047[Antibody production in B cells is initiated by antigen binding to the cell-surface B cell receptors (BCRs). The initial binding of antigen to the BCR induces a cascade of intracellular signaling events that results in B cell activation. Antigen binding to BCR triggers rapid internalization of the BCR–antigen complex. After internalization, the BCR–antigen complex is sorted into early endosomes and subsequently into major histocompatibility complex class II (MHCII)-containing late endosomes. Upon fusion with lysosomes, these compartments degrade the antigens into peptides that are loaded onto MHCII for presentation to helper T cells (FIG. 3). The engagement of specific helper T cells stimulates antibody production by the activated B cells. (FIG. 3)

[0048[An autoimmune disease occurs due to an immune response (e.g., antibody production by B cells) against to a normal body tissue and/or organ. About 80 types of autoimmune diseases are known in humans and almost any tissue and/or organ can be affected.

[0049[Clinically, the frequent autoimmune disease relapse in patients is due to the presence of a specific group of memory B cells that are present in a quiescent state in the patient's body and are circulating between the peripheral blood and peripheral lymph tissues and/or organs (e.g., lymph nodes). Upon re-encounter with an antigen for which they are specific, these memory B cells are quickly activated and subsequently differentiate into plasma cells that produce massive amounts of disease-causing antibodies (pathogenic antibodies) in a short period of time. In addition, these memory B cells serve as antigen presenting cells (APCs)

that present the processed antigen to antigen-specific helper T cells thus triggering and/or enhancing T cell mediated organ and/or tissue damage. These memory B cells and antigen-specific T cells possess unique and antigen-specific BCRs and T cell receptors (TCRs), respectively, both of which bind specifically to the antigen. Currently, there is no treatment that can specifically target this group of disease-causing memory B cells and antigen specific-T cells (autoimmune memory B cells and T cells). In particular, there is no treatment that can specifically target this group of autoimmune memory B cells and autoimmune T cells circulating between the peripheral blood and peripheral lymph tissues and/or organs (e.g., lymph nodes).

[0050[In some embodiments, the present disclosure is related to a new drug development platform that specifically targets this repertoire of autoimmune cells in the peripheral blood and the peripheral lymph organs of a subject/patient. In some embodiments, targets this repertoire of autoimmune cells in the peripheral blood and the peripheral lymph organs will result in a life-long treatment effect.

[0051[The part of an antigen responsible for the autoimmune antibody binding is a specific region called an epitope. As used herein "epitope" can be a natural peptide, a synthetic peptide, an artificial peptide, a biosimilar, an aptamer, a protein domain, or a combination thereof. In some embodiments, an epitope is conformational. In some embodiments, an epitope is non-conformational. In some embodiments, an epitope is both conformational and non-conformational. A peptide/protein epitope can be recombinantly expressed as well as obtained in large quantities using one or more techniques that well-known in the art of epitope synthesis and purification. An epitope has a high affinity for the disease-causing autoantibodies, as well as for the antigen-specific BCRs expressed on memory B cells, TCRs expressed on helper T cells, or both. In some embodiments, the epitope can be a non-peptide/protein epitope. Non-limiting examples include DNA, RNA, small molecules, and organic chemicals.

[0052[The present disclosure is related epitope-drug conjugates (EDCs), and compositions, methods and/or kits comprising EDCs. In some embodiments, the present disclosure is related to an EDC comprising a peptide epitope that targets antigen-specific BCRs expressed on memory B cells, TCRs expressed on helper T cells, or both (FIG. 21). In some embodiments, the EDC also targets APCs that phagocytose and/or endocytose the EDC (FIG. 21). As the platform is based on an epitope(s) that are specifically recognized by immune cells, the platform according to the present disclosure can be adapted for any autoimmune disease. FIG. 21 shows a schematic of a drug development platform for autoimmune disease treatment. FIG. 21 (top panel) shows design of EDC. FIG. 21 (middle panel) shows EDC binds to the antigen-specific receptors on the surface of the B cells and T cells. EDC can be internalized into the cells via BCR-mediated endocytosis.

[0053[In some embodiments, the EDC according to the present disclosure comprises an epitope, a linker, and a drug. In some embodiments, the linker is stable (i.e., not hydrolyzed or degraded) in the patient body and/or circulation outside a cell. In some embodiments, the linker is cleavable allowing separation of the epitope and drug. In some embodiments, the linker is cleaved inside a cell. In some embodiments, the linker is cleaved outside a cell. In some embodiments, the linker is cleaved both inside and outside a cell. In some embodiments, the linker is partially cleaved outside a cell and partially cleaved inside a cell. In some embodiments, the linker is partially cleaved outside a cell and completely cleaved inside a cell. In some embodiments, initial cleavage of the linker occurs outside a cell and final cleavage occurs inside a cell. Non-limiting examples of cleavable linkers include hydrazone linkers, disulfide-based linkers and peptide linkers. In some embodiments, disulfide-based linkers are selectively broken down inside a cell. In some embodiments, disulfide-based linkers are selectively broken down inside a cell because of higher intracellular concentration of thiols. In some embodiments, peptide linkers are selectively broken down inside a cell by intracellular enzymes.

[0054] In some embodiments, the linker is non-cleavable. Non-limiting examples of non-cleavable linkers include thioether linkers, PEG4Mal linker, and the like. In some embodiments, the linker is attached to one or more amino acids on the epitope. In some embodiments, the linker is attached to a cysteine residue on the epitope. In some embodiments, the linker is attached to a residue other than cysteine (e.g., lysine) on the epitope. In some embodiments, one or more sites on the epitope for linker attachment can be one or more solvent-accessible cysteine or lysine or both.

[0055[Other types of linkers, conjugation chemistries, and conjugation sites on epitopes for generation of EDCs are included within the scope of this disclosure. Non-limiting examples are disclosed in U.S. Pat. No. 9,156,854 and U.S. Pat No. 9,388,408, which are hereby incorporated by reference in their entireties. Other non-limiting examples of linkers include Imidoesters, Maleimides, Carbodiimide, Pyridyldithiol, Isocyanate, Isopeptag, SpyTag, SnoopTag and SNAP-tag.

[0056[In some embodiments, the linker comprises attachment sites for both the epitope and drug to join the two components. In some embodiments, the linker is cleavable. In some embodiments, the linker is cleavable from one or both of the epitope and the drug. In some embodiments, the linker is non-cleavable. In some embodiments, the linker is non-cleavable from one or both of the epitope and the drug.

[0057[In some embodiments, a free cysteine residue is introduced at the C-terminus of the epitope. The thio group of the free cysteine is then conjugated with a cleavable linker. In some embodiments, the cleavable linker is a valine-citrulline linker. In some embodiments, the valine-citrulline linker is pre-conjugated with a drug (e.g., duocarmycin analog). In some embodiments, a free cysteine residue is introduced at the C-terminus of the epitope and the thio group of the free cysteine is conjugated with a cleavable valine-citrulline linker pre-conjugated with a durg (e.g., duocarmycin analog).

[0058[In some embodiments, a short sequence of CXPXR is introduced to the C-terminus of the epitope. In some embodiments, the cysteine residue is converted to a formylglycine aldehyde tag. In some embodiments, the cysteine residue is converted to a formylglycine aldehyde tag using a formylglycine-generating enzyme. In some embodiments, the formylglycine aldehyde tag is then conjugated to a drug-linker by a non-cleavable linkage. In some embodiments, the formylglycine aldehyde tag is conjugated to the drug-linker by a non-cleavable linkage via oxime chemistry. In some embodiments, the formylglycine aldehyde tag is conjugated to the drug-linker by a non-cleavable linkage via Pictet-Spengler reaction. In some embodiments, the drug in the drug-linker is a cytotoxic reagent.

[0059[In some embodiments, a drug is conjugated to the epitope in a region outside of the BCR/TCR interaction site. In some embodiments, a drug is conjugated to the epitope in a region outside of the BCR/TCR interaction site to one or more lysine residues. In some embodiments, a drug is conjugated to the epitope in a region outside of the BCR/TCR interaction site to one or more lysine residues via amide bonds to an N-hydroxysuccinimide (NHS) ester appended to a drug-linker.

[0060[In some embodiments, epitopes can be engineered as described in Example 4 and Example 7. In some embodiments, one or more chemical conjugation sites are introduced into an epitope. In some embodiments, one or more chemical conjugation sites are non-natural amino acids. In some embodiments, one or more chemical conjugation sites include one or more cysteine residues.

[0061[In some embodiments, the epitope can be in its native amino acid sequence. In some embodiments, the native amino acid sequence of epitope may be modified. For example, glycosylation sites in an epitope (e.g., the extracellular portion of PLA2R is glycosylated) can add to the size, bulk, and conformational complexity of the epitope. Potential N-linked glycosylation sites (underlined Asn residues) in the first 272 amino acids of PLA2R (SEQ ID NO: 15) are shown in FIG. 20, in Dsg1 epitope (SEQ ID NO: 16) are shown in FIG. 31, and in Dsg3 epitope (SEQ ID NO: 17) are shown in FIG. 32. In some embodiments, the size,

bulk, conformational complexity of the epitope can be reduced by substituting potential glycosylation sites (e.g., at Asn70 and Asn89 in FIG. 20) with Gln or any other non-glycosylated amino acids that will not affect protein structure and/or conformation for autoantibody recognition.

[0062[In some embodiments, residues that are potentially glycosylated can be substituted using, for example, site directed mutagenesis. In some embodiments, residues that are potentially glycosylated can be substituted using, for example, site directed mutagenesis when using an expression vector to express an epitope. In some embodiments, an epitope can be directly synthesized. In some embodiments, when an epitope is directly synthesized, the peptide and/or protein synthesis can be customized such that the potentially glycosylated residues are replaced with non-glycosylated residues that will not affect protein structure and/or conformation for autoantibody recognition.

[0063[In some embodiments, additional modifications may be made to the epitope, for example, to attach a reagent (e.g., a drug) to the epitope, improve accessibility of the epitope to receptors on the cell surface, and/or improve protein expression and yield. For example, the epitope may be modified by inserting small (about 2 to 10 amino acids) N- or C-terminal peptide or both, making conservative and/or non-conservative substitutions, and/or adding one or more heterologous sequences to achieve a desired objective.

[0064[In some embodiments, one or more of epitopes provided herein are encoded by nucleic acids. In some embodiments, the epitope-encoding nucleic acid is a cDNA or an mRNA. In some embodiments, the epitope-encoding nucleic acid can be comprised within a protein expression vector. In some embodiments, the protein expression vector is a DNA vector or an RNA vector. In some embodiments, the protein expression vector is an adeno-associated viral (AAV) vector. In some embodiments, the protein expression vector is a mammalian cell expression vector. In some embodiments, the protein expression vector is an insect cell expression vector. In some embodiments, the epitope-encoding nucleic acid comprised within a protein expression vector is operably linked to regulatory elements to regulate the expression of the epitope.

[0065[Regulatory elements can include promoters, terminators, enhancers, etc. As used herein, “operably linked” refers to a regulatory element positively or negatively controlling the expression of a protein from a nucleic acid.

[0066[One or more of the proteins expression vectors provided herein as well as other protein expression vectors known to one of ordinary skill in the art can be used to obtain large quantities of one or more of the epitopes disclosed herein and fragments and variants

thereof for incorporation into one or more of the compositions, methods, and/or kits provided herein. In some embodiments, a variant of an epitope can have about 70% to about 99.99% identity to the epitope. In some embodiments, a variant of an epitope can have about 65, 70, 57, 80, 85, 90, 95, 96, 97, 98, 99, 99.25, 99.5, 99.75, 99.99% identity to the epitope, or a value with a range defined by any two of the aforementioned values.

[0067[In some embodiments, the protein expression vector introduces a tag on the encoded epitope. In some embodiments, one or more tags enable purification of the epitope. In some embodiments, the tag is on the N-terminal end. In some embodiments, the tag is on the C-terminal end. In some embodiments, the tag is on both N- and C-terminal ends. Non-limiting examples of tags include chitin binding protein, maltose binding protein, glutathione-S-transferase, thioredoxin, poly(NANP), FLAG, V5, Myc, HA, NE, biotin, biotin carboxyl carrier protein, GFP, Halo, Nus, Fc, AviTag, calmodulin, poly-Glu, E, S, SBP, Softag 1, Softag 3, Strep, TC, VSV, Ty and Xpress. In some embodiments, the tag is a poly-histidine (poly-His) tag.

[0068[In some embodiments, the protein expression vector additionally introduces a cleavage site between the epitope and the tag to allow for separation of the epitope from the tag. In some embodiments, the cleavage site is a proteolytic site. In some embodiments, the cleavage site is a non-proteolytic site. Non-limiting examples of proteolytic sites include sites for TEV protease, Factor Xa or enteropeptidase. In some embodiments, the proteolytic site is a thrombin cleavage site. Non-limiting examples of other protease and non-protease cleavage sites that are contemplated include foot-and-mouth disease virus (FMDV) protease, Arg-C proteinase, Asp-N endopeptidase, BNPS-Skatole, Caspases, Chymotrypsin-high specificity, Chymotrypsin-low specificity, Clostripain (Clostridiopeptidase B), CNBr, Enterokinase, Factor Xa, Formic acid, Glutamyl endopeptidase, GranzymeB, Hydroxylamine, Iodosobenzoic acid, LysC, LysN, NTCB (2-nitro-5-thiocyanobenzoic acid), Neutrophil elastase, Pepsin, Proline-endopeptidase, Proteinase K, Staphylococcal peptidase I, Thermolysin, Thrombin, Trypsin, and other site specific enzymes known to one of ordinary skill in the art.

[0069[One or more commercially available cell lines can be used to express the epitope. For example, in some embodiments, a poly-His-tagged epitope can be expressed in mammalian cells (e.g., HEK 293 cells) and purified from the cell culture medium using Ni-affinity purification and gel filtration columns. The poly-His tag can then be removed by proteolytic digestion (e.g., using thrombin), and the epitope further purified to homogeneity using gel filtration chromatography to remove the thrombin enzyme as well as the released poly-His tag. Epitope expression and purification can be tested on protein extracts using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (FIG. 23 and FIG. 28). Epitope expression can also be tested using Western blotting (FIG. 23 and FIG. 28) using one or

more antibodies and/or patient plasma and/or serum. The SDS-PAGE gel can be stained with one or more of Coomassie dye stains (FIG. 23 and FIG. 28), silver stains, Zinc stains, fluorescent dye stains, and functional group–specific stains.

[0070[As used herein, the "drug" in the EDC can also be referred to as a "treatment agent" (FIG. 21; top panel) or "agent." A drug includes agents that specifically used for the prevention and/or treatment of one or more diseases. The drug can be cytostatic, cytotoxic, immunosuppressive, or any agent that can be used for treatment purpose. For example, binding of EDC to TCR may result in the induction of immunotolerance by the drug or internalization of EDC into APCs may result in APC death (FIG. 21; bottom panel). In some embodiments, immune tolerance occurs by one or more known mechanisms. For example, the drug prevents release of one or more cytokines by the autoimmune B cells, T cells or both, or inhibits cytokine signaling in the autoimmune B cells, T cells or both. Non-limiting examples include clonal deletion, receptor editing, follicular exclusion, and anergy. In some embodiments, the drug inhibits antigen presentation by autoimmune B cells to T cells. In some embodiments, the drug inhibits one or more signaling pathways. Non-limiting examples include of IL-6 receptor signaling, NF-κB signaling, Toll-like receptor signaling, B cell receptor signaling, T cell receptor signaling, and inflammasome signaling. In some embodiments, one or more targets in the one or more signaling pathways may be targeted by the drug. Non-limiting examples include COX, CCR, histamine receptor, interleukin receptor, gp120/CD4, CXCR, PD-1/PD-L1, MALT, LTR, ROS, NOS, TLR, NADPH-oxidase, and Nrf2.

[0071[Non-limiting examples of drugs include Duocarmycin A, Duocarmycin B1, Duocarmycin B2, Duocarmycin C1, Duocarmycin C2, Duocarmycin D, Duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin, Cyclophosphamide, Rapamycin, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil or capecitabine, Monomethyl auristatin E (MMAE), anthracyclines, oxaliplatin, or bortezomib.

[0072[In some embodiments, the EDC can be based on an immunotoxin, which is an antibody-based targeting domain fused to a bacterial toxin for cell killing (Alewine, C., et al, The Oncologist, Vol. 20, pp. 176–185, 2015, which is hereby incorporated by reference in its entirety). Immunotoxin-based EDCs can kill cells by inhibiting protein synthesis and can target both dividing and non-dividing cells.

[0073[A drug can be a detection reagent, for example, fluorophores. Non-limiting examples include FITC, Hydroxycoumarin, Aminocoumarin, Methoxycoumarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer yellow, NBD, NBD-X, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613 (PE-Texas Red), Peridinin chlorophyll protein (PerCP),

TruRed (PerCP-Cy5.5 conjugate), FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhodamine B, Texas Red, Allophycocyanin (APC), and APC-Cy7 conjugates.

[0074[In some embodiments, FACS and/or microscopy-based assays can be designed for screening and identifying small molecules that can be used as the drug in the EDC. In some embodiments, a FACS-based assay (based on Example 1) can be used to screen one or more commercially available libraries of small molecules and identify small molecules that can be used as the drug in the EDC. In some embodiments, a microscopy-based assay (based on Example 2) can be used to screen one or more commercially available libraries of small molecules and identify small molecules that can be used as the drug in the EDC.

[0075[In some embodiments, the drug can be one or more of a chemical, a reagent, a protein or a peptide that can induce autoimmune cell death. In some embodiments, the drug can be one of more of antisense RNA, miRNA, siRNA or RNA fragment for RNAi that can interfere with antibody mRNA stability, turnover, and/or translation in the B cells. In some embodiments, the drug is a cytotoxic drug and has a cytotoxic effect on the B and T cells that result in cell death. In some embodiments, cell death occurs by one or more of programmed cell death, apoptosis, macroautophagy, autophagy, necrosis, necroptosis, mitotic catastrophe, activation-induced cell death, anoikis, cornification, excitotoxicity, ferroptosis, Wallerian degeneration, and immunogenic apoptosis.

[0076[Once the EDC binds to the antigen-specific receptor on the memory B cell surface, the protein complex is internalized via endocytosis, and subsequently the conjugated drug is released that triggers cellular effects. When the EDC binds to the antigen-specific receptor on the helper T cell surface, it induces cellular effects. Non-limiting examples of cellular effects include, cell growth arrest, cell death, apoptosis, autophagy, immune tolerance, etc. In come embodiments, the EDC is internalized by an APC. In some embodiments, an EDC internalized by an APC prevents the APC from presenting the antigens to T cells.

[0077[The interaction between EDC and BCR/TCR can be expressed in terms of "affinity," which can be defined as the strength of binding of a single EDC to its receptor. Affinity is expressed as the equilibrium dissociation constant (K_D), which is the concentration at which equilibrium exists between the rate of binding of the EDC to its receptor and the rate of dissociation of the EDC from the receptor. A smaller K_D value means a higher affinity and vice versa.

[0078[In some embodiments, affinity of EDC for BCRs and TCRs can range from about 10^{-7} to about 10^{-13}. In some embodiments, the affinity can range from about 10^{-4} to about

10^{-10}. In some embodiments, the affinity can range from about 10^{-9} to about 10^{-15}. In some embodiments, affinity can range from about 10^{-4} to about 10^{-10}. In some embodiments, affinity is about 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13}, 10^{-14}, 10^{-15}, 10^{-16}, 10^{-17}, or 10^{-18}, or a value within a range defined by any two of the aforementioned values.

[0079[In some embodiments, more than one EDC can be used in combination. In some embodiments, more than one EDC can be used, in which case a potentiated effect on autoimmune cells is observed. The potentiation can be additive or synergistic. A synergistic effect is greater than an additive effect. An additive effect is observed when the potentiation is equal to the sum of the individual effects of the different EDCs. A synergistic effect is observed when the potentiation is greater than the sum of the individual effects of the different EDCs. Synergistic effect, additive effect or both can be occur human patients, non-human patients, non-patient human volunteers, in vivo models, ex vivo models, in vitro models, etc. Potentiation can range from about <1 to about 100 fold. In some embodiments, the synergistic effect is about 3 to about 30 fold. In some embodiments, the potentiation ranges from <1, 1, >1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 fold, or within a range defined by any two of the aforementioned values.

[0080[Owing to the specificity of the epitope for BCRs and TCRs, EDCs specifically target only the disease-causing autoimmune cells (e.g., antigen-specific B and T cells) without affecting the function of the normal immune cells. Thus, the platform according to the present disclosure only targets the specific repertoire of the disease-causing autoimmune cells with no/minimal side effects or without significant side effects. Thus, disease progression and relapses are mitigated resulting in long-lasting protective effects.

[0081[The EDC can be of any molecular size range. For example, in some embodiments, the size can range from about 2.5 kDa to about 75 kDa. In some embodiments, the size can range from about 50 kDa to about 500 kDa. In some embodiments, the size can range from about 250 kDa to about 2500 kDa. In some embodiments, the size can be about 2.5, 5, 10, 25, 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, or 2500 kDa, or a value within a range defined by any two of the aforementioned values.

[0082[FIG. 22 shows a schematic of delivery of EDC to memory B cells residing in the peripheral lymph nodes. In order to efficiently deliver EDC to patients and to achieve maximal access of EDC to the memory B cells, EDCs according to the present disclosure are designed in the molecular range of about 14 kDa to about 70 kDa. When the molecular size of EDC is within a range of about 14 kDa to about 70 kDa, the EDC can be drained directly to the afferent lymph when administrated via subcutaneous and/or intradermal routes (Pape, K.A., et al;

Roozendaal, R., et al). Thus, in some preferred embodiments, the size of EDC ranges from about 14 kDa to about 70 kDa. In some preferred embodiments, the size of EDC is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 kDa, or a value within a range defined by any two of the aforementioned values.

[0083[When delivered via the subcutaneous and/or intradermal routes, EDC in the size range of about 14 kDa to about 70 kDa drains directly to the afferent lymph (FIG. 22). Thus, in some more preferred embodiments, the route of administration of EDC in the size range of about 14 kDa to about 70 kDa is subcutaneous, intradermal or both to deliver them directly to the afferent lymph.

[0084[When delivered via the subcutaneous and/or intradermal routes, EDC in the size range of about 14 kDa to about 70 kDa drains directly to the afferent lymph, and encounters and binds to memory B cells in the lymph nodes directly without the help of antigen presenting cells (FIG. 22). Therefore, in some more preferred embodiments, the route of administration of EDC in the size range of about 14 kDa to about 70 kDa is subcutaneous, intradermal or both to deliver them directly to the afferent lymph and deliver them to directly to memory B cells in the lymph nodes without the help of antigen presenting cells.

[0085[In some preferred embodiments, EDCs within a molecular weight range of about 14 kDa to about 70 kDa can be quickly drained to the lymph nodes to target the antigen-specific memory B cells (and T cells) when delivered via the subcutaneous and/or intradermal routes. However, EDCs can be delivered to patients via all possible delivery routes depending on need. For example, in some embodiments, EDC administration via one or more of topical, parenteral, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intramuscular, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathecal, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal routes is contemplated.

[0086[In some patients, the levels of circulating autoantibodies are high, for example, patients with active autoimmune disease. During active disease, autoantibodies are present in the patient's circulation and the patient is experiencing the effects of the disease. The circulating autoantibodies can neutralize the effects of an EDC, especially when administered intravenously, by binding to the epitope in the EDC. In addition, the autoantibodies may form immune complexes with EDCs resulting in unwanted side-effects. Thus, in some embodiments,

especially during active disease state and/or when high levels of autoantibodies are present in the subject's circulation, the preferred route of administration is one or more of subcutaneous, intradermal, or oral. In such patients, the subcutaneous and/or intradermal routes of delivery minimize the chance of EDC neutralization by the autoantibodies and maximize the chance of EDC reaching the memory B cells (and T cells) in the lymphoid tissues and/or organs (FIG. 22). Once the active disease stage has passed and the patient is in a state of remission with low levels of circulating antibodies, the patient can be given EDC via the intravenous route to target the autoimmune cells that are circulating the peripheral in addition to administering of the EDC via the intradermal and/or subcutaneous routes.

[0087[However, in some patients, it may be necessary to administer EDC via a combination of routes (e.g., Pemphigus vulgaris). In such patients, the EDC is delivered after the titers of autoantibodies in patient circulation are decreased. For example, after the titers of autoantibodies in patient circulation are decreased, the EDC can be delivered in a combined approach of intradermal, subcutaneous and intravenous routes.

[0088[In some embodiments, a patient's active disease is managed via alternative therapies until circulating autoantibody levels naturally reduce based on antibody half-life due to recycling, degradation, or both, following which the EDC is administered. In situations where the patient's active disease has been managed by alternative therapies, the EDC may be administered via a one or more of intradermal, subcutaneous, oral, or intravenous routes.

[0089[In some embodiments, EDC can be delivered to patients who were administered alternative therapies and are in remission. In such situations, EDC can be administered to eliminate any lingering populations of disease-causing memory B cells and T cells and thus prevent potential future disease relapses.

[0090[In some embodiments, the drug development platform according to the present disclosure can be used to diagnose, prevent and/or effectively treat any T cell and B cell-mediated autoimmune disease. In some embodiments, the drug development platform according to the present disclosure can be used to diagnose, prevent and/or effectively treat any antigen-specific autoimmune disease, including but not limited to, Addison's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome, Autoimmune angioedema, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune urticaria, Axonal & neuronal neuropathy (AMAN), Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bullous pemphigoid, Castleman disease (CD), Celiac disease,

Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss, Cicatricial pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis (EoE), Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes gestationis or pemphigoid gestationis (PG), Hypogammalglobulinemia, IgA Nephropathy, IgG4-related sclerosing disease, Immune thrombocytopenic purpura (ITP), Inclusion body myositis (IBM), Interstitial cystitis (IC), Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus, Lyme disease chronic, Meniere's disease, Microscopic polyangiitis (MPA), Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica, Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism (PR), PANDAS, Paraneoplastic cerebellar degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Pars planitis (peripheral uveitis), Parsonnage-Turner syndrome, Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia (PA), POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progesterone dermatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Raynaud's phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjogren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome (SPS), Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia (SO), Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome (THS), Transverse myelitis, Type 1 diabetes, Ulcerative colitis (UC), Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vitiligo, Wegener's granulomatosis (or Granulomatosis with Polyangiitis (GPA)).

[0091[In some embodiments, diagnosis, prevention and/or treatment of other immune diseases are contemplated including those in which the function of one or more immune cells is skewed resulting in immune system disease that is not necessarily an autoimmune disease. Thus, the drug development platform herein can also be used to diagnose, prevent, and/or effectively treat diseases that are not autoimmune diseases. In preferred embodiments, human autoimmune diseases are targeted by the platform. In some embodiments, autoimmune diseases of non-humans are targeted. Non-limiting examples of non-humans include dogs, cats, rabbit, mouse, guinea pig, monkey, cow, sheep goat, and zebra. In some embodiments, immune system diseases that are non-autoimmune diseases in human and non-humans are targeted by the platform of the present disclosure.

[0092[In some embodiments, the patient has had an autoimmune disease for about 1 month to about 5 years. In some embodiments, the patient has had an autoimmune disease for about 1, 2, 3 or 4 weeks, or within a range defined by any two of the aforementioned values, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or within a range defined by any two of the aforementioned values, or 2, 3, 4, 5, 6, 7, 8, 9 or 10 years, or within a range defined by any two of the aforementioned values.

[0093[In some embodiments, the patient has previously not received any treatment for an autoimmune disease. In some embodiments, the patient has previously received treatment (either immunosuppressive drugs or steroid hormones or both) for an autoimmune disease. In some embodiments, the patient has previously been successfully treated with either immunosuppressive drugs or steroid hormones or both for an autoimmune disease. In some embodiments, the patient has previously been successfully treated with either immunosuppressive drugs or steroid hormones or both for an autoimmune disease, however, the autoimmune disease has relapsed. In some embodiments, the patient has previously been unsuccessfully treated with either immunosuppressive drugs or steroid hormones or both for an autoimmune disease.

[0094[The age of the patient may range from about 20 years to about 95 years. In some embodiments, the age of the patient ranges from about 5 years to about 70 years. In some embodiments, the age of the patient is less than 5 years. In some embodiments, the age of the patient is more than 70 years. In some embodiments, the age of the patient is about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 years, or within a range defined by any two of the aforementioned values. In some embodiments, the patient is a male. In some embodiments, the patient is a female.

[0095[In some embodiments, the EDC is provided in the form of a composition. In some embodiments, the EDC composition can be formulated for delivery via one or more routes of administration herein. Compositions can be without limitations aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0096[Compositions can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. Injection solutions and suspensions can be prepared from one or more of sterile powders, granules, capsules and tablets. Other non-limiting compositions such as for aerosol- or nebulizer-based and delivery skin patch-based delivery are also contemplated. In some embodiments, the efficacy of the compositions may be tested in non-patient human volunteers, in vivo models, ex vivo models, in vitro models, before being administered to human patients, non-human patients, or both.

[0097[In some embodiments, an EDC is administered daily, weekly, biweekly or monthly. In some embodiments, the EDC is administered 1, 2, 3, 4, 5, 6, 7 or 8 times a day.

[0098[In some embodiments, the duration of treatment with an EDC ranges from about 2 weeks to about 4 months. In some embodiments, depending on the severity of the disease, the duration of treatment with an EDC is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 weeks, or within a range defined by any two of the aforementioned values.

[0099[In some embodiments, the treatment efficacy of treatment ranges from about 80% to about 95% as determined by the level of circulating autoantibodies and/or the number of epitope-specific immune cells (e.g., B and T cells) in the patient. In some embodiments, the treatment efficacy is about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, or within a range defined by any two of the aforementioned values as determined by the level of circulating autoantibodies and/or the number of epitope-specific immune cells (e.g., B and T cells) in the patient.

[0100[In some embodiments, the volume of the EDC composition ranges from about 0.1 ml to about 10 ml. In some embodiments, the volume of the EDC composition ranges from about 0.05 ml to about 100 ml. In some embodiments, the volume the EDC composition is about 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 50, 100, or 150 ml, or within a range defined by any two of the aforementioned values.

[0101[Owing to the very high affinity and specificity of EDCs, for example, for BCRs and TCRs, and accessibility to relatively inaccessible locations in the body, in some embodiments, effective treatment of an autoimmune disease is achieved at much lower dose of the drug in the EDC as compared to when the drug in the EDC is administered alone. Additionally, in some embodiments, the duration of treatment required with an EDC is much shorter as compared to when the drug in the EDC is administered alone. For example, in some embodiments, when the drug in the EDC is an anti-cancer/anti-tumor drug, the starting dose of EDC is about $1/10^{th}$ of the dose at which the drug in the EDC is used for solid tumor treatment.

[0102[As EDCs have much better access to BCR in peripheral circulation and peripheral lymphoid tissues/organs, the starting dose of the drug in the form of EDC can be much lower than what the dose for the drug would be on its own (e.g., for tumors/caners in relatively inaccessible locations in the body). For example, the starting dose of the drug in the form of EDC can be about $1/5^{th}$, $1/10^{th}$, $1/15^{th}$, $1/20^{th}$, $1/25^{th}$, $1/30^{th}$, $1/35^{th}$, $1/40^{th}$, $1/45^{th}$, $1/50^{th}$, $1/55^{th}$, $1/60^{th}$, $1/65^{th}$, $1/70^{th}$, $1/75^{th}$, $1/80^{th}$, $1/85^{th}$, $1/90^{th}$, $1/95^{th}$, $1/100^{th}$, $1/200^{th}$, $1/250^{th}$, $1/500^{th}$, $1/1000^{th}$, $1/2000^{th}$ or $1/5000^{th}$, or within a range defined by any two of the aforementioned values, of the dose at which the drug in the EDC would be is used for treatment of the disease.

[0103[In some embodiments, the one or more EDC-based treatment options provided herein are rapidly effective, require a much lower dose and cause minimal adverse effects as compared to treatment with immunosuppressive drugs and/or steroids.

[0104[In some embodiments, a unit dose of EDC can range from about 0.001 mg/kg to about 5 mg/kg. In some embodiments, a unit dose of EDC can range from about 0.01 mg/kg to about 50 mg/kg. In some embodiments, a unit dose of EDC for targeting B cells can range from about 0.001 mg/kg to about 50 mg/kg. In some embodiments, a unit dose of EDC for targeting T cells can range from about 0.001 mg/kg to about 50 mg/kg. In some embodiments, a unit dose of EDC can is about 0.001, 0.01, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg/kg, or a value within a range defined by any two of the aforementioned values.

[0105[In some embodiments, the EDC is administered as a single daily dose. In some embodiments, the EDC is administered as more than one dose per day. In some embodiments, the number of doses per day ranges from one to six. In some embodiments, the number of doses per day is 1, 2, 3, 4, 5, 6, 7, or 8.

[0106[In some embodiments, the EDCs herein can be used to remove and/or eliminate circulating autoimmune cells during plasmapheresis, apheresis, and leukoreduction.

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Diagnosis and post-treatment patient monitoring

[0107[Disease diagnosis and monitoring post-treatment disease progression are critical to ascertaining patient treatment options, patient response to treatment, and patient disease management.

[0108[In some instances, diagnosis and post-treatment patient monitoring of disease progression are performed using painful and invasive methods. For example, diagnosis and post-treatment patient monitoring of membranous nephropathy (MN) progression relies entirely on kidney biopsies, which are invasive and may cause severe kidney bleeding and many other side effects.

[0109[In some embodiments, provided herein are EDCs for non-invasive and/or minimally invasive diagnosis and post-treatment monitoring autoimmune diseases.

[0110[In some embodiments, the EDCs for non-invasive and/or minimally invasive diagnosis and post-treatment monitoring of autoimmune disease comprise one or more epitopes provided herein. The epitope can be in solution or immobilized on a substrate, or both. The substrate can be any of a variety of substrates such as columns, beads, microspheres, test strips or multi-well plates known to be useful for assays for diagnosis and post-treatment monitoring. In some embodiments, the diagnosis and post-treatment monitoring of autoimmune disease is based on one or more assays. In some embodiments, the assay can be one or more of an immunoassay such as an enzyme-linked immunosorbent assay (ELISA), FACS, Enzyme-Linked ImmunoSpot (ELISPOT), radioimmunoassay, magnetic immunoassay.

[0111[In some embodiments, one or more epitopes provided herein can be attached to a substrate (e.g., an ELISA plate) using standard procedures. A patient sample (e.g., blood, serum and/or plasma) can be collected during a visit to a clinic using standard clinical procedures. The patient samples can be added to the ELISA plate at different dilutions (e.g., $1/10^{th}$, $1/100^{th}$, $1/1000^{th}$, etc.) with appropriate control samples. After standard incubation, the ELISA plate can be read using a standard plate reader.

[0112[In some embodiments, there is a detectable increase in anti-epitope autoantibodies in the patient sample relative the control sample. In some embodiments, a detectable increase in anti-epitope autoantibodies by at least 10% relative to the control sample indicates the presence of an autoimmune disease in the patient. In some embodiments, the detectable increase in anti-epitope autoantibodies in the patient sample is greater than 10% relative to the control sample. In some embodiments, the detectable increase in anti-epitope autoantibodies in the patient sample ranges from about 5% to about 15% relative to the control sample. In some embodiments, the detectable increase in anti-epitope autoantibodies in the

patient sample is about 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100% relative to the control sample, or within a range defined by any two of the aforementioned values.

[0113[In some embodiments, there is a detectable increase in anti-epitope autoantibodies in the patient sample relative to a control sample. The increase in anti-epitope autoantibodies in a patient sample is detectable using one or more of the composition, methods and/or kits for diagnosis provided herein. As used herein, "control sample" refers to a sample that is either representative of normal levels of anti-epitope autoantibodies, or obtained from a subject known to be free of the autoimmune disease.

[0114[In some embodiments, the patient sample can be, for example, a bodily fluid (e.g., one or more of blood, plasma, serum, urine, cerebrospinal fluid, and lymph), a biopsy sample of a tissue and/or an organ affected by an autoimmune disease. For example, in some embodiments, a kidney biopsy may be performed in a patient with MN. In some embodiments, a biopsy of a mucocutaneous lesion may be performed in a patient with Pemphigus vulgaris.

[0115[A microscopy-based assay for diagnosis and post-treatment monitoring of an autoimmune disease can comprise obtaining patient blood samples. Using one or more EDCs herein, the blood sample can be analyzed as is or one or more specific populations of cells (e.g., total B cells or total T cells) may be isolated using one or more cell purification techniques (e.g., FACS) known in the art prior to analysis for the presence of autoimmune cells in the patient. For example, one or more EDCs herein can be used to detect the presence of autoantibody-producing B cells in the patient sample (Example 5; FIG. 24, and Example 8; FIG. 29). One or more EDCs herein can also be used for detecting the levels of autoantibodies in a patient.

[0116[In some embodiments, the assay can also be adapted for monitoring the status of an autoimmune disease in a patient and/or responsiveness to one or more of the treatment options provided herein. For example, a blood, serum and/or plasma sample can be collected from a patient at a first time point. The first time point can be a patient's, first visit to the clinic and the patient has not been diagnosed with an autoimmune disease and has never been previously treated for the autoimmune disease.

[0117[In some embodiments, the first time point can be before the initiation of one or more treatment options provided herein with the patient having previously undergone other forms of treatment. In some embodiments, after the initiation of one or more treatment options provided herein, a blood, serum and/or plasma sample can then be collected from a patient at a second time point as well as at additional subsequent time points.

[0118[In some embodiments, the amount of anti-epitope autoantibody-producing B cells in the patient sample can be compared at the various time points. In some embodiments, a decrease in the amount of autoantibody-producing B cells in the patient sample at the second and/or subsequent time points relative to the first time point is indicative of amelioration of disease. In some embodiments, a decrease in the amount of autoantibody-producing B cells in the patient sample at the second and/or subsequent time points relative to the first time point is indicative of elimination of autoantibody-producing B cells and 100% treatment of the disease. In some embodiments, elimination of autoimmune cells and amelioration of disease can range from about 50% to 100%. In some embodiments, elimination of autoimmune cells and amelioration of disease is about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. In some embodiments, the elimination of autoimmune cells is 100% after just one round of treatment. In some embodiments, when the elimination of autoimmune cells is low after one round of treatment (e.g., below 50%), one or more additional rounds of treatment may be provided to eliminate the autoimmune cells and/or the patient may be administered one or more additional therapies.

[0119[In some embodiments, the efficacy of an EDC may be tested in vitro using patient samples prior to administering the EDC to patients. For example, the efficacy of an EDC is tested on isolated B cells from patient peripheral blood mononuclear cells (PBMCs) (FIG. 25; Example 6, and FIG. 30; Example 9). An EDC comprising a fluorophore (e.g., FITC) is used detect the presence of autoantibody-producing B cells in patient samples (left panels in FIG. 25 (MN patient samples) and FIG. 30 (Pemphigus vulgaris patient samples) prior to exposing the samples to an EDC comprising a cytotoxic drug. The patient samples are then treated with an EDC comprising a cytotoxic drug (e.g., MMAE). The EDC comprising the fluorophore is then used determine the efficacy of the EDC comprising a cytotoxic drug of autoantibody-producing B cells in patient samples (middle panels in FIG. 25 (MN patient samples) and FIG. 30 (Pemphigus vulgaris patient samples)).

[0120[In some embodiments, the efficacy of the EDC comprising a cytotoxic drug in eliminating patient autoantibody-producing B cells is 100% (middle panels in FIG. 25 (MN patient samples) and FIG. 30 (Pemphigus vulgaris patient samples)). In some embodiments, the efficacy ranges from about 50% to 100%. In some embodiments, the efficacy is about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. In some embodiments, when the efficacy after a first round of treatment is not 100%, one or more additional rounds of treatment can be performed and a reassessment of efficacy can be performed after each round of treatment.

[0121[In some embodiments, the one or more EDCs comprising a fluorophore are used as diagnostic tools to determine the number of epitope specific cells in a patient. For example, FIG. 24 shows the use of an EDC based on PLA2R epitope and FITC as a diagnostic tool for determine the presence of autoimmune B cells in the peripheral circulation of patients with MN. FIG. 29 shows the use of an EDC based on Dsg epitope and FITC as a diagnostic tool for determine the presence of autoimmune B cells in the peripheral circulation of patients with PV.

[0122[In some embodiments, the one or more EDCs comprising a fluorophore are used as diagnostic tools to determine the number of epitope specific autoimmune cells in a patient at a first time point. If the number of epitope specific autoimmune cells in the patient at a first time point is above a predetermined threshold (indicating that the patient requires one or more therapies for the disease), the patient is administer one or more EDCs comprising a drug either by itself or in combination with one or more adjunct therapies. The number of epitope specific autoimmune cells is reassessed at a second time using the one or more EDCs comprising a fluorophore. If the number of epitope specific autoimmune cells at the second time point is below the predetermined threshold, the treatment is discontinued. If the number of epitope specific autoimmune cells at the second time point is above the predetermined threshold, the treatment is continued and a subsequent assessment performed.

[0123[The presence of autoimmune cells in a patient can be detected in blood, other biological fluids, and tissue and organ biopsies (e.g., collected during surgery). The EDCs can be used to detect any cell population that can bind an epitope (e.g., B cells, T cells, other cells in PBMCs, and cells in other biological fluids).

[0124[In some embodiments, the platform herein can be adapted to any peptide, protein domain, small molecules, and other ligands (e.g., ligand domains or peptide ligands that bid specific receptors).

[0125[In some embodiments, the level/numbers of autoantibody-producing B cells in the patients can be calculated using standard automated computer programs. In some embodiments, the level of autoantibody-producing B cells can be compared before and after treatment with one or more treatment options provided herein. In some embodiments, one or more epitopes provided herein can be produced and purified in large quantities, and standard commercial grade ELISA plates can be manufactured and used as a routine procedure for diagnosing and post-treatment patient monitoring in a clinical laboratory.

[0126[In some embodiments, the assay is non-invasive and/or minimally invasive, simple, time efficient, cost effective and can be performed routinely in a clinical laboratory. For

example in some embodiments, the assay can be performed without the need for an invasive kidney biopsy as used for MN patients.

[0127[In some embodiments, a PLA2R epitope-specific ELISA assay can be designed for screening and identifying small molecules for MN treatment. For examples, in some embodiments, small molecules can be identified that specifically block the binding of PLA2R autoantibodies to PLA2R. For example, an embodiment of the ELISA plate provided herein can be used to screen one or more commercially available libraries of small molecules.

[0128[In some embodiments, the one or more commercially available libraries of small molecules can comprise without limitation metabolites (e.g., of alkaloids, glycosides and lipids), peptides, natural phenols (e.g., flavonoids), polyketides, terpenes, steroids and tetrapyrroles.

[0129[In some embodiments, any of the composition and/or methods described herein is provided as one or more kits. The kit can comprise one or more polypeptides, antibodies, probes and or other assay reagents described herein. The kit can include the solid support to which assay reagents may be bound or immobilized. The reagents can optionally be labelled with a detectable marker. The kit can further comprise one or more containers for containing, storing and/or transporting the polypeptides, antibodies, probes and other reagents described herein.

[0130[In some embodiments, the compositions, methods, and/or kits that specifically target and eliminate epitope-specific immune cells (e.g., B and T cells) are provided. In some embodiments, one or more patients are selected and treated using the compositions, methods, and/or kits provided herein to specifically target and eliminate epitope recognizing immune cells (e.g., B and T cells) from the patient.

Membranous Nephropathy (MN)

[0131[Membranous Nephropathy (MN) (also referred to as primary MN or idiopathic MN) is a common glomerular disease. Incidence of MN is high in patients over the age of 40. Frequent disease relapse is the major challenge of clinical treatment. There are no effective treatments except to use high dose of steroid hormones and immunosuppressant and patients progress to kidney failure in 5-10 years under current management. There are 4,000 - 6,000 new cases/year in US, 10,000 new cases/year in Europe, and 80,000 new cases/year worldwide.

[0132[MN is an autoimmune glomerular disease. The disease causing mechanism of MN was recently determined to be due to binding to a membrane receptor, phospholipase A2

receptor (PLA2R) on the surface of the kidney podocytes of circulating autoimmune antibodies (autoantibodies) generated by autoantibody-producing B cells. Over 70% patients are caused by anti-PLA2R autoantibodies. MN often relapses leading to kidney failure in 5-10 years.

[0133[Current clinical treatments for MN use either immunosuppressive drugs (e.g., Cyclosporine, Rituximab, Chlorambucil, Tacrolimus, Cyclophosphamide, Mycophenolate mofetil) or steroid hormones (e.g., corticosteroids). However, both of these treatment options are non-specific and produce significant side effects. Often disease relapse occurs when the dose of these treatments are reduced. Moreover, in many patients these treatments are not effective. Therefore, there remains a strong need for a specific treatment for the MN without harmful side effects.

[0134[The disease causing mechanism of MN was recently determined to be due to circulating autoantibodies binding to PLA2R on the surface of the kidney podocytes. Additionally, polymorphisms M292Vand H300D in C-type lectin-like domain 1 (CTLD1) and G1106S in the linker region between CTLD6 and CTLD7 may correlate to the occurrence of MN in patients (Kao, L., et al.).

[0135[The level of the antibody in the plasma of patients with MN is directly correlated with the severity of the disease and a patient's response to the medical treatment. Thus, there is a need to remove autoantibody-producing B cells from the patient to treat the disease while simultaneously avoiding harmful side effects.

[0136[PLA2R is a large integral membrane protein with a molecular weight of about 180- 185 kDa (SEQ ID NO: 14). A proposed topology of PLA2R based on the mannose receptor is provided in FIG. 1. PLA2R contains a large glycosylated extracellular portion that interacts with ligand, a single transmembrane domain and a short cytoplasmic tail (FIG. 1). The large extracellular portion can be further divided into 10 domains: a cysteine rich domain (CysR), a fibronectin-like type II domain (FnII), and 8 repeated C-type lectin-like domains (CTLD) in tandem (FIG. 1).

[0137[FIG. 1 indicates the position of amino acids demarcating the various domains (indicated by arrows) of PLA2R. The amino acids sequences of some of the domains are provided in SEQ ID NO: 7 – SEQ ID NO: 12 (FIG. 12 – FIG. 17). The PLA2R epitope (SEQ ID NO: 13), which is recognized by autoantibodies in MN patients, is located in the extracellular portion of the receptor, is conformational and sensitive to reduction, and has a very high affinity for the autoantibodies.

[0138[However, the PLA2R epitope (FIG. 18; SEQ ID NO: 13) when expressed on its own does not fold properly into the correct conformation. Inclusion of the first two domains

allows for proper conformational folding of the antigen. The full-length PLA2R protein (FIG. 19; SEQ ID NO: 14) also cannot be expressed and purified in large scale as it is not biochemically stable. However, domains 1-3 domain and up to domains 1-6 are suitable for expressing and obtaining large amounts of protein. Domains 1-5 provide for optimal protein expression. For example, properly folded conformation can be obtained by using the sequence provided in SEQ ID NO: 9.

[0139[The production of anti-PLA2R autoantibodies in MN patients is due to activation and expansion of a specific B cell population that carries specific BCRs that recognize an epitope in PLA2R. The specific B cell population is activated when engaged by a specific helper T cell population that carries a T cell receptor specific for the same PLA2R epitope. These specific B and T cells represent only a small fraction of the total repertoire of B and T cells.

Treating MN by targeting B and T cells

[0140[Removing the anti-PLA2R autoantibodies from patients may alleviate the pathological effects of the autoantibodies. However, removing autoantibodies from a patient still leaves the patient vulnerable to disease relapse. This is because the PLA2R autoantibody-producing B cells continue to produce the autoantibodies despite removal of disease-causing autoantibodies from the patient blood. Therefore, specifically targeting PLA2R-epitope recognizing immune cells (e.g., B and T cells) can eliminate the pathogenic autoantibody production without affecting the normal function of other immune cells.

[0141[In some embodiments, the compositions, methods, and/or kits specifically target and eliminate PLA2R-epitope recognizing immune cells. In some embodiments, the compositions, methods, and/or kits specifically target and eliminate PLA2R-epitope recognizing B cells. In some embodiments, the compositions, methods, and/or kits specifically target and eliminate PLA2R-epitope recognizing T cells. In some embodiments, the compositions, methods, and/or kits specifically target and eliminate PLA2R-epitope recognizing B and T cells. In some embodiments, the EDC comprises one or more PLA2R fragments provided herein, a linker and a drug.

[0142[Current treatments for MN are non-specific with significant side-effects and frequent disease relapse. The EDCs herein for MN are specific with minimum/no side-effects, and eliminate disease relapse.

[0143[The patient presents at least one clinical symptom of MN. The symptoms of MN include one or more of nephrotic syndrome, edema (swelling in any area of the body),

proteinuria, foamy appearance of urine (due to large amounts of protein), urination (excessive at night), fatigue, poor appetite and weight gain.

[0144[In some embodiments, the patient has had MN for about 1 month to about 5 years. In some embodiments, the patient has had MN for about 1, 2, 3 or 4 weeks, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or 2, 3, 4, 5, 6, 7, 8, 9 or 10 years, or within a range defined by any two of the aforementioned values.

[0145[In some embodiments, the patient has previously not received any treatment for MN. In some embodiments, the patient has previously received treatment (either immunosuppressive drugs or steroid hormones or both) for MN. In some embodiments, the patient has previously been successfully treated with either immunosuppressive drugs or steroid hormones or both for MN. In some embodiments, the patient has previously been unsuccessfully treated with either immunosuppressive drugs or steroid hormones or both for MN.

[0146[In some embodiments, the patient may be subject to a kidney biopsy to confirm that the patient has MN. In some embodiments, the patient is subject to a kidney biopsy even if there is a detectable increase in anti-PLA2R autoantibodies in the patient sample relative to the control sample. In some embodiments, the patient is not subject to a kidney biopsy if there is a detectable increase in anti-PLA2R in the patient sample relative to the control sample.

[0147[Full-length PLA2R (FIG. 19; SEQ ID NO: 14) is difficult to purify and therefore has limited application in clinical settings. Thus, in some embodiments, the compositions, methods, and/or kits for removing autoantibody-producing B cells from a patient sample comprise a purified fragment of full-length PLA2R. In some embodiments, the fragment comprises CysR, FnII and one or more CTLDs (FIG. 2).

[0148[In some embodiments, the fragment comprises at least domains 1-5 of PLA2R. In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 1 (about 658 amino acids in length) (FIG. 6). In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 2. In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 2 (FIG. 7) and about 5% to about 95% of the sequence provided in SEQ ID NO: 3 (FIG. 8). In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 2 (FIG. 7) and about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% of the sequence provided in SEQ ID NO: 3 (FIG. 8).

[0149[In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 4 (about 805 amino acids in length) (FIG. 9). In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 5 (FIG. 10). In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 5 (FIG. 10) and about 5% to about 95% of the sequence

provided in SEQ ID NO: 6 (FIG. 11). In some embodiments, the sequence of the fragment is as provided in SEQ ID NO: 5 and about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% of the sequence provided in SEQ ID NO: 6 (FIG. 11).

[0150[In some embodiments, the compositions, methods, and/or kits specifically target and eliminate PLA2R-epitope recognizing immune cells comprise one or more fragments of PLA2R provided herein as a carrier for one or more drugs (EDC) (FIG. 4). The PLA2R fragments would specifically target the drug to the specific B cells and the corresponding helper T cells that are responsible for anti-PLA2R autoantibody production in patients. The EDC can bind to the specific receptors on the surface B and T cells and enter the cells via endocytosis.

[0151[In some embodiments, a free cysteine residue is introduced at the C-terminus of the PLA2R fragment. The thio group of the free cysteine is then conjugated with a cleavable linker. In some embodiments, the cleavable linker is a valine-citrulline linker. In some embodiments, the valine-citrulline linker is pre-conjugated with a drug. In some embodiments, the drug is a duocarmycin analog. In some embodiments, a free cysteine residue is introduced at the C-terminus of the PLA2R fragment and the thio group of the free cysteine is conjugated with a cleavable valine-citrulline linker pre-conjugated with a duocarmycin analog.

[0152[In some embodiments, a short sequence of CXPXR is introduced to the C-terminus of the PLA2R fragment. In some embodiments, the cysteine residue is converted to a formylglycine aldehyde tag. In some embodiments, the cysteine residue is converted to a formylglycine aldehyde tag using a formylglycine-generating enzyme. In some embodiments, the formylglycine aldehyde tag is then conjugated to a drug-linker by a non-cleavable linkage. In some embodiments, the formylglycine aldehyde tag is conjugated to the drug-linker by a non-cleavable linkage via oxime chemistry. In some embodiments, the formylglycine aldehyde tag is conjugated to the drug-linker by a non-cleavable linkage via Pictet-Spengler reaction. In some embodiments, the drug in the drug-linker is a cytotoxic reagent.

[0153[In some embodiments, a drug is conjugated to the PLA2R fragment in a region outside of the BCR interaction site. In some embodiments, a drug is conjugated to the PLA2R fragment in a region outside of the BCR interaction site to one or more lysine residues. In some embodiments, a drug is conjugated to the PLA2R fragment in a region outside of the BCR interaction site to one or more lysine residues via amide bonds to an N-hydroxysuccinimide (NHS) ester appended to a drug-linker.

[0154[For example, in some embodiments, the EDC is administered into the patient circulation via the intravenous route. In some embodiments, binding of the EDC to the PLA2R-recognizing autoantibody-producing B cells triggers rapid internalization of the receptor-EDC

complex via endocytosis (FIG. 5). In some embodiments, binding of the EDC to the PLA2R-recognizing T cells triggers rapid internalization of the receptor-EDC complex via endocytosis. In the endosome, the internalized receptor-EDC complex is digested causing release of the conjugated drug. In some embodiments, the released drug then causes death of the PLA2R-recognizing autoantibody-producing B cells (FIG. 5). In some embodiments, the released drug then causes death of the PLA2R-recognizing T cells. In some embodiments, the drug causes death of the B and T cells by apoptosis. In some embodiments, the drug causes death of the B and T cells by non-apoptotic mechanisms.

Pemphigus vulgaris

[0155[Pemphigus vulgaris (PV) is a potential life-threatening-autoimmune blistering disease characterized by intraepithelial blister formation caused by loss of cell–cell adhesion. PV affects both of the skin and mucous membranes and is mediated by circulating pathogenic autoantibodies directed against keratinocyte cell surface molecules desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3).

[0156[The binding of autoantibodies to desmoglein impairs the integrity of desmosome that results in a loss of cell-to-cell adhesion causing blistering, and in addition, triggers a cellular process that results in acantholysis. Patients with the mucocutaneous form of PV have pathogenic anti-Dsg1 and anti-Dsg3 autoantibodies. Patients with the mucosal form of PV have only anti-Dsg3 autoantibodies.

[0157[Dsg3 and Dsg1 are both transmembrane proteins with a similar molecular structure, of which each comprises 5 extracellular cadherin (EC) domains, a single-pass transmembrane region, and an intracellular tail (FIG. 26). The dominant epitope in Dsg3 is located in the EC1-3 domains (FIG. 26) that bind to all anti-Dsg3 pathogenic autoantibodies. The Dsg3 EC1-3 domains contain 334 amino acids. The dominant epitope in Dsg1 is located in the EC1-2 domains (FIG. 26) that bind to all anti-Dsg1 pathogenic autoantibodies. and the Dsg1 EC1-2 contains 221 amino acids respectively. The calcium binding sites in the epitopes are critical for autoantibody binding (FIG. 26) (Ohyama, B., et al.).

[0158[In some embodiments, EDCs based on epitopes in Dsg1 are provided. FIG. 31 shows the amino acid sequence of an embodiment of an epitope of Dsg1 (SEQ ID NO: 16; 336 amino acids) used for developing Dsg1 epitope-based EDC according to the present disclosure. In some embodiments, EDCs based on epitopes in Dsg3 are provided. FIG. 32 shows the amino acid sequence of an embodiment of an epitope of Dsg3 (SEQ ID NO: 17; 450

amino acids) used for developing Dsg3 epitope-based EDC according to the present disclosure. In some embodiments, EDCs based on epitopes in Dsg1 and Dsg3 are provided.

[0159[Schematics of embodiments of EDCs based on epitopes in Dsg1 and Dsg3 are shown in FIG 27. Development of EDCs based on epitopes in Dsg1 and Dsg3 for treatment of PV is described in Example 7.

[0160[In some embodiments, residues that are potentially glycosylated can be substituted using, for example, site directed mutagenesis when using an expression vector to express a Dsg1 fragment or Dsg3 fragment. In some embodiments, when a Dsg1 or Dsg3 protein fragment is directly synthesized, the protein synthesis can be customized such that the potentially glycosylated residues are replaced with non-glycosylated residues that will not affect protein structure and/or conformation for autoantibody recognition.

[0161[In some embodiments, additional modifications may be made to the Dsg1 or Dsg3 fragment, for example, to attach a reagent (e.g., a drug) to the Dsg1 or Dsg3 fragment, improve accessibility of the Dsg1 or Dsg3 epitope to receptors on the cell surface, and/or improve protein expression and yield. For example, a Dsg1 or Dsg3 fragment may be modified by inserting small (about 2 to 10 amino acids) N- or C-terminal peptide or both, making conservative and/or non-conservative substitutions, and/or adding one or more heterologous sequences to achieve a desired objective.

[0162[In some embodiments, one or more of the Dsg1 or Dsg3 fragments provided herein are encoded by nucleic acids. In some embodiments, the Dsg1 or Dsg3 encoding nucleic acid is a cDNA or an mRNA. In some embodiments, the Dsg1 or Dsg3 encoding nucleic acid can be comprised within a protein expression vector. In some embodiments, the protein expression vector is a DNA vector or an RNA vector. In some embodiments, the protein expression vector is an adeno-associated viral (AAV) vector. In some embodiments, the protein expression vector is a mammalian cell expression vector. In some embodiments, the protein expression vector is an insect cell expression vector. In some embodiments, the Dsg1 or Dsg3 fragment encoding nucleic acid comprised within a protein expression vector is operably linked to regulatory elements to regulate the expression of the PLA2R fragment.

[0163[Regulatory elements can include promoters, terminators, enhancers, etc. As used herein, "operably linked" refers to a regulatory element positively or negatively controlling the expression of a protein from a nucleic acid.

[0164[One or more of the proteins expression vectors provided herein and others known to one of ordinary skill in the art can be used to obtain large quantities of one or more of

the Dsg1 or Dsg3 fragments provided herein for incorporation into one or more of the compositions, methods, and/or kits provided herein.

[0165[In some embodiments, the protein expression vector introduces a tag on the encoded Dsg1 or Dsg3 fragment. In some embodiments, the tag is on the N-terminal end. In some embodiments, the tag is on the C-terminal end. In some embodiments, the tag is on both ends. Non-limiting examples of tags include chitin binding protein, maltose binding protein, glutathione-S-transferase, thioredoxin, poly(NANP), FLAG, V5, Myc, HA, NE, biotin, biotin carboxyl carrier protein, GFP, Halo, Nus, Fc, AviTag, calmodulin, poly-Glu, E, S, SBP, Softag 1, Softag 3, Strep, TC, VSV, Ty and Xpress. In some embodiments, the tag is a poly-histidine (poly-His) tag.

[0166[In some embodiments, the protein expression vector additionally introduces a cleavage site between the Dsg1 or Dsg3 fragment and the tag. In some embodiments, the cleavage site is a proteolytic site. Non-limiting examples of proteolytic sites include sites for TEV protease, Factor Xa or enteropeptidase. In some embodiments, the proteolytic site is a thrombin cleavage site. Other cleavage sites

[0167[For example, in some embodiments, a poly-His-tagged Dsg1 or Dsg3 fragment can be expressed in mammalian cells (e.g., HEK 293 cells) and purified from the cell culture medium using Ni-affinity purification. The poly-His tag can then be removed by proteolytic digestion (e.g., using thrombin), and the Dsg1 or Dsg3 fragment further purified using gel filtration chromatography to remove the thrombin enzyme and the released poly-His tag.

[0168[In some embodiments, autoantibodies can accumulate in the dermal (the targeting cell junctions in the dermal compartment) and/or subcutaneous compartments, for example, in a patient with PV. In such situations, if EDC is delivered subcutaneously, the EDC may be neutralized right at the injection site and not make it to the lymph nodes. Therefore, owing the potential neutralization of one or more EDC herein by the autoantibodies in the dermal and/or subcutaneous compartments, the PV patient is initially treated with one or more immunosuppressive agents (e.g. Rituxan) in order to lower and/or eliminate the autoantibodies. Although the immunosuppressive agents do not specifically target the autoantibodies in the dermal and/or subcutaneous compartments, the immunosuppressive agents suppress the immune system, and therefore, suppress the production of autoantibodies. This results in inhibition of production of new antibodies and elimination of the circulating autoantibodies (the circulating autoantibodies are recycled and/or degraded based on their half-lives). The PV patient can then be administered one or more EDCs herein via the intravenous, subcutaneous and/or intradermal

routes to target the autoimmune memory B cells in the lymph nodes to prevent disease relapse. Thus, in some embodiments, the one or more EDCs provided herein can be used in conjunction with one or more immunosuppressive therapies to prevent disease relapse. For example, PV has a high frequency of relapse and repeated Rituxan treatment causes fatal infections. Thus, administration of one or more EDCs herein following administration of Rituxan can prevent fatal infections in PV patients. Additionally, Rituxan cannot reach the autoimmune memory B cells in the lymph nodes. In contrast, the EDCs can reach the autoimmune memory B cells in the lymph nodes. Therefore, in addition to preventing relapse, the EDCs can cure the patient of PV by eliminating the autoimmune memory B cells in the lymph nodes.

EXAMPLES

[0169[The following examples are non-limiting and presented merely to illustrate the present invention and to assist one of ordinary skill in the art in making and using the invention. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1

[0170[Blood from a patient with MN is collected. Total B cells are isolated from the blood. FACS is used to identify and separate cells that express the receptor for PLA2R epitope. EDC is added to the cells that carry the receptor for PLA2R epitope. Cells that do not express the receptor for PLA2R epitope are used as control. The EDC is added to each cell population at a dose $1/1000^{th}$ the ordinary dose of the drug in the EDC. The cells populations are incubated overnight with the EDC. Cells that express PLA2R epitope receptor are killed by the EDC whereas cells that do not express the receptor are unaffected and survive. Cells can be stained with trypan blue to assess the effectiveness of the EDC in killing cells that express the receptor for PLA2R epitope.

Example 2

[0171[Blood from a patient with MN is collected. Cells that express the receptor for PLA2R epitope are labeled with a fluorescently-tagged PLA2R epitope. Labeled and unlabeled cells are separated using microscopy. Unlabeled cells are used as control. The EDC is added to each cell population at a dose $1/1000^{th}$ the ordinary dose of the drug in the EDC. The cells populations are incubated overnight with the EDC. Cells that express PLA2R epitope receptor are killed by the EDC whereas cells that do not express the receptor are unaffected and survive.

Cells can be stained with trypan blue to assess the effectiveness of the EDC in killing cells that express the receptor for PLA2R epitope.

Example 3

[0172[In one example, following treatment with one or more treatment options provided herein, a patient with MN recovered in about 1 month as compared to about 6 months with previous treatment regimens. Patient recovery was measured by protein levels in urine.

Example 4 - Engineering of a modified PLA2R-epitope for site-specific chemical conjugation

[0173[PLA2R-epitope contains at minimum 3 domains, namely, the N-terminal cysteine rich domain (CysR), the fibronectin-like type II domain (FnII), and the C-type lectin-like domain 1 (CTLD1) (FIG. 1, FIG. 2, and FIG. 23). The nature of interaction(s) of the epitope with autoantibodies and/or B cell receptor (BCR) has remained unclear. It is critical that conjugation of a chemical to the epitope does not interfere with the antibody-antigen interaction.

[0174[To achieve this, a specific site in PLA2R-epitope for conjugation was developed. Specifically, a free cysteine was engineered into the C-terminal tail of the epitope to develop a specific site for chemical conjugation. A TEV cleavage site and a His-tag (6 Histidine residues) were also engineered into the epitope protein C-terminal to the introduced cysteine for protein purification. FIG. 23 (top) shows a schematic of the design of a modified PLA2R-epitope construct for protein purification and drug conjugation. FIG. 23 (middle) shows a schematic of how a drug (cytotoxic agent) is conjugated to the purified PLA2R epitope.

[0175[Assessment of protein expression showed that modified PLA2R-epitope was well-expressed and strongly reacted to the anti-PLA2R autoantibodies, indicating the introduced cysteine had no effect on the epitope folding. The construct was then expressed in the HEK293 cells and the epitope protein was purified using nickel and gel filtration columns followed with TEV cleavage to remove the His-tag. The protein was then coupled with a fluorescent agent (eg. FITC) or a cytotoxic reagent (eg. Monomethyl auristatin E (MMAE)).

[0176[FIG. 23 (top) shows the design of a modified PLA2R-epitope construct for protein purification and drug conjugation. A site-specific conjugation site was introduced into PLA2R epitope followed with a TEV cleavage site and a His-tag in the design of PLA2R EDC. FIG. 23 (middle) shows how a drug is conjugated to the purified PLA2R-epitope. The purified epitope protein was conjugated with a cytotoxic agent in a site-specific manner. FIG. 23 (bottom) shows the purity of PLA2R-epitope protein after purification. The conjugated PLA2R epitope proteins were purified and resolved on SDS-PAGE. The epitope was analyzed on a 7%

SDS-PAGE which showed a single protein band at the correct molecular weight (about 37 kDa) (FIG. 23; bottom left).

[0177[In a separate experiment (FIG. 23; bottom right), equal amount of epitope, FITC conjugated epitope and MMAE conjugated epitope proteins were resolved on a SDS-PAGE under the non-reducing condition, transferred to a membrane, and probed by a patient serum containing anti-PLA2R autoantibodies. The unconjugated and the conjugated PLA2R-epitope proteins were resolved on a 4-20% SDS-PAGE under the non-reducing condition and probed with a patient serum containing anti-PLA2R autoantibodies (FIG. 23, bottom right). Results showed that the drug conjugated PLA2R-epitope bound strongly to the autoantibodies as strongly as the unconjugated PLA2R-epitope (FIG. 23; bottom right).

[0178[Western blotting with anti-PLA2R autoantibodies showed that the PLA2R epitope strongly reacted with anti-PLA2R autoantibodies indicating that the introduced cysteine had not affected the epitope's folding (FIG. 23 (bottom right; Western blot)). The construct was then expressed in the HEK293 cells and the epitope protein was purified using nickel and gel filtration columns followed with TEV cleavage to remove the His-tag. The protein was then coupled with a fluorescent agent (eg. FITC) or a cytotoxic reagent (eg. Monomethyl auristatin E (MMAE)) (FIG. 23).

Example 5 - Assessment of PLA2R-epitope-FITC binding to the memory B cells isolated from PMN patients

[0179[PMN is a B cell-mediated autoimmune disease. The pathogenic autoantibodies are secreted by the plasma cells derived from a group of memory B cells that possess unique B cell receptors (BCR) specifically bind to the PLA2R-epitope. To distinguish this group of memory B cells and to assess if the PLA2R epitope drug conjugate could effectively bind to the BCRs on the surface of this group of memory B cells, a binding assay was performed using PLA2R-epitope-FITC analysis on the total B cells isolated from PMN patients' blood samples. Total B cells isolated from patient peripheral blood were cultured overnight in the RPMI media supplemented with fetal bovine serum and antibiotics at 37 °C, 5% CO_2. Cells were then dispersed by pipetting up and down, collected and washed twice with ice-cold PBS. Cells were subsequently incubated with PLA2R-epitope-FITC in PBS for 1 h at 4 °C. Cells were washed 3 times with ice-cold PBS and imaged under a fluorescent microscope at the excitation wave length 488 nm and emission wavelength 520 nm. FIG. 24 shows a population of B cells that were labeled with PLA2R-epitope-FITC, indicating the presence of a specific population of PLA2R epitope-binding memory B cells in PMN patients, and the strong binding of PLA2R-epitope-FITC to the BCRs on the surface of these memory B cells.

Example 6 - Assessment of the efficacy of PLA2R-epitope-MMAE on the memory B cells isolated from PMN patients

[0180[The efficacy of the PLA2R-epitope-MMAE on eliminating the PLA2R-epitope specific memory B cells isolated from PMN patients was assessed. MMAE is a potent cytotoxic agent that blocks tubulin polymerization causing cell death. MMAE has been used to develop an antibody-drug conjugate, Brentuximab vedotin (trade name Adcetris) for lymphoma treatment, and was approved by FDA in 2011. Total B cells isolated from patient peripheral blood were cultured overnight in RPMI medium and then split equally into two wells on a 12 well cell culture plate (2 ml/per well). One well of cells was incubated with MMAE, and the other was incubated with PLA2R-epitope-MMAE for 24 h in a cell culture incubator. Cells from both of the wells were then collected, washed with ice cold PBS, and incubated with PLA2R-epitope-FITC for 1 h at 4 °C. Cells were washed 3 times with ice-cold PBS and imaged under a fluorescent microscope at the excitation wave length 488 nm and emission wavelength 520 nm.

[0181[Preincubation with MMAE alone resulted in a number of B cells being stained by the PLA2R-epitope-FITC (FIG. 25). In contrast, preincubation with PLA2R-epitope-MMAE showed no staining with PLA2R-epitope-FITC indicating the specific targeting and eliminating of the PLA2R-epitope specific memory B cells by PLA2R-epitope-MMAE (FIG. 25). Light microscope images of PLA2R-epitope-MMAE treated B cells also indicated that EDC produced little effect on most of the B cells (FIG. 25).

Example 7 – Development of an EDC treatment for Pemphigus vulgaris

[0182[In order to preserve the calcium binding sites in Dsg3 epitope, which are critical for autoantibody binding, Dsg3 EC1-4 domains (450 amino acids; FIG. 32; SEQ ID NO: 17) were selected as the epitope for drug conjugation. In order to develop a specific conjugation site in Dsg3 EC1-4, all the endogenous cysteines (underlined in FIG. 32) were replaced with a structural similar amino acid, serine. In addition, 2 endogenous N-glycosylation sites (Asn61 and Asn131; underlined in FIG. 32) were replaced with a structural similar amino acid, glutamine. The same approach was adopted for designing the Dsg1 epitope for drug conjugation, with the modified Dsg1 EC1-3 domains (336 amino acids) were selected (FIG. 31; SEQ ID NO: 16). The epitopes generated were named Dsg1 EC1-3 and Dsg3 EC1-4. The molecular weight of the Dsg1 EC1-3 epitope was about 35 kDa and the molecular weight of the Dsg3 EC1-4 epitope was about 60 kDa.

[0183[A free cysteine was then engineered into the C-terminal tail of each of the epitopes to develop a specific site for chemical conjugation. A TEV cleavage site and a His-tag (6 His) were also engineered into the epitope protein after the introduced cysteine for protein purification (FIG. 27).

[0184[The engineered epitopes were expressed in the HEK 293 cells and purified from the culture media using nickel affinity and gel filtration columns followed with TEV cleavage to remove the His-tag (FIG. 28, left panel; Coomassie Blue). The epitopes were then coupled with either a fluorescent agent (e.g., FITC) or a cytotoxic reagent (e.g., MMAE)

[0185[Because autoantibodies to Dsg1 and Dsg3 preferentially bind the non-denatured Dsg 1 or Dsg3 proteins, the purified FITC or MMAE conjugated forms of the two epitopes prior to TEV treatment were mixed with a mucocutaneous PV patient serum in PBS buffer to form immune complexes and then immunoprecipitated with the protein G beads. Equal amounts of the purified conjugated forms of Dsg1 and Dsg3 epitopes (prior to TEV cleavage) were mixed with a mucocutaneous PV patient serum in PBS buffer for 2 h at 4 °C and immunoprecipitated with protein G beads. The beads were extensively washed and the bound epitopes were eluted with SDS-sample buffer, resolved on a SDS-PAGE, transferred to a membrane, and probed by an anti-His tag antibody (FIG 28, middle and right panels; Western blot). Western blot results showed that the engineered and the chemical conjugated epitopes of Dsg1 and Dsg3 reacted strongly with the autoantibodies and were immunoprecipitated well by the protein G beads, indicating that engineering and chemical conjugation of the epitope proteins had no interference with the autoantibody-antigen interactions.

Example 8 - Assessment of Dsg-epitope-FITC binding to the memory B cells isolated from mucocutaneous PV patients

[0186[The ability of Dsg-epitope-FITC binding to the specific BCRs in the memory B cells isolated from mucocutaneous PV patients' blood samples was assessed. Peripheral blood mononuclear cells were first isolated from the whole blood using density centrifugation (Histopaque-1077), followed with total B cell isolation using a B cell negative isolation kit. The isolated total B cells were then cultured overnight in the RPMI media supplemented with fetal bovine serum and antibiotics at 37 °C, 5% CO_2. Cells were then processed, stained with Dsg-epitope-FITC in PBS for 1 h at 4 °C and imaged under a fluorescent microscope at the excitation wave length 488 nm and emission wavelength 520 nm. FIG. 29 shows, Dsg-epitope-FITC strongly stained a group of B cells, indicating the strong binding of Dsg-epitope-FITC to the BCRs on the surface of these memory B cells.

Example 9 - Assessment of the efficacy of Dsg-epitope-MMAE on the memory B cells isolated from mucocutaneous PV patients

[0187[The efficacy of the Dsg1 and Dsg3 epitope conjugated to MMAE to eliminate Dsg1 and Dsg3-epitope specific memory B cells, respectively, isolated from the mucocutaneous PV patients was assessed. Total B cells isolated from patient peripheral blood were cultured overnight in RPMI medium and then split equally into two wells on a 12 well cell culture plate (2 ml/per well). One well of cells was incubated with MMAE, and the other was incubated with Dsg1 and Dsg3-epitope-MMAE for 24 h in a cell culture incubator. Cells from both of the wells were then collected, washed with ice cold PBS, and incubated with Dsg1 and Dsg3-epitope-FITC for 1 h at 4 °C. Cells were washed 3 times with ice-cold PBS and imaged under a fluorescent microscope at the excitation wave length 488 nm and emission wavelength 520 nm.

[0188[Preincubation with MMAE alone resulted in a number of B cells being stained by Dsg1 and Dsg3-epitope-FITC (FIG. 30). In contrast, preincubation with Dsg1 and Dsg3-epitope-MMAE showed no staining with Dsg1 and Dsg3-epitope-FITC indicating the specific targeting and eliminating of the Dsg1 and Dsg3-epitope specific memory B cells by Dsg1 and Dsg3-epitope-MMAE (FIG. 30). Light microscope images of Dsg 1 and Dsg3-epitope-MMAE treated B cells also indicated that EDC produced little effect on most of the B cells (FIG. 30).

[0189[As used herein, the section headings are for organizational purposes only and are not to be construed as limiting the described subject matter in any way. All literature and similar materials cited in this application, including but not limited to, patents, patent applications, articles, books, treatises, and internet web pages are expressly incorporated by reference in their entirety for any purpose. When definitions of terms in incorporated references appear to differ from the definitions provided in the present teachings, the definition provided in the present teachings shall control. It will be appreciated that there is an implied "about" prior to the temperatures, concentrations, times, etc discussed in the present teachings, such that slight and insubstantial deviations are within the scope of the present teachings herein.

[0190[In this application, the use of the singular includes the plural unless specifically stated otherwise. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting.

[0191[As used in this specification and claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

[0192[As used herein, "about" means a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0193[Although this invention has been disclosed in the context of certain embodiments and examples, those skilled in the art will understand that the present invention extends beyond the specifically disclosed embodiments to other alternative embodiments and/or uses of the invention and obvious modifications and equivalents thereof. In addition, while several variations of the invention have been shown and described in detail, other modifications, which are within the scope of this invention, will be readily apparent to those of skill in the art based upon this disclosure. It is also contemplated that various combinations or sub-combinations of the specific features and aspects of the embodiments may be made and still fall within the scope of the invention. It should be understood that various features and aspects of the disclosed embodiments can be combined with, or substituted for, one another in order to form varying modes or embodiments of the disclosed invention. Thus, it is intended that the scope of the present invention herein disclosed should not be limited by the particular disclosed embodiments described above.

[0194[It should be understood, however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

[0195[The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner. Rather, the terminology is simply being utilized in conjunction with a detailed description of embodiments of the systems, methods and related components. Furthermore, embodiments may comprise several novel features, no single one of which is solely responsible for its desirable attributes or is believed to be essential to practicing the inventions herein described.

[0196[Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention.

[0197[All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

[0198[As used herein, a “detectable marker” or “label” is a molecule attached to, or synthesized as part of a reagent. This molecule should be uniquely detectable and will allow the reagent to be detected as a result. These detectable moieties are often radioisotopes, chemiluminescent molecules, enzymes, haptens, or even unique oligonucleotide sequences.

[0199[As used herein, "polypeptide" includes proteins, fragments of proteins, and peptides, whether isolated from natural sources, produced by recombinant techniques or chemically synthesized. Polypeptides typically comprise at least about 6 amino acids. Shorter polypeptides, e.g., those less than about 50 amino acids in length, are typically referred to as “peptides”.

[0200[A polypeptide of the invention can, in some embodiments, comprise a variant of a native protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native protein in one or more substitutions, deletions, additions and/or insertions, such that the therapeutic efficacy of the polypeptide is not substantially diminished. In other words, the efficacy may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described above) to the identified polypeptides. As is known in the art, variants can also be selected to optimize affinity of the polypeptide for a binding partner.

[0201[Preferably, a variant contains conservative substitutions. A “conservative substitution” is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr; (2) Cys, Ser, Tyr, Thr; (3) Val, Ile, Leu, Met, Ala, Phe; (4) Lys, Arg, His; And (5)

Phe, Tyr, Trp, His. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

[0202[The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner. Rather, the terminology is simply being utilized in conjunction with a detailed description of embodiments of the systems, methods and related components. Furthermore, embodiments may comprise several novel features, no single one of which is solely responsible for its desirable attributes or is believed to be essential to practicing the inventions herein described.

[0203] It should be understood, however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those ordinary skill in the art.

References

[0204[All references cited in this disclosure are incorporated herein by reference in their entireties.

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[0209[Ohyama, B., et al., J Invest Dermatol, Vol. 132, pp. 1158–1168, 2012.

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WHAT IS CLAIMED IS:

1. A method of treating a patient with membranous nephropathy (MN), the method comprising:

identifying a patient with MN; and

administering to the patient a complex comprising a PLA2R epitope and a drug, wherein the epitope is comprised within a PLA2R fragment,

thereby eliminating or reducing an anti-PLA2R autoantibody producing B cell population in the patient.

2. The method of claim 1, wherein the PLA2R epitope is as provided in SEQ ID NO: 13.

3. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 1.

4. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2.

5. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2 and at least about 5% of the sequence provided in SEQ ID NO: 3.

6. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 4.

7. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5.

8. The method of claim 1, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5 and at least about 5% of the sequence provided in SEQ ID NO: 6.

9. The method of any of the foregoing claims, wherein the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine.

10. The method of any of the foregoing claims, wherein an efficacy of eliminating the anti-PLA2R autoantibody producing B cell population ranges from about 70% to about 100%.

11. The method of any of the foregoing claims, wherein the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-PLA2R autoantibody producing B cell population.

12. A complex comprising a PLA2R epitope and a drug, wherein the epitope is comprised within a PLA2R fragment.

13. The complex of claim 12, wherein the PLA2R epitope is as provided in SEQ ID NO: 13.

14. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 1.

15. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2.

16. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 2 and at least about 5% of the sequence provided in SEQ ID NO: 3.

17. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 4.

18. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5.

19. The complex of claim 12, wherein the sequence of the PLA2R fragment is as provided in SEQ ID NO: 5 and at least about 5% of the sequence provided in SEQ ID NO: 6.

20. The complex of any one of claims 12-19, wherein the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine.

21. The complex of any one of claims 12-20, wherein the drug is linked to the PLA2R fragment via a valine-citrulline linker.

22. A method of delivering a drug to a subject having autoimmune B cells or T cells, the method comprising:

providing the drug in an epitope-drug conjugate (EDC) comprising an epitope conjugated to the drug via a linker, the epitope being recognized by receptors on the autoimmune B cells or T cells in the subject, the drug having an activity that blocks the B cells from stimulating other cells; and

intradermally or subcutaneously administering the EDC to the subject.

23. The method of claim 22, wherein the autoimmune B cells or T cells are circulating in the blood of the subject.

24. The method of claim 22, wherein the autoimmune B cells or T cells are in lymph nodes of the subject.

25. The method of any one of claims 22-24, wherein the drug kills the B cell or the T cell.

26. The method of claim 25, wherein the drug is selected from the group consisting of Duocarmycin A, Duocarmycin B1, Duocarmycin B2, Duocarmycin C1, Duocarmycin C2, Duocarmycin D, Duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil or capecitabine, Monomethyl auristatin E (MMAE), anthracyclines, oxaliplatin, or bortezomib.

27. The method of any one of claims 22-24, wherein the drug blocks release of cytokines from the B cell or the T cell or block cytokine signaling in the B cell or the T cell.

28. The method of claim 27, wherein the drug is selected from the group consisting of Rapamycin, Ciclosporin, Tacrolimus, Mycophenolate, Fingolimod, Imatinib, Temsirolimus, Sorafenib, Sunitinib, Pirfenidone, Src family tyrosine kinase inhibitors (Dasatinib, Saracatinib, Bosutinib, Bafetinib), MEK kinase inhibitors (Selumetinib, Trametinib, and Refametinib).

29. The method of any one of claims 22-28, wherein the subject is not in an acute phase of active autoimmune disease.

30. The method of claim 29, wherein the subject has been treated with an immunosuppressant such that the patient is not in the acute phase of active autoimmune disease.

31. The method of any one of claim 22-30, wherein the EDC has a molecular weight of 14-70 kDa.

32. A method of treating a patient with Pemphigus vulgaris (PV), the method comprising:

identifying a patient with PV; and

administering to the patient a complex comprising a desmoglein 1 or a desmoglein 3 epitope and a drug, wherein the epitope is comprised within a desmoglein 1 or desmoglein 3 fragment,

thereby eliminating or reducing an anti- desmoglein 1 or anti- desmoglein 3 autoantibody producing B cell population in the patient.

33. The method of claim 32, wherein the desmoglein 1 epitope is as provided in SEQ ID NO: 16.

34. The method of claim 32, wherein the sequence of the desmoglein 1 fragment is as provided in SEQ ID NO: 16.

35. The method of claim 32, wherein the desmoglein 3 epitope is as provided in SEQ ID NO: 17.

36. The method of claim 32, wherein the sequence of the desmoglein 3 fragment is as provided in SEQ ID NO: 17.

37. The method of any one of claims 32-36, wherein the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more

Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine.

38. The method of any one of claims 32-37, wherein an efficacy of eliminating the anti-desmoglein 1 autoantibody producing B cell population ranges from about 70% to about 100%.

39. The method of any one of claims 32-38, wherein an efficacy of eliminating the anti-desmoglein 3 autoantibody producing B cell population ranges from about 70% to about 100%.

40. The method of any one of claims 32-37, wherein the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-desmoglein 1 autoantibody producing B cell population.

41. The method of any one of claims 32-40, wherein the complex also eliminates a T cell population, wherein the T cell population provides T cell help to the anti-desmoglein 3 autoantibody producing B cell population.

42. A epitope drug complex (EDC) comprising a drug and a desmoglein 1 epitope or desmoglein 3 epitope, wherein the desmoglein 1 epitope is comprised within a desmoglein 1 fragment and wherein the desmoglein 3 epitope is comprised within a desmoglein 3 fragment.

43. The epitope drug complex of claim 42, wherein the desmoglein 1 epitope is as provided in SEQ ID NO: 16.

44. The epitope drug complex of claim 42, wherein the sequence of the desmoglein 1 fragment is as provided in SEQ ID NO: 16.

45. The epitope drug complex of claim 42, wherein the desmoglein 3 epitope is as provided in SEQ ID NO: 17.

46. The epitope drug complex of claim 42, wherein the sequence of the desmoglein 3 fragment is as provided in SEQ ID NO: 17.

47. The complex of any one of claims 42-46, wherein the drug is selected from the group consisting of antisense RNA, miRNA, siRNA or RNA fragment for RNAi, one or more Duocarmycin analogues, or cytotoxic drug such as adozelesin, bizelesin, carzelesin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Epirubicin, cisplatin, 5-fluorouracil and capecitabine.

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FIG. 1

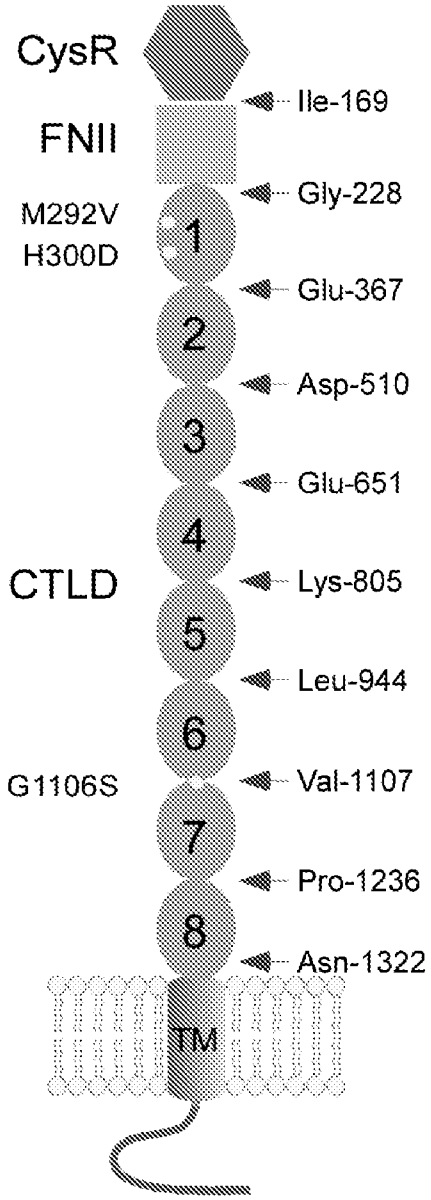
CysR
FNII
M292V
H300D
CTLD
G1106S
1
2
3
4
5
6
7
8
TM
Ile-169
Gly-228
Glu-367
Asp-510
Glu-651
Lys-805
Leu-944
Val-1107
Pro-1236
Asn-1322

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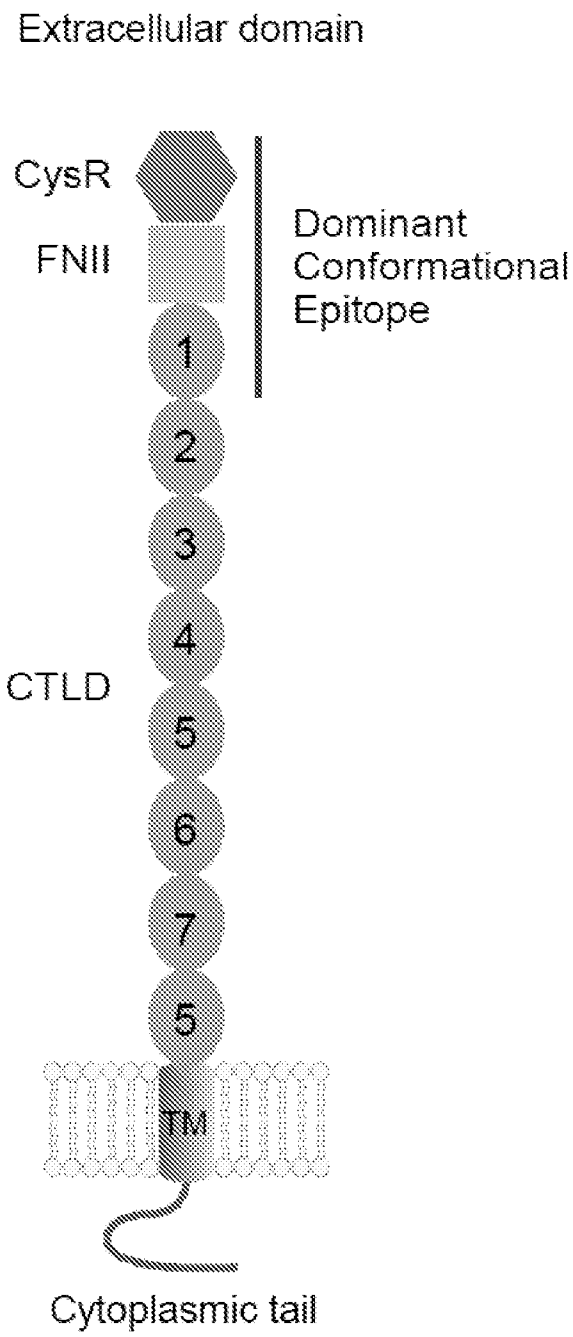
FIG. 2


Extracellular domain
CysR
FNII
Dominant
Conformational
Epitope
1
2
3
4
CTLD
5
6
7
5
TM
Cytoplasmic tail

3/32
FIG. 3

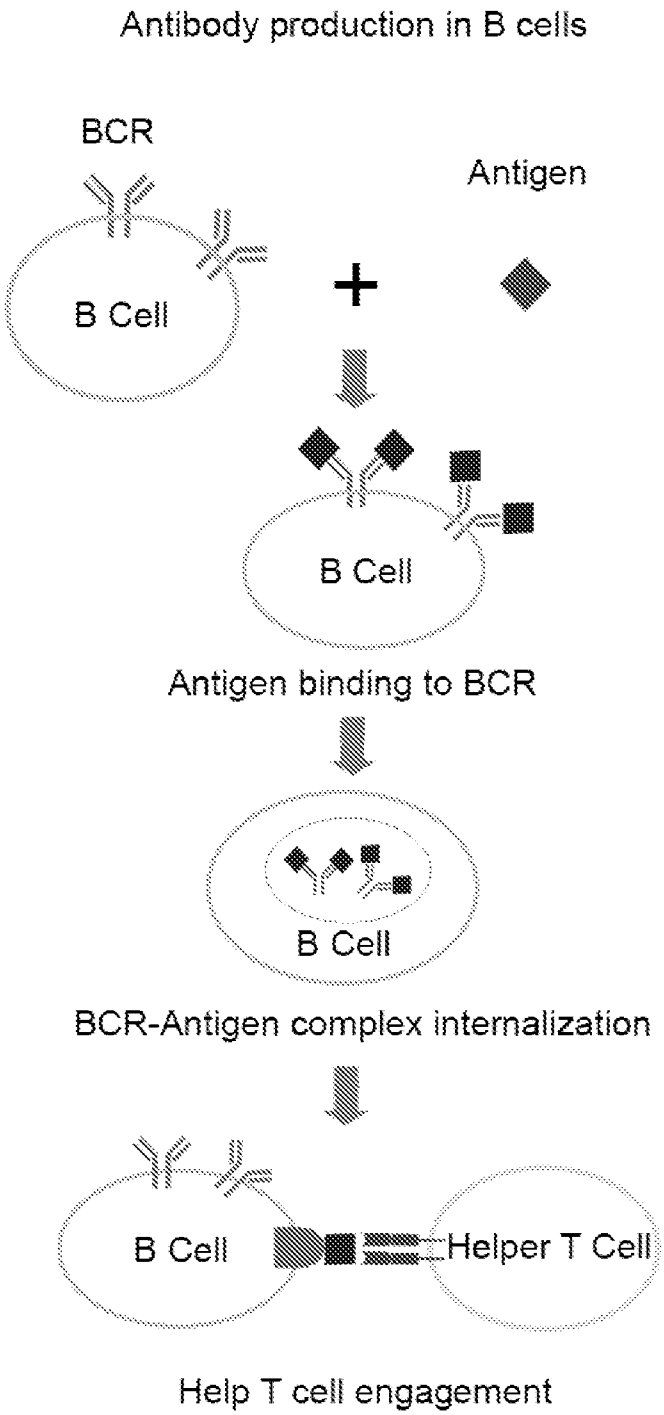

Antibody production in B cells
BCR
Antigen
B Cell
B Cell
Antigen binding to BCR
B Cell
BCR-Antigen complex internalization
B Cell
Helper T Cell
Help T cell engagement

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FIG. 4

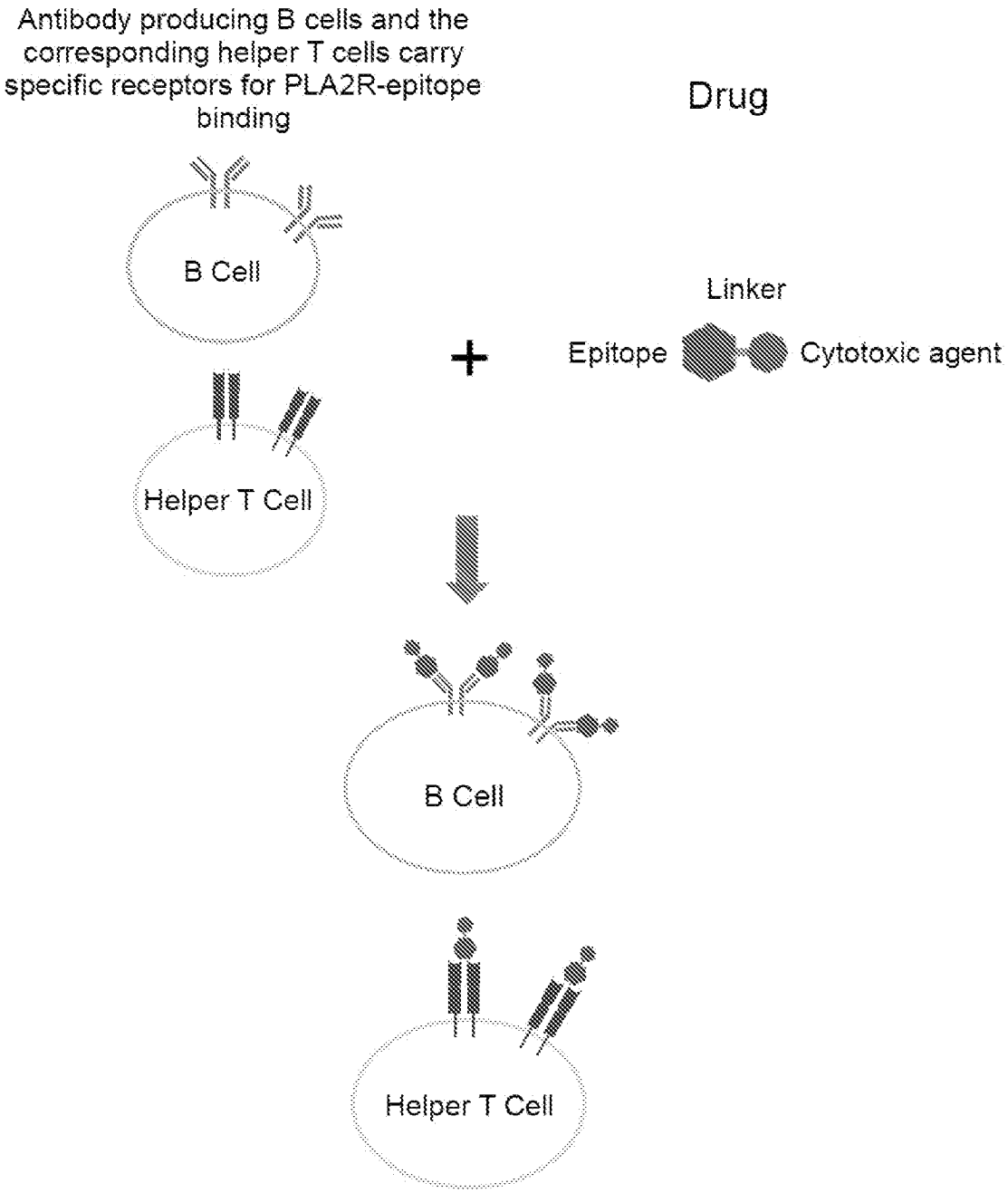
Antibody producing B cells and the corresponding helper T cells carry specific receptors for PLA2R-epitope binding
Drug
B Cell
Linker
Epitope
Cytotoxic agent
Helper T Cell
B Cell
Helper T Cell

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FIG. 5

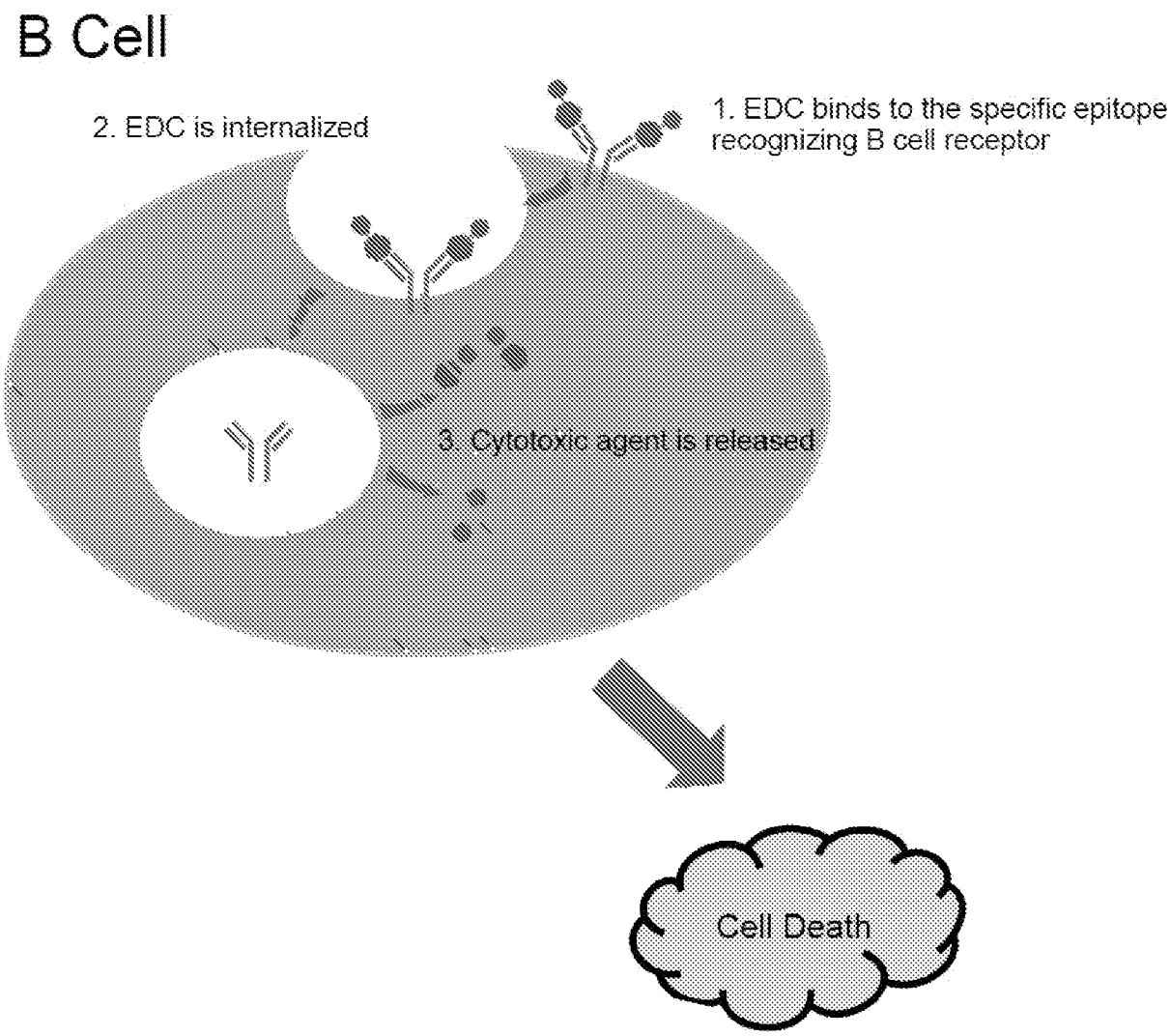
B Cell
2. EDC is internalized
1. EDC binds to the specific epitope recognizing B cell receptor
3. Cytotoxic agent is released
Cell Death

FIG. 6

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQ
KEEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSN
KIPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNC
EERLFYICKKAGHVLSDAESGCQEGWERHGGFCYKIDTVLRSFDQASSG
YYCPPALVTITNRFEQAFITSLISSVVKMKDSYFWIALQDQNDTGEYTWKP
VGQKPEPVQYTHWNTHQPRYSGGCVAMRGRHPLGRWEVKHCRHFKA
MSLCKQPVENQEKAEYEER **(SEQ ID NO: 1)**

FIG. 7

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHE **(SEQ ID NO: 2)**

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FIG. 8

IVEKDAWKYYATHCEPGWNPYNRNCYKLQKEEKTWHEALRSCQADNSA
LIDITSLAEVEFLVTLLGDENASETWIGLSSNKIPVSFEWSNDSSVIFTNWH
TLEPHIFPNRSQLCVSAEQSEGHWKVKNCEERLFYICKKAGHVLSDAESG
CQEGWERHGGFCYKIDTVLRSFDQASSGYYCPPALVTITNRFEQAFITSLI
SSVVKMKDSYFWIALQDQNDTGEYTWKPVGQKPEPVQYTHWNTHQPR
YSGGCVAMRGRHPLGRWEVKHCRHFKAMSLCKQPVENQEKAEYEER
(SEQ ID NO: 3)

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FIG. 9

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQ
KEEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSN
KIPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNC
EERLFYICKKAGHVLSDAESGCQEGWERHGGFCYKIDTVLRSFDQASSG
YYCPPALVTITNRFEQAFITSLISSVVKMKDSYFWIALQDQNDTGEYTWKP
VGQKPEPVQYTHWNTHQPRYSGGCVAMRGRHPLGRWEVKHCRHFKA
MSLCKQPVENQEKAEYEERWPFHPCYLDWESEPGLASCFKVFHSEKVL
MKRTWREAEAFCEEFGAHLASFAHIEEENFVNELLHSKFNWTEERQFWI
GFNKRNPLNAGSWEWSDRTPVVSSFLDNTYFGEDARNCAVYKANKTLL
PLHCGSKREWICKIPRDVKPK **(SEQ ID NO: 4)**

FIG. 10

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVE **(SEQ ID NO: 5)**

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FIG. 11

KDAWKYYATHCEPGWNPYNRNCYKLQKEEKTWHEALRSCQADNSALIDI
TSLAEVEFLVTLLGDENASETWIGLSSNKIPVSFEWSNDSSVIFTNWHTLE
PHIFPNRSQLCVSAEQSEGHWKVKNCEERLFYICKKAGHVLSDAESGCQ
EGWERHGGFCYKIDTVLRSFDQASSGYYCPPALVTITNRFEQAFITSLISS
VVKMKDSYFWIALQDQNDTGEYTWKPVGQKPEPVQYTHWNTHQPRYS
GGCVAMRGRHPLGRWEVKHCRHFKAMSLCKQPVENQEKAEYEERWPF
HPCYLDWESEPGLASCFKVFHSEKVLMKRTWREAEAFCEEFGAHLASFA
HIEEENFVNELLHSKFNWTEERQFWIGFNKRNPLNAGSWEWSDRTPVVS
SFLDNTYFGEDARNCAVYKANKTLLPLHCGSKREWICKIPRDVKPK **(SEQ ID NO: 6)**

FIG. 12

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTI **(SEQ ID NO: 7)**

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FIG. 13

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVG **(SEQ ID NO: 8)**

FIG. 14

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVE **(SEQ ID NO: 9)**

FIG. 15

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQ
KEEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSN
KIPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNC
EERLFYICKKAGHVLSD **(SEQ ID NO: 10)**

FIG. 16

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQ
KEEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSN
KIPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNC
EERLFYICKKAGHVLSDAESGCQEGWERHGGFCYKIDTVLRSFDQASSG
YYCPPALVTITNRFEQAFITSLISSVVKMKDSYFWIALQDQNDTGEYTWKP
VGQKPEPVQYTHWNTHQPRYSGGCVAMRGRHPLGRWEVKHCRHFKA
MSLCKQPVENQE **(SEQ ID NO: 11)**

FIG. 17

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSCQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQ
LDEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRS
RDCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQ
KEEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSN
KIPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNC
EERLFYICKKAGHVLSDAESGCQEGWERHGGFCYKIDTVLRSFDQASSG
YYCPPALVTITNRFEQAFITSLISSVVKMKDSYFWIALQDQNDTGEYTWKP
VGQKPEPVQYTHWNTHQPRYSGGCVAMRGRHPLGRWEVKHCRHFKA
MSLCKQPVENQEKAEYEERWPFHPCYLDWESEPGLASCFKVFHSEKVL
MKRTWREAEAFCEEFGAHLASFAHIEEENFVNELLHSKFNWTEERQFWI
GFNKRNPLNAGSWEWSDRTPVVSSFLDNTYFGEDARNCAVYKANKTLL
PLHCGSKREWICKIPRDVKPK **(SEQ ID NO: 12)**

FIG. 18

CDTIWEKDLNSHICYQFNLLSSLSWSEAHSSCQMQGGTLLSITDETEENFI REHMSSKTVEVWMGLNQLDEHAGWQWSDGTPLNYLNWSPEVNFEPFV EDHCGTFSSFMPSAWRSRDCESTLPYICKKYLNHIDHEIVE **(SEQ ID NO: 13)**

FIG. 19

MLLSPSLLLLLLLGAPRGCAEGVAAALTPERLLEWQDKGIFVIQSESLKKCI
QAGKSVLTLENCKQANKHMLWKWVSNHGLFNIGGSGCLGLNFSAPEQP
LSLYECDSTLVSLRWRCNRKMITGPLQYSVQVAHDNTVVASRKYIHKWIS
YGSGGGDICEYLHKDLHTIKGNTHGMPCMFPFQYNHQWHHECTREGRE
DDLLWCATTSRYERDEKWGFCPDPTSAEVGCDTIWEKDLNSHICYQFNL
LSSLSWSEAHSSQMQGGTLLSITDETEENFIREHMSSKTVEVWMGLNQL
DEHAGWQWSDGTPLNYLNWSPEVNFEPFVEDHCGTFSSFMPSAWRSR
DCESTLPYICKKYLNHIDHEIVEKDAWKYYATHCEPGWNPYNRNCYKLQK
EEKTWHEALRSCQADNSALIDITSLAEVEFLVTLLGDENASETWIGLSSNK
IPVSFEWSNDSSVIFTNWHTLEPHIFPNRSQLCVSAEQSEGHWKVKNCE
ERLFYICKKAGHVLSDAESGCQEGWERHGGFCYKIDTVLRSFDQASSGY
YCPPALVTITNRFEQAFITSLISSVVKMKDSYFWIALQDQNDTGEYTWKPV
GQKPEPVQYTHWNTHQPRYSGGCVAMRGRHPLGRWEVKHCRHFKAM
SLCKQPVENQEKAEYEERWPFHPCYLDWESEPGLASCFKVFHSEKVLM
KRTWREAEAFCEEFGAHLASFAHIEEENFVNELLHSKFNWTEERQFWIG
FNKRNPLNAGSWEWSDRTPVVSSFLDNTYFGEDARNCAVYKANKTLLPL
HCGSKREWICKIPRDVKPKIPFWYQYDVPWLFYQDAEYLFHTFASEWLN
FEFVCSWLHSDLLTIHSAHEQEFIHSKIKALSKYGASWWIGLQEERANDEF
RWRDGTPVIYQNWDTGRERTVNNQSQRCGFISSITGLWGSEECSVSMP
SICKRKKVWLIEKKKDTPKQHGTCPKGWLYFNYKCLLLNIPKDPSSWKN
WTHAQHFCAEEGGTLVAIESEVEQAFITMNLFGQTTSVWIGLQNDDYET
WLNGKPVVYSNWSPFDIINIPSHNTTEVQKHIPLCALLSSNPNFHFTGKW
YFEDCGKEGYGFVCEKMQDTSGHGVNTSDMYPMPNTLEYGNRTYKIINA
NMTWYAAIKTCLMHKAQLVSITDQYHQSFLTVVLNRLGYAHWIGLFTTDN
GLNFDWSDGTKSSFTFWKDEESSLLGDCVFADSNGRWHSTACESFLQG
AICHVPPETRQSEHPELCSETSIPWIKFKSNCYSFSTVLDSMSFEAAHEFC
KKEGSNLLTIKDEAENAFLLEELFAFGSSVQMVWLNAQFDGNNETIKWFD
GTPTDQSNWGIRKPDTDYFKPHHCVALRIPEGLWQLSPCQEKKGFICKM
EADIHTAEALPEKGPSHSIIPLAVVLTLIVIVAICTLSFCIYKHNGGFFRRLAG
FRNPYYPATNFSTVYLEENILISDLEKSDQ **(SEQ ID NO: 14)**

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FIG. 20

MDSYLLMWGLLTFIMVPGCQAELCDDDPPEIPHATFKAMAYKEGTMLNC
ECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQ
PEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVG
QMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQF
PGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQVAV
AGCVFLLISVLLLSGLTWQRRQRKSRRTI **(SEQ ID NO: 15)**

Position	Residue	Score	Prediction
48	NCE	-0.32701759	Non-glycosylated
70	NSS	0.37511271	Potential Glycosylated
78	NQC	-0.35326057	Non-glycosylated
89	NTT	0.24039211	Potential Glycosylated
133	NEA	-0.58545335	Non-glycosylated

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FIG. 21

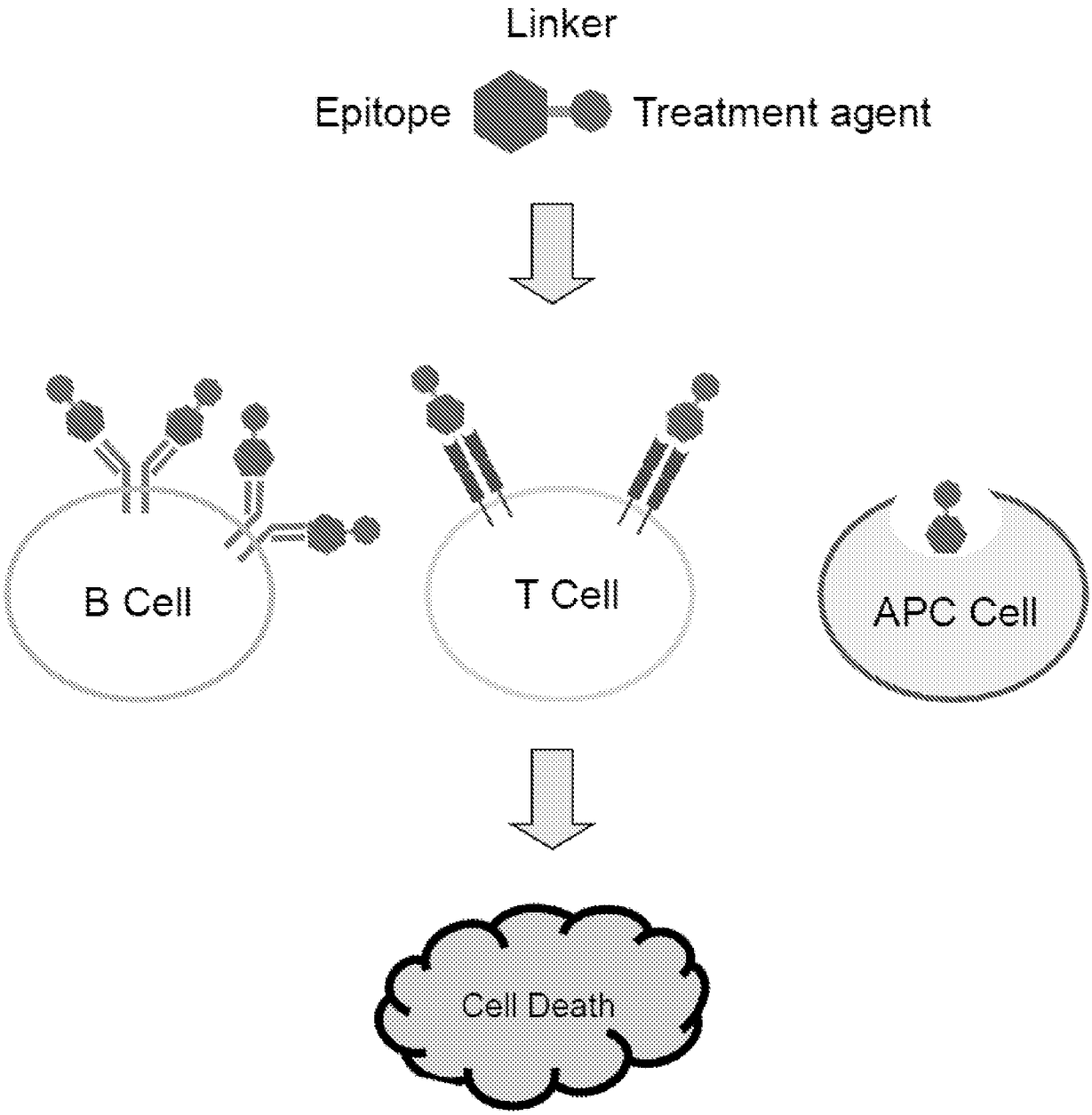
Linker
Epitope
Treatment agent
B Cell
T Cell
APC Cell
Cell Death

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FIG. 22

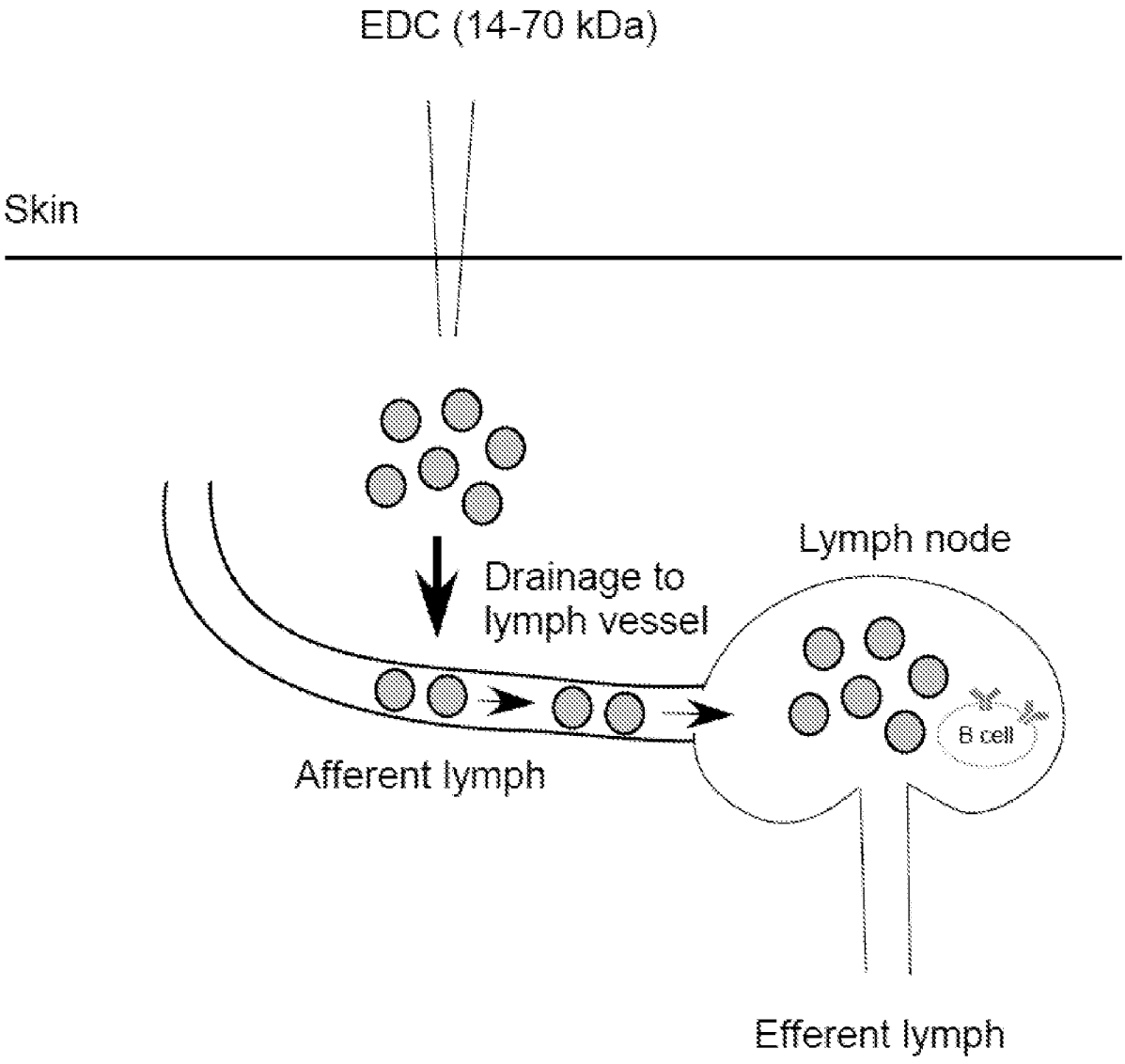
EDC (14-70 kDa)
Skin
Drainage to lymph vessel
Lymph node
B cell
Afferent lymph
Efferent lymph

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FIG. 23

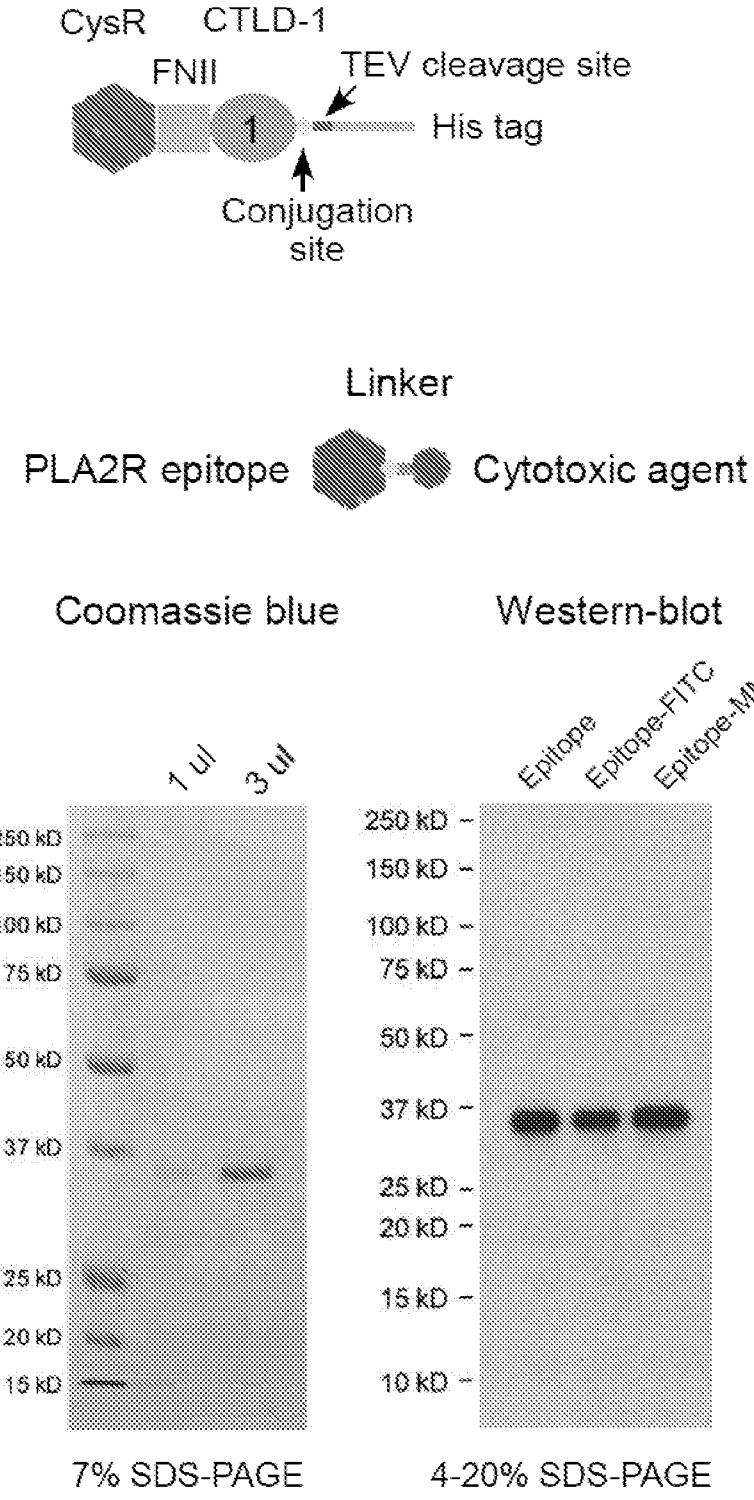
CysR
CTLD-1
FNII
TEV cleavage site
1
His tag
Conjugation site
Linker
PLA2R epitope
Cytotoxic agent
Coomassie blue
Western-blot
1 ul
3 ul
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD
15 kD
Epitope
Epitope-FITC
Epitope-MMAE
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD
15 kD
10 kD
7% SDS-PAGE
4-20% SDS-PAGE

FIG. 24

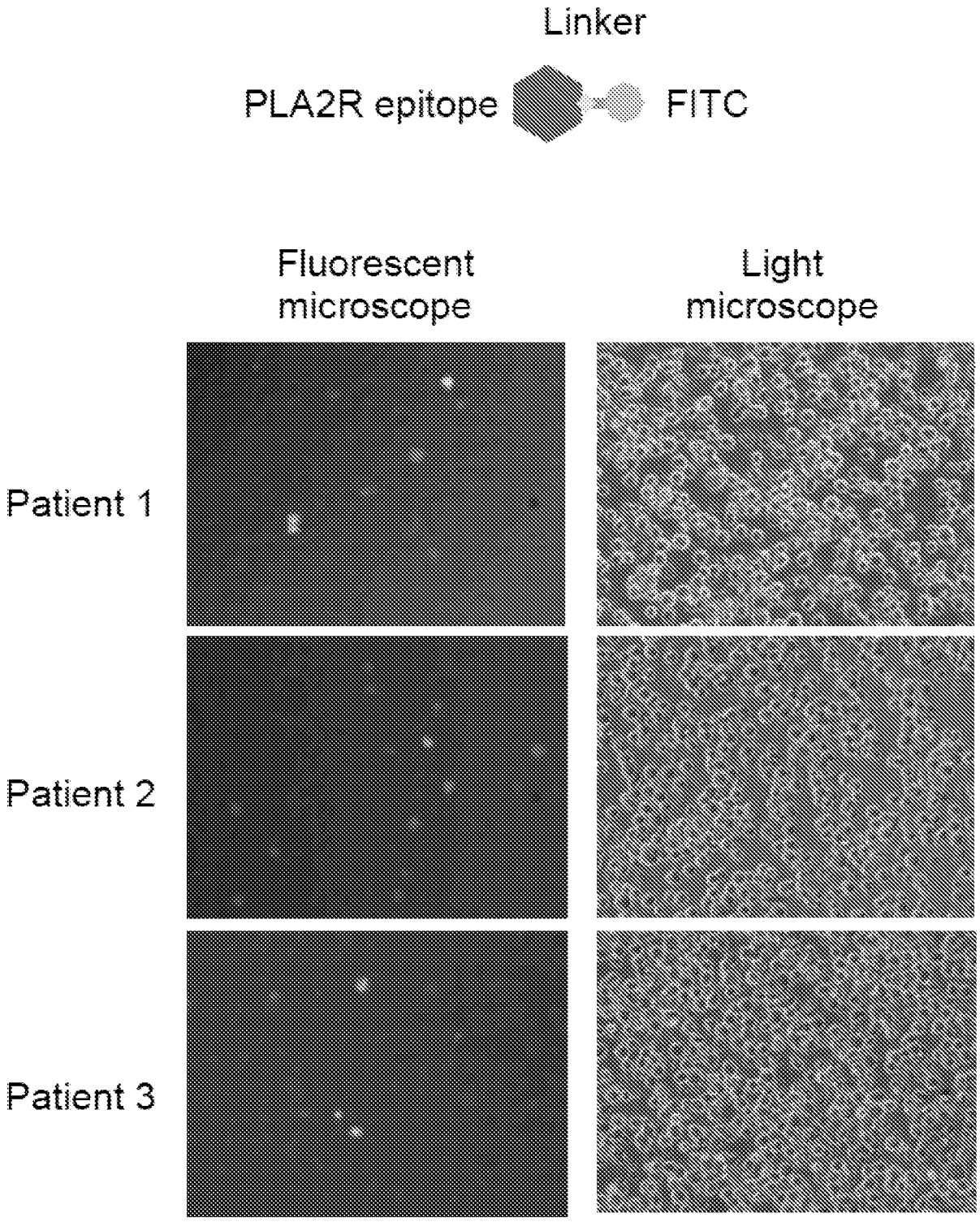
Linker
PLA2R epitope
FITC
Fluorescent microscope
Light microscope
Patient 1
Patient 2
Patient 3

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FIG. 25

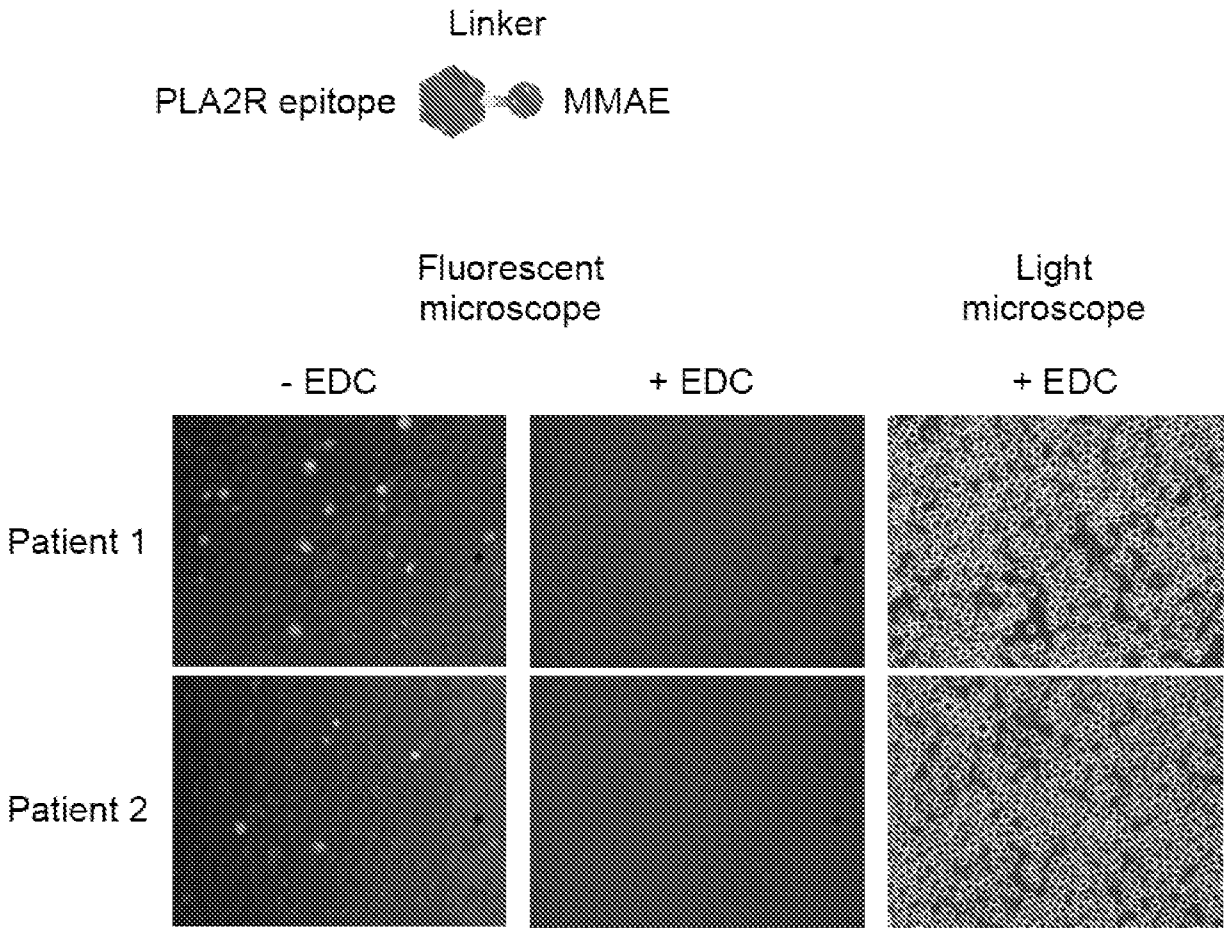
Linker
PLA2R epitope
MMAE
Fluorescent microscope
Light microscope
- EDC
+ EDC
+ EDC
Patient 1
Patient 2

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FIG. 26

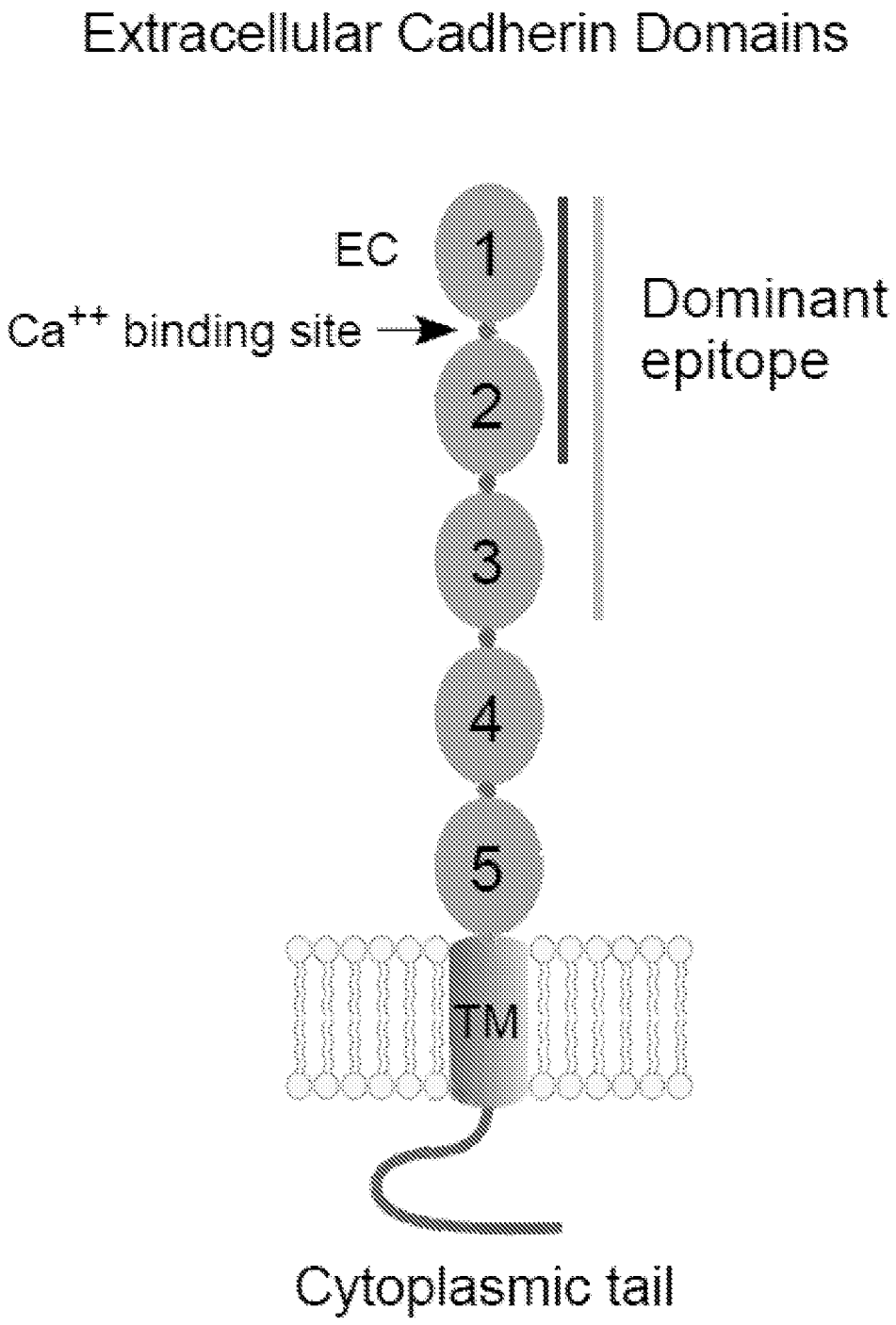
Extracellular Cadherin Domains
EC
1
Ca++ binding site
Dominant epitope
2
3
4
5
TM
Cytoplasmic tail

FIG. 27

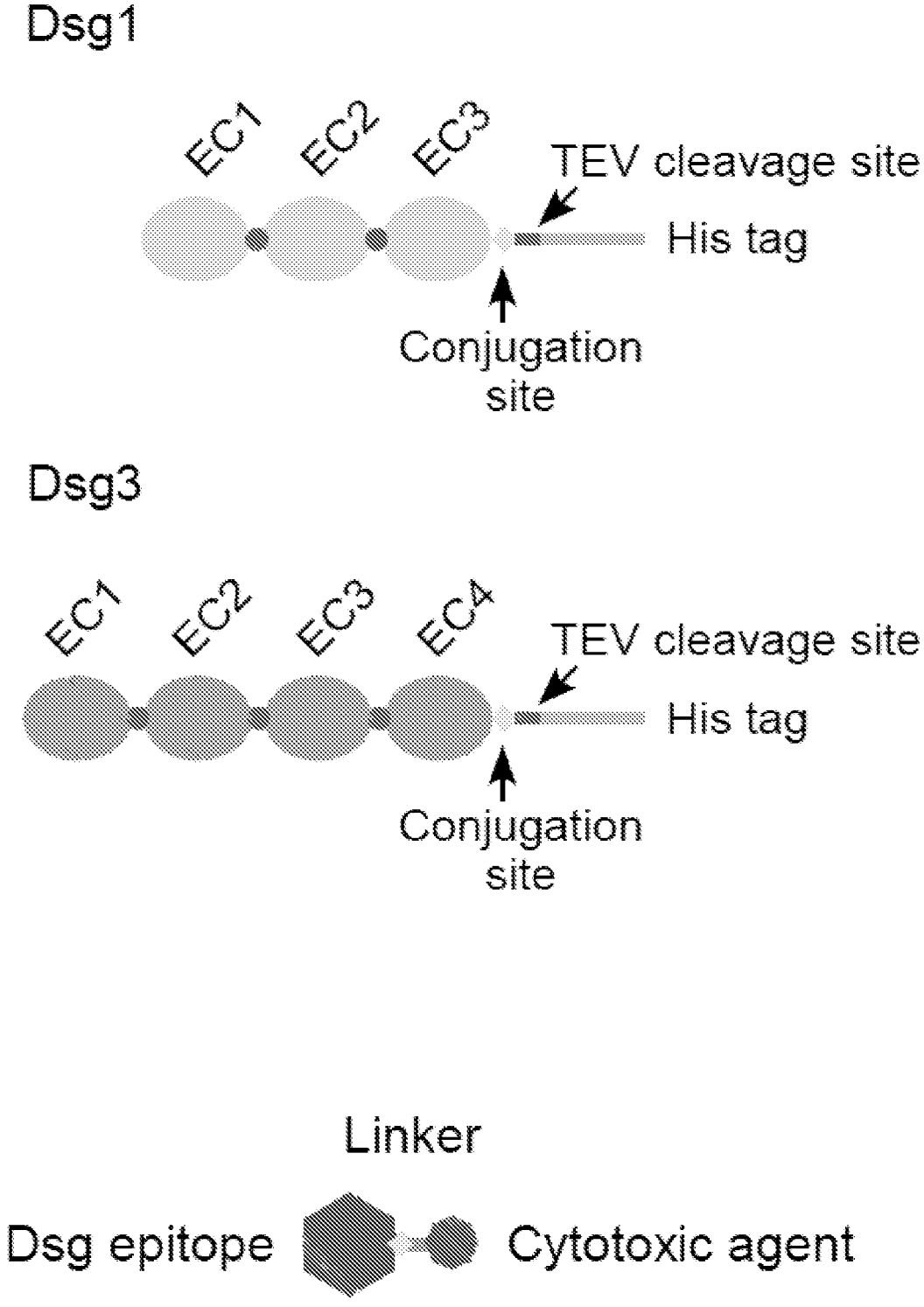
Dsg1
EC1
EC2
EC3
TEV cleavage site
His tag
Conjugation site
Dsg3
EC1
EC2
EC3
EC4
TEV cleavage site
His tag
Conjugation site
Linker
Dsg epitope
Cytotoxic agent

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FIG. 28

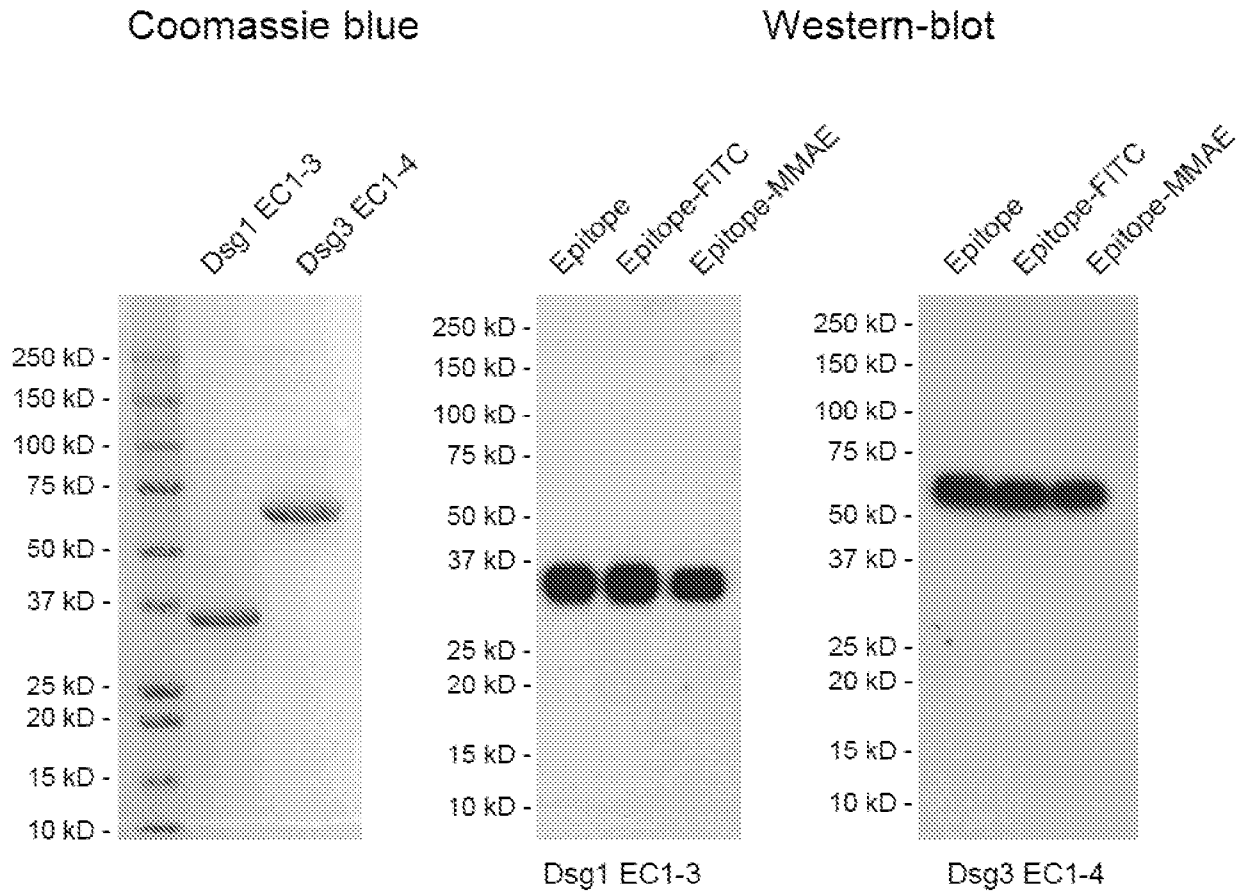
Coomassie blue
Western-blot
Dsg1 EC1-3
Dsg3 EC1-4
Epitope
Epitope-FITC
Epitope-MMAE
250 kD -
150 kD -
100 kD -
75 kD -
50 kD -
37 kD -
25 kD -
20 kD -
15 kD -
10 kD -
Dsg1 EC1-3
Dsg3 EC1-4

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FIG. 29

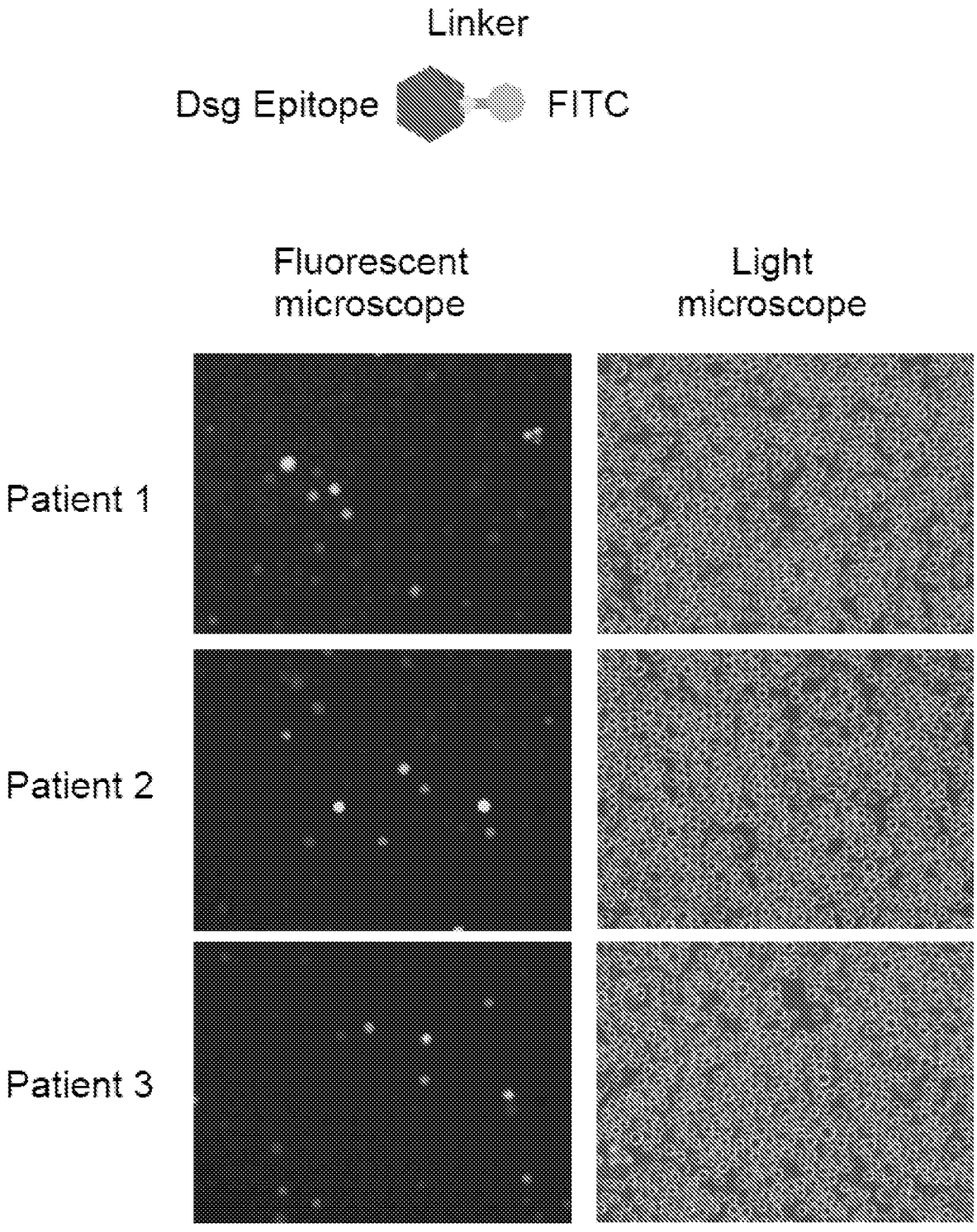
Linker
Dsg Epitope
FITC
Fluorescent microscope
Light microscope
Patient 1
Patient 2
Patient 3

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FIG. 30

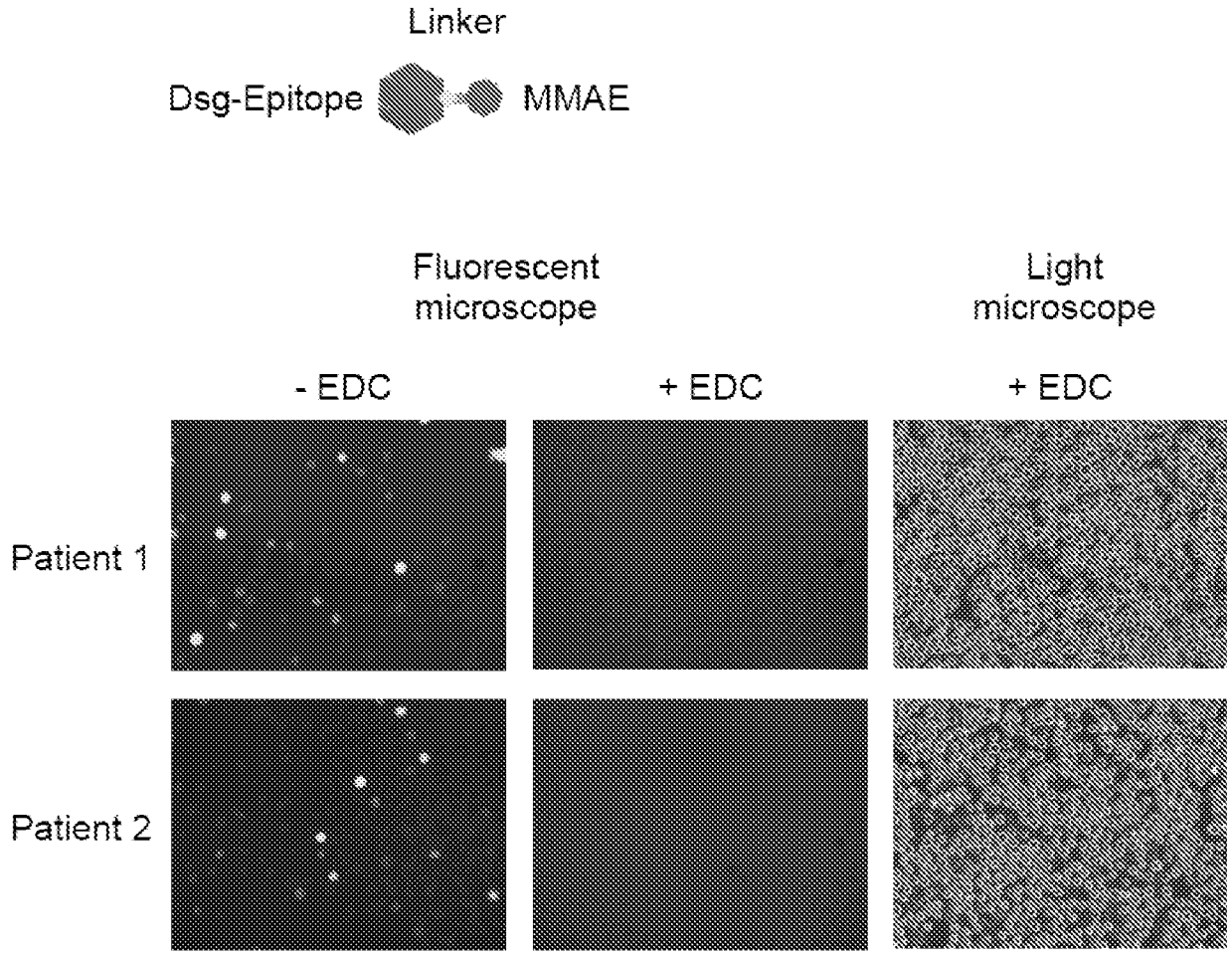
Linker
Dsg-Epitope
MMAE
Fluorescent microscope
Light microscope
- EDC
+ EDC
+ EDC
Patient 1
Patient 2

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FIG. 31

EWIKFAAACREGEDNSKRNPIAKIHSDCAANQQVTYRISGVGIDQPPYGIF
VINQKTGEINITSIVDREVTPFFIIYCRALNSMGQDLERPLELRVRVLDINDN
PPVFSMATFAGQIEENSNANTLVMILNATDADEPNNLNSKIAFKIIRQEPSD
SPMFIINRNTGEIRTMNNFLDREQYGQYALAVRGSDRDGGADGMSAECE
CNIKILDVNDNIPYMEQSSYTIEIQENTLNSNLLEIRVIDLDEEFSANWMAVI
FFISGNEGNWFEIEMNERTNVGILKVVKPLDYEAMQSLQLSIGVRNKAEF
HHSIMSQYKLKASAISVTVLNVIEGPVF **(SEQ ID NO: 16)**

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FIG. 32

EWVKFAKPCREGEDNSKRNPIAKITSDYQATQKITYRISGVGIDQPPFGIF
VVDKNTGDINITAIVDREETPSFLITCRALNAQGLDVEKPLILTVKILDINDNP
PVFSQQIFMGEIEENSASNSLVMILNATDADEPNHLNSKIAFKIVSQEPAG
TPMFLLSRNTGEVRTLTNSLDREQASSYRLVVSGADKDGEGLSTQCECN
IKVKDVNDNFPMFRDSQYSARIEENILSSELLRFQVTDLDEEYTDNWLAVY
FFTSGNEGNWFEIQTDPRTNEGILKVVKALDYEQLQSVKLSIAVKNKAEFH
QSVISRYRVQSTPVTIQVINVREGIAFRPASKTFTVQKGISSKKLVDYILGT
YQAIDEDTNKAASNVKYVMGRNDGGYLMIDSKTAEIKFVKNMNRDSTFIV
NKTITAEVLAIDEYTGKTSTGTVYVRVPDFNDNCPTAVLEK **(SEQ ID NO: 17)**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/46626

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/68, G01N 33/68, G01N 33/53, G01N 33/53 (2017.01)
CPC - G01N 33/564, G01N 33/53, G01N 2333/918

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0177534 A1 (SALANT et al.) 21 July 2011 (21.07.2011) abstract; para [0005]; [0013]; [0017]; [0061]-[0070]; [0105]-[0114]; [0176]; [0202]; [0208]; [0217]; SEQ ID NO: 1.	1-9, 22-28
A	WO 2015/185949 A1 (THE UNIVERSITY OF MANCHESTER) 10 December 2015 (10.12.2015) abstract; claims 1-5; SEQ ID NOs: 1-6, 13, 16, 17.	1, 22

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

* Special categories of cited documents:
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"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
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"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
20 November 2017	11 DEC 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/46626

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/46626

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

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

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

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

3. ☒ Claims Nos.: 10, 11, 21, 29-31, 38-41
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-9 and 22-28, directed to a method of treating a patient with membranous nephropathy with a PLA2R epitope drug conjugate.

Group II, claims 12-20, directed to a complex comprising a PLA2R epitope and a drug.

Group III, claims 32-37 and 42-47, directed to a epitope drug complex (EDC) comprising a drug and a desmoglein 1 epitope or desmoglein 3 epitope, and a method of use thereof to treat Pemphigus vulgaris (PV).

--continued on first extra sheet--

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 22-28

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/46626

--continued from Box III: Observations where unity of invention is lacking--

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

Special technical features:

Group I has the special technical feature of treaing membranous nephropathy (MN) by administering a a PLA2R epitope drug complex, that is not required by Groups II or III.

Group II has the special technical feature of a complex comprising a PLA2R epitope and a drug, that is not required by Groups I or III.

Group III has the special technical feature of treating PV with a drug and a desmoglein 1 epitope or desmoglein 3 epitope, that is not required by Groups I or II.

Common technical features:

Groups I and III share the common technical feature of a method of treating a patient with an autoimmune disorder, having autoimmune B cells or T cells, the method comprising: identifying a patient with said disorder; and administering to the patient a complex comprising a peptide epitope and a drug, wherein the epitope is comprised within a peptide fragment, thereby eliminating or reducing an autoantibody producing B cell population in the patient.

Groups I and II further share the common technical feature of a complex comprising a PLA2R epitope and a drug, wherein the epitope is comprised within a PLA2R fragment.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are made obvious by reference US 2011/0177534 A1 to Salant et al., (hereinafter Salant).

Salant teaches a method of treating a patient with membranous nephropathy (para [0013] "provided herein is a method of treatment of membranous nephropathy in a subject"), the method comprising:
identifying a patient with MN (para [0005] "a method of diagnosing MN in a subject"); and
administering to the patient a PLA2R epitope and a drug (para [0013] "provided herein is a method of treatment of membranous nephropathy in a subject, the method comprising administering an effective amount of PLA2R or fragments thereof or a vector expressing a PLA2R or fragments thereof"), wherein the epitope is comprised within a PLA2R fragment (para [0013] "amount of PLA2R or fragments thereof"; [0017] "the fragments suitable for treatment or adsorption are fragments comprising the CTLDs or CRDs 4, 5 6 of PLA2R"), thereby eliminating or reducing an anti-PLA2R autoantibody producing B cell population in the patient (abstract "Therapeutic methods include removal of the auto-antibodies by absorbance or administration of soluble PLA2R or fragments to sequester the auto-antibodies"; note: anti-PLAR2 auto-antibodies are produced by B cells).
Salant does not specifically teach the PLA2R epitope and drug are administered as a complex comprising a PLA2R epitope and a drug. However, Salant does teach "the composition comprising a PLA2R or fragments thereof is administered in combination with immunosuppressive therapies including, but not limited to, azathioprine, infliximab, omalizumab, daclizumab, adalimumab, eculizumab, efalizumab, natalizumab, and omalizumab" (para [0208]) and when the PLA2R epitope is delivered in an expression vector, "simultaneous delivery of a second gene using the recombinant AAV virions, wherein the second gene is capable of providing an ancillary therapeutic effect when expressed within the transduced cell" (para [0217]). Since Salant further teaches "a cocktail of several peptides is used for treatment. Envisioned peptides can be fused with other proteins for longer serum half-life, tandemly linked peptides or circular peptides", it would have been obvious to one of ordinary skill in the art to have fused a known immunosuppressive peptide with the taught PLA2R peptide fragments, during the ordinary course of experimentation, in order to enhance peptide stability while additionally enhancing therapeutic effect with the additional immunosuppressive peptide.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I, II and III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 10, 11, 21, 29-31 and 38-41 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

专利名称(译)	自身免疫疾病的诊断，预防和/或治疗		
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优先权	62/374382 2016-08-12 US		
其他公开文献	EP3497452A4		
外部链接	Espacenet		

摘要(译)

组合物，方法和试剂盒用于通过检测，靶向和/或消除表位特异性自身免疫细胞来诊断，预防和/或治疗自身免疫性疾病。该组合物包含表位和允许检测，靶向和/或消除表位特异性自身免疫细胞的试剂的缀合物。