

TOLERIZING IMMUNOMODULATORY COMBINATION

TECHNICAL FIELD

[0001] The disclosure relates to immunomodulatory agent-lipid conjugates in combination with nucleic acids encoding epitopes targeted by autoreactive T cells.

BACKGROUND

[0002] There are no cures for autoimmune diseases, such as type 1 diabetes (T1D), Multiple Sclerosis (MS), Hashimoto's disease, celiac disease, and many others.

[0003] T1D is a devastating disease. T1D results from autoimmune attack of the insulin-producing beta cells within pancreatic islets, by T cells which recognize specific proteins present in beta cells and islets; this results in loss of beta-cells, near-absolute insulin deficiency, and chronically elevated blood sugar levels. People with T1D require multiple daily injections of insulin (or other sources of exogenous insulin) to live, the discovery of which converted T1D from an acutely fatal disease, to one that is chronic and manageable. Importantly however, insulin is not a cure. T1D patients on insulin still suffer devastating consequences from chronically elevated blood sugar levels; T1D is the leading cause of limb amputation, blindness, kidney failure, cardiovascular disease, and decreases life span by 15 years. In addition, every time a patient injects insulin, they are at risk of overdosing, sending blood sugar levels into the hypoglycemic range, a potentially fatal medical emergency. The difficulty in properly regulating blood sugar levels with insulin injections arises because exogenous insulin cannot mimic the fine-tuned, glucose-dependent insulin secretion achieved by beta-cells; thus, a cure to diabetes lies in a method to protect the patient's own beta-cells from their autoreactive T cells, thereby preventing the underlying cause of the disease. Such a method would involve re-training the immune system in a way to stop inappropriate attack of self-peptides (autoantigens) and promote so-called immune tolerance, whereby the immune system ignores autoantigens.

[0004] Inducing antigen specific immune tolerance (ASIT) involves changing the function of only the T cells that react to a particular antigen (e.g. islet autoantigen reactive T cells in T1D) while not changing the function of other T cells. The physical interaction through MHC:peptide complexes on antigen presenting cells (APCs) and their cognate T cell receptor (TCR) on T cells

underpins this, allowing APCs to instruct the corresponding T cell response to a particular antigen. APCs presenting antigen only, or antigen along with regulatory signals induce a tolerogenic T cell response, whereas presentation of antigen and costimulatory molecules induces an effector T cell response. ASIT therapies aim to induce or mimic the former to dampen antigen-specific effector T cells and/or enhance antigen-specific suppressive T cells (e.g. Tregs), and typically involve administration of protein/peptide autoantigen which is then taken up by APCs and presented to autoreactive T cells. Antigen administration has not succeeded in clinical trials.

[0005] The major hurdles to achieving ASIT in T1D are: (1) multiple autoantigens involved in disease, limiting efficacy of a single antigen approach; (2) HLA restriction, limiting the utility of any given epitope to a subset of people with T1D; (3) lack of durable or robust immune tolerance induction; (4) limited scalability and feasibility, for example, complex administration requirements or bespoke cell therapies that are costly and difficult to manufacture. These challenges are evident in the numerous failed ASIT trials in T1D.

[0006] ASIT is likewise unavailable for other autoimmune diseases, such as MS, Hashimoto's disease, celiac disease, and others.

[0007] There is therefore a need in the art for compositions that tolerize a subject's T cell response to antigens involved in autoimmune diseases or conditions.

SUMMARY

[0008] One potential reason for failure of previous ASIT products in T1D and other autoimmune diseases is that the tolerance signal provided by antigen alone is too weak or insufficient. The choice of antigen is also very important as the disease involves multiple autoantigens. Lipid nanoparticles (LNPs) have revolutionized vaccines by enabling delivery of mRNA-encoded protein antigens to immune cells. Using mRNA to encode protein antigens enables use of antigens in the form of full-length proteins (encompassing the full breadth of possible epitopes of an antigen) and multiple protein antigens, as demonstrated in multi-valent mRNA vaccines. The major challenge for LNPs in immune tolerance, is the very property that makes them excellent vaccines: their natural immune stimulatory property acts as an adjuvant. Indeed, even with modified mRNA bases that diminish the inflammatory response to mRNA itself, injection of mRNA-carrying LNPs induces an acute inflammatory response largely due to the ionizable lipid component. To enable ASIT in T1D and other autoimmune diseases, a stronger tolerogenic signal

than antigen-only is necessary, and to harness the power of LNPs to deliver multiple antigens in mRNA form, the immune stimulatory nature of LNPs must be mitigated.

[0009] This disclosure provides a multi-cargo delivery system (e.g. LNPs), encapsulating a combination of immunomodulatory small molecule prodrugs along with nucleic acid(s) (e.g. mRNA)-encoded epitope(s) targeted by autoreactive T cells in an autoimmune disease or condition, e.g. T1D. In more detail, the immunomodulatory combination comprises: a first lipid conjugate comprising a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, wherein the first immunomodulatory agent is a corticosteroid; a second lipid conjugate comprising a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, wherein the second immunomodulatory agent is an mTOR/mTORC inhibitor; the nucleic acid(s) encodes one or more epitopes, wherein each epitope is an epitope of an autoantigen and/or an antigen that triggers recognition of an autoantigen by autoreactive T cells in an autoimmune disease. The first lipid conjugate, the second lipid conjugate, and the nucleic acid(s) are co-formulated together in a delivery vehicle, or are formulated in two or more separate delivery vehicles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1A shows gated plots (gated on live cells) from pancreatic lymph nodes of mice assessed for APC markers, CD11b and CD11c, by flow cytometry 48 hours post-administration of DiO (3,3'-Diiodo-4,4'-dimethoxy-5,5'-diphenylmethane Perchlorate) labelled lipid nanoparticles (LNPs, DSPC/Chol/DSPE-PEG at 54/45/1 mol:mol). Mice received 2 injections, 24 hours apart, of LNP injected at a dose of 600 mg/kg i.p.

[0011] FIG. 1B shows counts of the DiO positive APCs (gated on CD11b+CD11c+ cells) in the pancreatic lymph nodes of the mice injected with the DiO-labelled LNPs.

[0012] FIG. 1C shows gated plots (gated on live cells) from pancreatic islets of the mice assessed for APC markers, CD11b and CD11c, by flow cytometry 48 hours post-administration of DiO-labelled lipid nanoparticles (DSPC/Chol/DSPE-PEG at 54/45/1 mol:mol).

[0013] FIG. 1D shows counts of the DiO positive APCs (gated on CD11b+CD11c+ cells) in the pancreatic islets of the mice injected with the DiO-labelled LNPs.

[0014] FIG. 2A shows the DiO positive cell counts in islet macrophages (CD11b+CD11c+) from mice injected with PBS control, DSPC/Chol/DSPE-PEG LNP (DSPC/Chol/DSPE-PEG at 54/45/1 mol:mol) or ionizable LNP (A002/DSPC/Chol/PEG-DMG at 50/10/38.5/1.5 mol:mol). Mice were injected with 150 mg/kg of the LNP formulations loaded with DiO, and 24 hours later, islets, pancreatic lymph nodes and splenocytes were isolated.

[0015] FIG. 2B is a bar graph that depicts the percentage of DiO positive APCs (CD11b+CD11c+ cells) in islets, pancreatic lymph nodes and spleen, from the DSPC/Chol LNP (horizontal hatches) and the ionizable LNP- (diagonal hatches) injected mice.

[0016] FIG. 3 is a bar graph that shows the percentage of lipid conjugate (prodrug) remaining in LNPs (DSPC/Chol/Prodrug/DSPE-PEG at 49/40/10/1 mol:mol) co-formulated with dexamethasone (D045) and calcitriol (D053, D068, D083) lipid conjugates (prodrugs) after 2 hours incubation in human plasma (hPlasma).

[0017] FIG. 4 is a bar graph showing mean uninflamed islets/section for various lipid conjugate prodrugs in combination with diabetes-relevant mRNA-encoded antigen.

[0018] FIG. 5 shows histograms of mode-normalized cell counts vs fluorescence of indicated marker, indicating attenuation of LPS-induced maturation of BMDCs.

[0019] FIG. 6 shows histograms of mode-normalized cell counts vs fluorescence of indicated marker, indicating attenuation of cell surface activation markers in human monocyte-derived DC's.

[0020] FIG. 7 shows two bar graphs of diabetes-relevant antigen increase of tolerogenic T cell markers *in vivo*.

[0021] FIG. 8 shows three bar graphs of cytokine/chemokine response in NOD mice measured over time in response to treatment with LNPs carrying immunomodulator prodrugs and mRNA.

[0022] FIG. 9 is a graph showing protection from diabetes in NOD mice treated with LNPs and mRNA encoding diabetes-relevant antigens. Mice were treated with 6 injections.

[0023] FIG. 10 is a graph showing protection from diabetes in NOD mice at an advanced stage of disease treated with LNPs and mRNA encoding diabetes-relevant antigens. Mice were treated with 3 injections using various routes of administration.

[0024] FIG. 11 is a graph showing protection from diabetes in NOD mice treated at the onset of disease with LNPs and mRNA encoding diabetes-relevant antigens.

[0025] FIG. 12 is a graph showing protection from diabetes in NOD mice at treated with LNPs and mRNA encoding diabetes-relevant antigens, comparing different configurations of antigens.

[0026] FIG. 13 is a graph showing protection from experimental autoimmune encephalitis in MOG35-55 induced mice (model for multiple sclerosis) treated with LNPs and MOG mRNA.

[0027] FIG. 14 is a graph showing protection from experimental autoimmune thyroiditis induced in SJL/J mice (model Hashimoto's thyroiditis) treated with LNPs and a multi-epitope Tg mRNA construct. Panel A shows thyroiditis score for mice receiving LNPs carrying thyroglobulin (Tg) mRNA and D034/D097 compared to buffer-treated controls and compared to mice treated with either Tg mRNA only LNPs or irrelevant antigen (Ova) +D034/D097 LNPs. Panel B shows T regulatory cells (CD25+Foxp3+) as a % of CD4 T cells in the spleen.

DETAILED DESCRIPTION

[0028] This disclosure relates to immunomodulatory combinations (alternatively referred to as vaccine formulations), uses thereof and methods thereof. The immunomodulatory combination comprises (i) a first lipid conjugate comprising a first immunomodulatory agent (a corticosteroid) covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, (ii) a second lipid conjugate comprising a second immunomodulatory agent (e.g. an mTOR/mTORC inhibitor) covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, and (iii) one or more nucleic acids encoding one or more epitopes (in some embodiments, a plurality of epitopes, e.g. a full length antigen(s) or synthetic series of epitopes) targeted by autoreactive T cells (e.g. in T1D or other autoimmune diseases). The first lipid conjugate, the second lipid conjugate, and the one or more nucleic acids are co-formulated together in a delivery vehicle, or are formulated in two or three separate delivery vehicles. The one or more epitopes comprise one or more class II epitopes. In some embodiments, the one or more epitopes comprises epitopes from a plurality of autoantigens and/or antigens that trigger recognition of autoantigens by autoreactive T cells in an autoimmune disease or condition. For autoimmune diseases/conditions triggered by self-antigens (e.g. T1D), the epitopes are from autoantigens. Certain autoimmune diseases are triggered by non-self (foreign) antigens (e.g. celiac

disease, IBD), and in such embodiments, the epitopes trigger autoreactive T cells because they resemble epitopes found in autoantigens; in such embodiments, epitopes may be selected from foreign antigens, self-antigens, or a combination of foreign and self-antigens. In some embodiments, the one or more epitopes comprises a full length autoantigen and/or a full length antigen that triggers autoreactive T cells in an autoimmune disease or condition. Methods/uses of the immunomodulatory combinations are also disclosed.

[0029] In other aspects, this disclosure relates to mRNA constructs comprising: (i) 5'UTR sequence; (ii) a sequence encoding a signal peptide; (iii) a multi-epitope coding sequence encoding a multi-epitope polypeptide, the multi-epitope polypeptide comprising three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, wherein epitopes in the multi-epitope polypeptide are separated from each other by cleavable linkers, and wherein the epitopes comprise epitopes from a plurality of autoantigens; (iv) 3'UTR sequence; and optionally, a polypeptide comprising an endolysosomal trafficking sequence motif positioned 5' to the 3'UTR sequence.

[0030] Definitions

[0031] "Vaccine formulation" refers to any pharmaceutical formulation that comprises one or more of the same or different delivery vehicles as described herein to treat, prevent and/or ameliorate an antigen-induced disorder in a subject. The term includes formulations prepared in any suitable pharmaceutically acceptable salt and/or excipient.

[0032] "Immunomodulatory agent" refers to an agent that can alter an immune response in a subject. In one non-limiting embodiment, the immunomodulator is an immunostimulant that enhances an immune response in a subject. In another non-limiting embodiment, the immunomodulator is an immunosuppressant that prevents or reduces an immune response in a subject. Immunomodulators can regulate myeloid cells (monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) or lymphoid cells (T cells, B cells and Natural Killer (NK) cells) and any further differentiated cells thereof.

[0033] “Antigen” has the usual meaning in the art, and refers to a molecule or molecular complex that can bind to a specific antibody or T cell receptor to modify the immune system. In some embodiments, the antigen is a foreign or non-foreign protein, polypeptide, peptide, lipoprotein, glycolipid, polynucleotide, or polysaccharide.

[0034] “Delivery vehicle” refers to any suitable particle in which an immunomodulatory agent-lipid conjugate (e.g. a prodrug) can be formulated. Non-limiting examples include lipid nanoparticles, liposomes, and the like.

[0035] “Lipophilic moiety” with reference to a moiety linked to an immunomodulatory agent as part of lipid conjugate (e.g. a prodrug) or a lipophilic moiety of an ionizable or permanently charged lipid includes, without limitation, a lipid or other lipophilic group that imparts sufficient hydrophobicity to the immunomodulatory agent, prodrug or lipid to facilitate formulation thereof into a suitable delivery vehicle.

[0036] “Scaffold moiety” refers to a hydrocarbon chain of a lipophilic moiety of a lipid conjugate, prodrug or an ionizable or permanently charged lipid upon which one or more hydrocarbon chains are linked via one or more respective biodegradable groups.

[0037] “Prodrug”, “lipid prodrug”, “immunomodulatory agent-lipid conjugate”, “lipid conjugate”, “drug-lipid conjugate” or “prodrug conjugate” as used herein refers to the immunomodulatory agent linked to the lipophilic moiety via any suitable linkage or linker, including covalent and non-covalent bonds. In some embodiments, the linkage or linker is covalently attached. The immunomodulatory agent may be activated upon release from the lipophilic moiety.

[0038] The terms “treat”, “treating”, “treatment”, and the like refer to managing, alleviating, or curing a disease or medical condition. This includes not only improving existing symptoms of the disease or condition, but also averting or delaying the onset or progression of a disease or condition. In some embodiments, the compositions in this disclosure avert or delay the onset or progression of a disease or condition. In some embodiments, the compositions in this disclosure manage, alleviate, or cure a disease or medical condition. In some embodiments, the compositions of this disclosure improve existing symptoms of the disease or condition.

[0039] Lipid conjugates

[0040] The first lipid conjugate and the second lipid conjugate each comprises an immunomodulatory agent that is linked to a lipophilic moiety.

[0041] Immunomodulatory agents exert their immunomodulatory effects in a subject by targeting various molecules of upstream and downstream immune pathways. Upstream are targets directly modified by the agents, and downstream are the key pathways through which immunomodulatory agents inhibit inflammation. Many of these agents converge/overlap on the same downstream pathways. Upstream targets include, without limitation: Glucocorticoid receptor, mammalian target of rapamycin (mTOR), COX1/COX2 (by direct acetylation as by ASA or inhibition of expression as by salicylate), Vitamin D receptor, JAK1, JAK2, JAK3, TYK2, and Calcineurin. Downstream targets include, without limitation: inhibition of NF-kappaB complex expression/activity, inhibition of AP-1 expression/activity, inhibition of p38 MAP Kinase pathway, and inhibition of NFAT family.

[0042] The first lipid conjugate comprises a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker. The first immunomodulatory agent is a corticosteroid. In some embodiments, the corticosteroid is alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, fluclorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortol, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol. In some embodiments, the corticosteroid is dexamethasone or a derivative thereof, optionally dexamethasone.

[0043] The second lipid conjugate comprises a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker. The second immunomodulatory agent is an mTOR/mTORC inhibitor. In some embodiments, the mTOR/mTORC inhibitor is sirolimus (also called rapamycin), everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-

0812, Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1, Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxtalisib (XL765), VS-5585 (SB2343), W922, WAY-600, WYE-125132 (WYE-132), WYE-354, WYE-687, or derivatives or metabolized forms of the foregoing (e.g. seco-sirolimus, seco-temsirolimus, etc.). In some embodiments, the mTOR/mTORC inhibitor is sirolimus or a derivative thereof, optionally sirolimus.

[0044] The lipophilic moiety of the lipid conjugate imparts sufficient hydrophobicity to the immunomodulatory agent to facilitate formulation thereof in a suitable delivery vehicle. Examples of suitable lipid moieties include those described in WO 2020/191477, which is incorporated herein by reference.

[0045] The lipid conjugates may be formulated in the same or separate delivery vehicles, together or separate from the one or more nucleic acids encoding the plurality of epitopes. In some embodiments, the two or more immunomodulatory agents may be formulated so that the two (or more) agents are stably retained within the same delivery vehicle at molar ratios that are additive or synergistic. In some embodiments, the immunomodulatory combination comprises a plurality of lipid conjugates, wherein the plurality of lipid conjugates comprise 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 immunomodulatory agents, wherein each immunomodulatory agent of the plurality of lipid conjugates is different, and wherein each lipid conjugate of the plurality of lipid conjugates is independently formulated in a separate delivery vehicle from the other components of the immunomodulatory combination or is co-formulated with one or more of the other components of the immunomodulatory combination. Additive or synergistic effects between the two or more immunomodulatory agents may be determined by any suitable technique, including the Chou Talalay method known to those of skill in the art. In some embodiments, the two lipid conjugates are co-formulated in the same delivery vehicle. The lipid conjugates herein are particularly

amenable to formulation in delivery vehicles at high encapsulation efficiencies, such as up to 90% encapsulation efficiency or more. In some embodiments, the immunomodulatory combination further comprises a third lipid conjugate comprising a third immunomodulatory agent. In some embodiments, the third immunomodulatory agent targets a different immune pathway than the immunomodulatory agent of the first and/or second lipid conjugate. In yet further embodiments, the immunomodulatory combination further comprises four or more lipid conjugates. In some embodiments, the third and/or fourth immunomodulatory agent target COX1/COX2 (by direct acetylation as by ASA or inhibition of expression as by salicylate), Vitamin D receptor, JAK1, JAK2, JAK3, TYK2, calcineurin, inhibition of NF-kappaB complex expression/activity, inhibition of AP-1 expression/activity, inhibition of p38 MAP Kinase pathway, and/or inhibition of NFAT family.

[0046] The immunomodulatory agent is covalently linked with lipid moieties such as fatty acids, glycerides, phospholipids or other hydrophobic moieties, including those produced by organic synthesis. Linkage of the lipophilic moiety to the immunomodulatory agent typically increases the hydrophobicity of the immunomodulatory agent.

[0047] The LogP of the lipid conjugate may be sufficient to impart a desired degree of hydrophobicity to the immunomodulatory agent. In one embodiment, the predicted cLogP of the lipid conjugate is between 5-20, 6-18, 9-18, or 9-17, optionally 9-17. In alternative embodiments, each lipid conjugate independently has a cLogP value of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0048] In some embodiments, the lipophilic moiety is linked to the immunomodulatory agent by a cleavable linkage or through a cleavable linker. The immunomodulatory agent may be bioactive when linked to the lipophilic moiety or bioactive upon cleavage therefrom after administration to a subject. In this regard, the lipid conjugate may comprise a biodegradable linker/linkage that is cleavable upon administration of the lipid conjugate to a subject.

[0049] The biodegradable groups may be independently selected from linkages comprising one or more functional groups selected from an ester, amide, amidine, hydrazone, disulfide, ether, carbonate, carbamate, thionocarbamate, guanidine, guanine, oxime, isourea, acylsulfonamide, phosphoramidate, phosphonamide, phosphoramidate, phosphate, phosphonate, phosphodiester, phosphate phosphonooxymethylether, N-Mannich adduct, N-acyloxyalkylamine, sulfonamide,

imine, azo, carbon-based functional groups including an alkane, alkene or alkyne, methylene (CH₂) or urea.

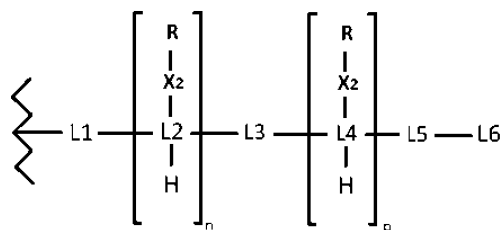
[0050] In one embodiment, the lipophilic moiety may be derived from a precursor fatty acid or other lipophilic molecule having, for example, 5 to 30 carbon atoms, 14 to 20 carbon atoms or 16 to 18 carbon atoms, optionally 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms.

[0051] In some embodiments, the lipophilic moiety is a linear or branched lipophilic chain with up to 3, 4, 5 or 6 biodegradable groups. In one embodiment, at least one of the biodegradable groups is selected from at least one of an ester, amide, amidine, hydrazone, disulfide, ether, carbonate, carbamate, thionocarbamate and combinations thereof. In one embodiment, the biodegradable group is an ester that is cleavable by an esterase *in vivo*.

[0052] In some embodiments, the lipid conjugate has the formula M-X1-L wherein M is the immunomodulatory agent, X1 is a linker or linkage, and L is the lipophilic moiety.

[0053] In some embodiments, the lipophilic moiety has the structure of **Formula I**:

Formula I:



[0054] L is represented by L1 + L2 + L3 + L4 + L5 + L6 (i.e. L1-L2_n-L3-L4_p-L5-L6) and wherein L comprises or consists of 2-100, 2-75, 2-80, 3-60, 4-50, 5-45, 5-40, 6-60, 7-60, 8-60, 9-60, 10-60, 11-60, 12-60, 13-60, 14-60, or 15-60 carbon atoms and 0, 1, 2, 3, 4, 5, or 6 cis or trans C=C double bonds. In alternative embodiments, L consists of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67,

68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 carbon atoms and 0, 1, 2, 3, 4, 5, or 6 cis or trans C=C double bonds.

[0055] L1 is a carbon chain having 0-40, 1-40, 1-35, 2-30, 3-30, 4-30, 5-30, 6-30, 7-30, or 8-30 (alternatively 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40) carbon atoms and 0-5, 1-5, 2-5, 3-5, or more than 5 cis or trans C=C double bonds (alternatively, 0, 1, 2, 3, 4, or 5 C=C double bonds).

[0056] L2 and L4 are each a carbon atom.

[0057] L3 is 0-20, 1-20, 3-20, 4-20, or 5-20 (alternatively 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) carbon atoms and has 0, 1, or 2 cis or trans C=C double bonds;

[0058] L5 is 0-20, 1-20, 3-20, 4-20, or 5-20 (alternatively 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) carbon atoms and comprises 0, 1, or 2 cis or trans C=C double bonds;

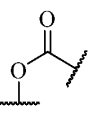
[0059] L6 is -CH₃, =CH₂ or H;

[0060] Each R is independently a linear or branched hydrocarbon chain having 0-40, 1-40, 2-40, 3-30, 4-30, 5-30, 6-30, 7-30, 8-30, 9-30, or 10-30 (alternatively 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40) carbon atoms and 0, 1, 2, or 3 cis or trans C=C double bonds, optionally 1-2 cis or trans C=C double bonds, wherein if one or more of R is branched, each branch point optionally includes an X2 functional group or is a carbon atom. In some embodiments, each R is 3-30 carbon atoms and 0, 1, 2, or 3 cis or trans C=C double bonds, optionally 1-2 cis or trans C=C double bonds, wherein if one or more of R is branched, each branch point optionally includes an X2 functional group or is a carbon atom.

[0061] n is 0-8 or 1-8. In alternative embodiments, n is 0, 1, 2, 4, 5, 6, 7, or 8. In some embodiments, n is 1. p is 0-8 or 1-8. In alternative embodiments, p is 0, 1, 2, 4, 5, 6, 7, or 8. In some embodiments, p is 1. In some embodiments, n + p is ≥ 1 , optionally 1-8, 2-6, or 2-4. In alternative embodiments, n+p is 1, 2, 4, 5, 6, 7, or 8.

[0062] Each X2, if present, is independently an ester, amide, amidine, hydrazone, ether, carbonate, carbamate, thionocarbamate, guanidine, guanine, oxime, isourea, acylsulfonamide, phosphoramidate, phosphonamide, phosphoramidate, phosphate, phosphonate, phosphodiester,

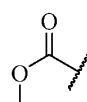
phosphate phosphonooxymethylether, N-Mannich adduct, N-acyloxyalkylamine, sulfonamide, imine, azo, alkane, alkene, or alkyne, methylene (CH₂), or urea. In some embodiments, each X₂ is

ester (optionally ).

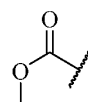
[0063] In some embodiments, the lipid conjugate comprises a scaffold moiety. The scaffold moiety in one embodiment is represented by L of Formula I above and at least one R is present as a hydrocarbon side chain, wherein n + p is 1 or 1 to 8 or 1 to 7, or 1 to 6 or 1 to 5 or 1 to 4 or 1 to 3.

[0064] In some embodiments, the lipid conjugate comprises one lipophilic moiety, optionally having the structure of Formula I. In some embodiments, the lipid conjugate comprises two lipophilic moieties, each lipophilic moiety independently having the structure of Formula I, which may be the same or different, and each lipophilic moiety linked to the immunomodulatory agent through a separate linkage or linker, or linked to the same linkage or linker. In certain embodiments, each linkage is an ester. In some embodiments, the lipid conjugate comprises more than two lipophilic moieties.

[0065] In some embodiments, each lipophilic moiety of Formula I is independently defined by: L₁ is a carbon chain having 3 to 30 carbon atoms, and 0 to 3 cis or trans C=C double bonds, n is 0,

L₃ is absent, p is 1, X₂ is carbonate or ester (optionally ), R is a linear or branched carbon chain having 1-20, 2-20, or 3-20 (optionally 2-20) carbon atoms and 0 to 3 cis or trans C=C double bonds, L₅ is a carbon chain having 1 to 10 carbon atoms and comprises 0 to 1 cis or trans C=C double bond, and L₆ is -CH₃; or L is a carbon chain having 5 to 20 carbon atoms and zero C=C double bonds.

[0066] In some embodiments, each lipophilic moiety of Formula I is independently defined by: L₁ is a carbon chain having 5 to 20 carbon atoms (optionally 8 to 15 carbon atoms), and 1 or 2 cis or trans C=C double bonds (optionally 1 cis or trans C=C double bond), optionally wherein L₁ is –

C₆₋₉–C=C–C–, n is 0, L₃ is absent, p is 1, X₂ is carbonate or ester (optionally ), R is a

linear or branched carbon chain having 1-20, 2-20, or 3-20 (optionally 2-20) carbon atoms and 0 to 2 cis or trans C=C double bonds (optionally wherein R is $-C_{1-6}$ or $-C_{5-8}-C=C-C-C=C-C_{4-6}$), L5 is a carbon chain having 1 to 10 carbon atoms and comprises 0 to 1 cis or trans C=C double bond (optionally zero C=C double bonds), and L6 is $-CH_3$; or L is a carbon chain having 5 to 15 carbon atoms and zero C=C double bonds.

[0067] L1 is linked to the immunomodulatory agent by a cleavable linker or cleavable linkage, represented by X1. X1 may be biodegradable, meaning that it can be cleaved after administration to a subject. Without being limiting, an ester bond is capable of being hydrolyzed by an esterase after administration to a patient, thereby releasing the immunomodulatory agent from the lipophilic moiety. However, other X1 linkages can be utilized for tailored drug release based on their release characteristics when exposed to the environment at a disease site.

[0068] In some embodiments, X1 is cleavable by an esterase, alkaline phosphatase, amidase, peptidase or may be cleavable upon exposure to a reducing environment, and/or a high or low pH.

[0069] In some embodiments, X1 is a cleavable linker. A wide variety of chemical linkers are known to those of skill in the art. A linker may have 1 to 12 carbon atoms and at least one cleavable functional group. In some embodiments, the linker has at least two functional groups, a first functional group for conjugating one end of the linker to the immunomodulatory agent and a second functional group for conjugating another end of the linker to a carbon atom on L of Formula I, optionally L1. The two functional groups may each be independently selected from an ester, amide, amidine, hydrazone, ether, carbonate, carbamate, thionocarbamate, guanidine, guanine, oxime, isourea, acylsulfonamide, phosphoramidate, phosphonamide, phosphoramidate, phosphate, phosphonate, phosphodiester, phosphate phosphonooxymethylether, N-Mannich adduct, N-acyloxyalkylamine, sulfonamide, imine, azo, carbon-based functional groups such as an alkane, alkene or alkyne, methylene (CH_2) or urea. In some embodiments, the two functional groups are each ester. In some embodiments, X1 is succinate.

[0070] The linker may provide enhanced release of the immunomodulatory agent through the introduction of a biodegradable group. A linker having one or more ester bonds may be capable of being hydrolyzed by an esterase after administration to a patient, thereby releasing the immunomodulatory agent from the lipid conjugate. Similar to a linkage resulting from direct reaction between the immunomodulatory agent and L, a linker introducing a hydrazone bond

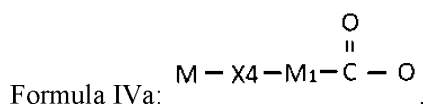
between the immunomodulatory agent and the lipophilic moiety can impart pH sensitive release of the immunomodulatory agent from the lipid conjugate.

[0071] In some embodiments, X1 is selected from one of the following linkers: aminohexanoic acid, polyglycine, polyamide, polyethylene, or a functionalized polymer having a carbon backbone that is one to twelve carbon atoms in length.

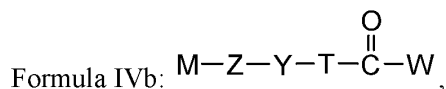
[0072] In some embodiments, the lipid conjugate has the formula M-X1-L, wherein M is the immunomodulatory agent (as defined above), X1 is a linker comprising a functional group, and L is the lipophilic moiety (as defined above). The functional group selected from an ester, amide, amidine, hydrazone, ether, carbonate, carbamate, thionocarbamate, guanidine, guanine, oxime, isourea, acylsulfonamide, phosphoramidate, phosphonamide, phosphoramidate, phosphate, phosphonate, phosphodiester, phosphate phosphonooxymethylether, N-Mannich adduct, N-acyloxyalkylamine, sulfonamide, imine, azo or urea. In some of these embodiments, the functional group is ester. In some embodiments where the lipid conjugate has the formula M-X1-L, the groups M-X1- has one of Formula IV, IVa, or IVb:

Formula IV: M-X4-M₁-X5,

wherein a X4 and X5 are independently selected from the functional group and M₁ is an optional spacer group linked to the X4 and X5 functional groups and has 0 to 12 carbon atoms or is CH₂, CH₂CH₂, N-alkyl, N-acyl, O or S, wherein X4 and X5 are individually optionally repeating units of 1 to 20, or wherein X4-M₁-X5 unit is an optionally repeating unit of 1 to 20;

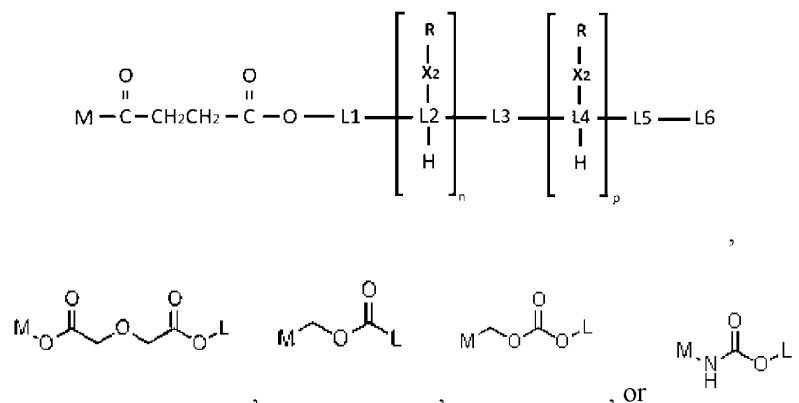


wherein X4 is selected from an ester, amide, hydrazone, ether, carbonate, carbamate or phosphodiester group, and M₁ is a spacer region of the linker having 0 to 12 carbon atoms or is CH₂, CH₂CH₂, N-alkyl, N-acyl or O;



wherein Z is O or N, Y is CH₂, CH₂CH₂ or C=O, T is 0 to 6 carbon atoms, and W is O or N.

[0073] In some embodiments, the lipid-conjugate has one of the following structures:



wherein M is the immunomodulatory agent (as defined above), L is the lipophilic moiety (as defined) above, and L1, L2, L3, L4, L5, L6, X2, R, n and p are as defined above.

[0074] However, it will be understood that the foregoing is merely exemplary. Additional examples of linkers are provided in U.S. Patent No. 5,149,794, which is incorporated herein by reference. Non-limiting examples of linkers described in U.S. Patent No. 5,149,794 include aminohexanoic acid, polyglycine, polyamides, polyethylenes, and short functionalized polymers having a carbon backbone that is one to twelve carbon atoms in length.

[0075] Yet further examples of linkers suitable for use in the lipid conjugates described herein are provided in the following references:

1. Rautio *et al.*, "The expanding role of prodrugs in contemporary drug design and development" *Nature Reviews Drug Discovery* 2018, 17, 559.
2. Irby *et al.*, "Lipid-drug conjugate for enhancing drug delivery" *Molecular Pharmaceutics* 2017, 14, 1325.
3. Sun *et al.*, "Chemotherapy agent-unsaturated fatty acid prodrugs and prodrug-nanoplatfroms for cancer chemotherapy" *Journal of Controlled Release* 2017, 264, 145.
4. Walther *et al.*, "Prodrugs in medicinal chemistry and enzyme prodrug therapies" *Advanced Drug Delivery Reviews* 2017, 118, 65.

5. Hu *et al.*, "Glyceride-mimetic prodrugs incorporating self-immolative spacers promote lymphatic transport, avoid first-pass metabolism and enhance oral bioavailability" *Angewandte Chemie International Edition* 2016, 55, 13700.
6. Blencowe *et al.*, "Self-immolative linkers in polymeric delivery systems" *Polymer Chemistry* 2011, 2, 773.

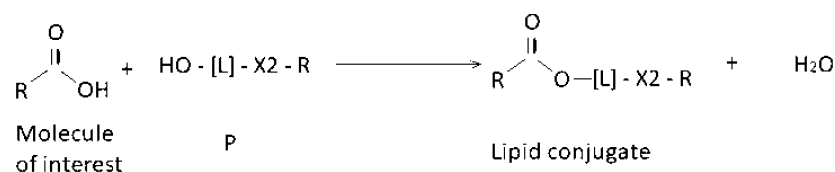
[0076] Each of the foregoing references is incorporated herein by reference in its entirety.

[0077] In one embodiment, at least the second functional group conjugating one end of the linker to L1 is an ester or an amide linkage. In another embodiment, a functional group on the linker can be hydrolyzed by an enzyme such as an esterase. In a further embodiment, both functional groups on the linker are ester linkages.

[0078] Manufacture of various lipid conjugates for use in immunomodulatory combinations is known in the art and has been previously described. For example, see WO/2020/191477 and WO 2023/035068, which are incorporated by reference herein in their entirety. Synthetic procedures for several lipid conjugates are described in the Examples.

[0079] Briefly, the immunomodulatory agent can be attached to the lipid moiety by conjugation to a reactive group on scaffold L or to a linker group to form chemical linkage/linker X1. In one embodiment, the immunomodulatory agent loses a hydroxyl group or a hydrogen atom upon conjugation with the lipophilic moiety (e.g. Formula I) or a linker to form the lipid conjugate. The immunomodulatory agent may be derived from a chemical structure that contains one or more reactive functional groups such as $-(C=O)O$, $-OH$, $-NH_2$, $-NHR$, $-PO_3H_2$, among others known to those of skill in the art, without limitation to the orientation of the atoms.

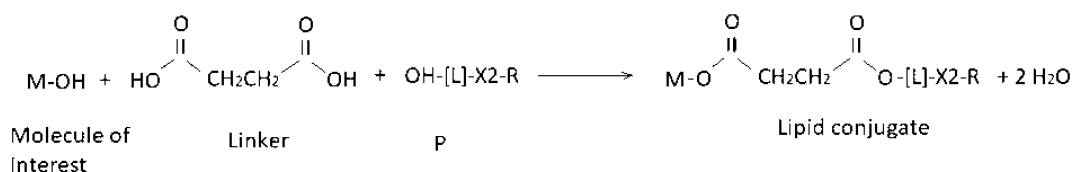
[0080] For example, the lipid conjugate may be formed (directly or via one or more intermediates) by a conjugation between a $(C=O)OH$ group on the immunomodulatory agent and a hydroxyl group on precursor scaffold P. The general reaction is shown below for a molecule of interest (e.g. an immunomodulatory agent):



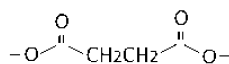
[0081] In the above exemplary embodiment, X1 is an ester and has the following structure:



[0082] In another illustrative example, the immunomodulatory agent may have a hydroxyl group (-OH) that reacts with a carboxyl group ((C=O)OH) in a linker. A second carboxyl group ((C=O)OH) on the linker may react with a hydroxyl group on a carbon atom on a precursor scaffold P via a condensation reaction. The following reaction depicts the use of succinic acid as a linker. The use of such a linker results in a lipid conjugate that has two ester groups according to the following reaction:



[0083] In the above non-limiting example, X1 has the following structure:



[0084] It should be appreciated that the above reaction may proceed in two steps. That is, the immunomodulatory agent may first be conjugated to the linker and the resultant drug-linker conjugate subsequently reacted with the precursor scaffold P to produce a lipid conjugate reaction product.

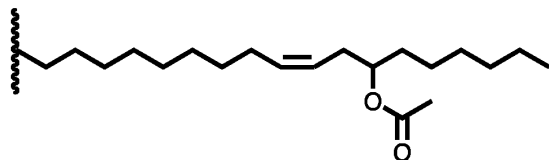
[0085] The foregoing is provided simply for illustrative purposes as a variety of different linkers besides succinic acid can be used to produce the lipid conjugates. For example, but without limitation, the X1 linker may be -X³-R^L-X⁴- wherein X³ and X⁴ are each independently selected from ester, amide, amidine, hydrazone, ether, carbonate, carbamate, thionocarbamate, guanidine,

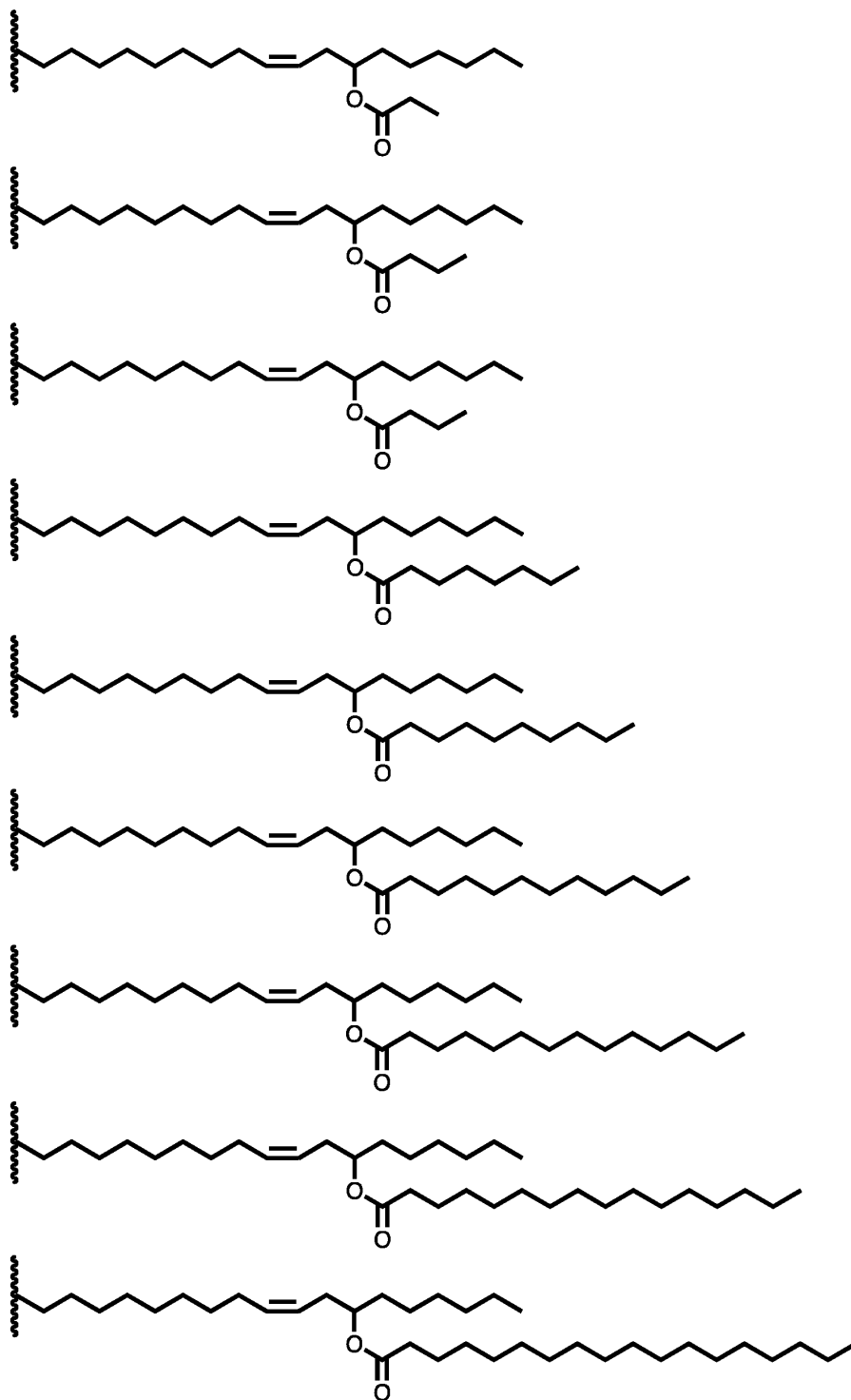
guanine, oxime, isourea, acylsulfonamide, phosphoramidate, phosphonamide, phosphoramidate, phosphate, phosphonate, phosphodiester, phosphate phosphonooxymethylether, N-Mannich adduct, N-acyloxyalkylamine, sulfonamide, imine, azo, alkene, or alkyne, or urea, wherein at least one of X^3 and X^4 is cleavable, and R^L is a linear, branched, and/or cyclic C_x alkylenyl, alkenylenyl and/or alkynylenyl, wherein x is 1-30, wherein any carbon bonded to two other carbons is optionally independently replaced by N, S, or O, and carbons are optionally independently substituted with oxo, hydroxyl, sulfhydryl, -SeH, halogen, guanidino, amine, amide, urea, carboxylic acid, sulfonic acid, sulfinic acid, or phosphoric acid. In some embodiments R^L is -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-CH₂-, -CH₂-CH₂-CH₂-CH₂-, -CH₂-CH₂-CH₂-CH₂-CH₂-, or -CH₂-CH₂-CH₂-CH₂-CH₂-. In some embodiments, X^3 and X^4 are ester and/or amide, and optionally both are ester. In some embodiments, X1 is succinic acid (succinate).

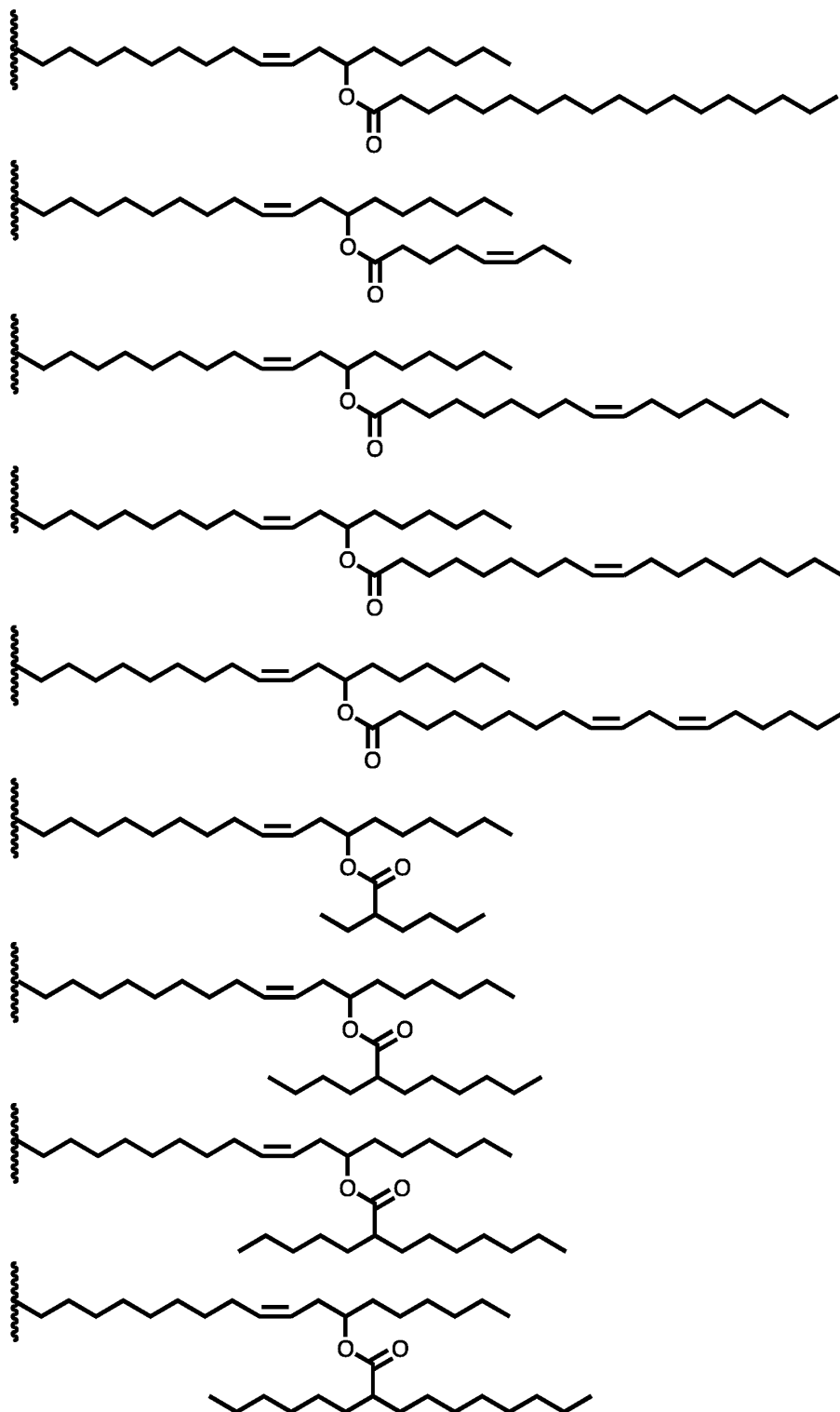
[0086] In another example, the immunomodulatory agent or a linker may have a carboxyl group ((C=O)O) for conjugation with an amine group of L to form an amide or amide-containing X1 between the immunomodulatory agent and L. As discussed below, other reactions between functional groups on a drug or a linker with a scaffold L can be envisaged by those of skill in the art to produce a lipid conjugate linking immunomodulatory agent and lipophilic moiety via X1.

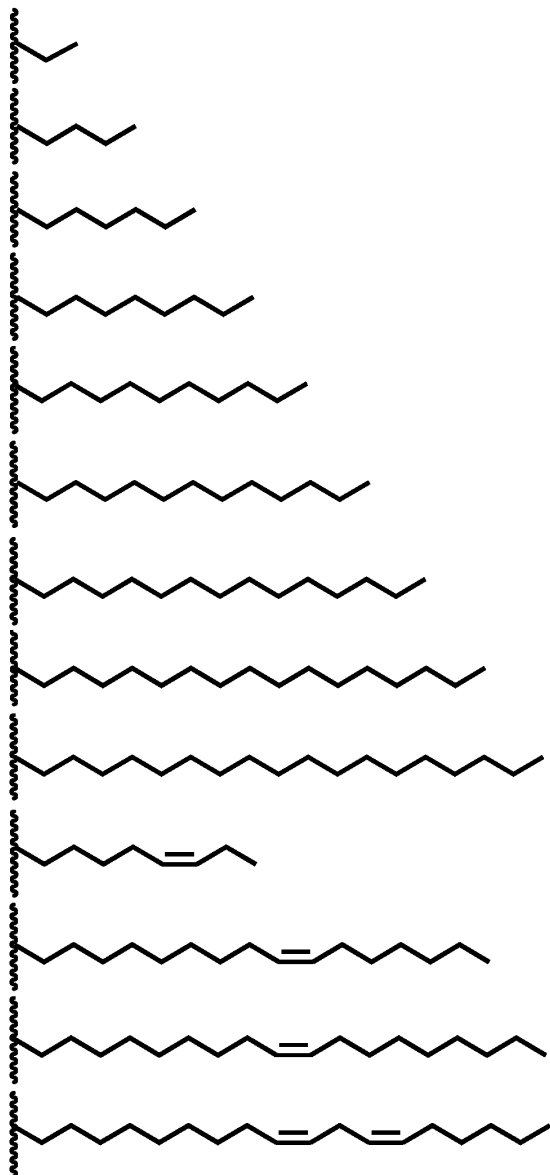
[0087] Certain immunomodulatory agents may comprise more than one reactive functional group for linkage to precursor scaffold P. In such embodiments, a protecting group may be employed during the synthesis of the drug-lipid conjugate as would be appreciated by those of skill in the art to selectively conjugate a given group on the drug to the scaffold L and leave another group unconjugated.

[0088] In some embodiments, each lipophilic moiety is independently selected from those depicted below, with the caveat that conjugation to a corticosteroid requires more than 14 carbons in the lipophilic moiety, optionally wherein all lipid conjugates have a cLogP value of 5-20:

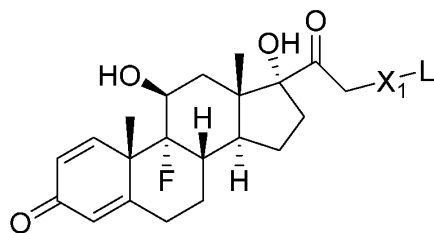






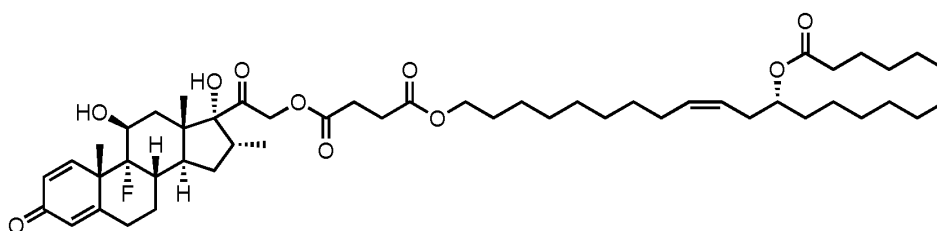


[0089] In some embodiments, the first lipid conjugate comprises dexamethasone and has the structure of Formula XV, or a pharmaceutically acceptable salt thereof, wherein X1 (i.e. the first cleavable linker or the first cleavable linkage) is as defined herein (optionally succinate), and L is a lipophilic moiety, optionally wherein L has Formula I:



(Formula XV).

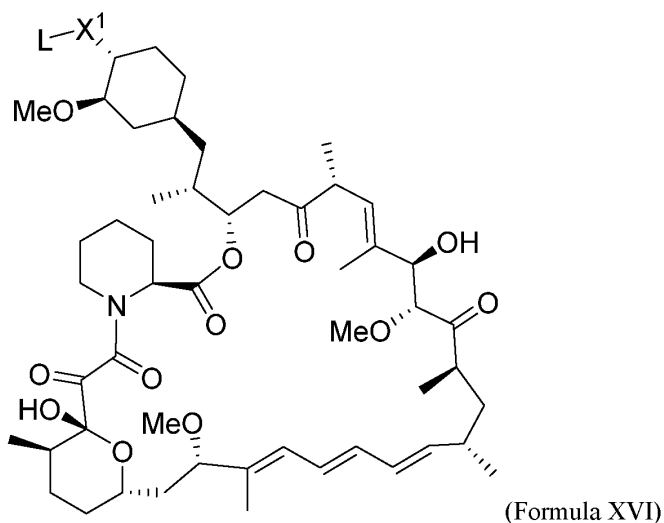
[0090] In some embodiments, the first lipid conjugate has the structure of INT-D034 or is a pharmaceutically acceptable salt thereof:



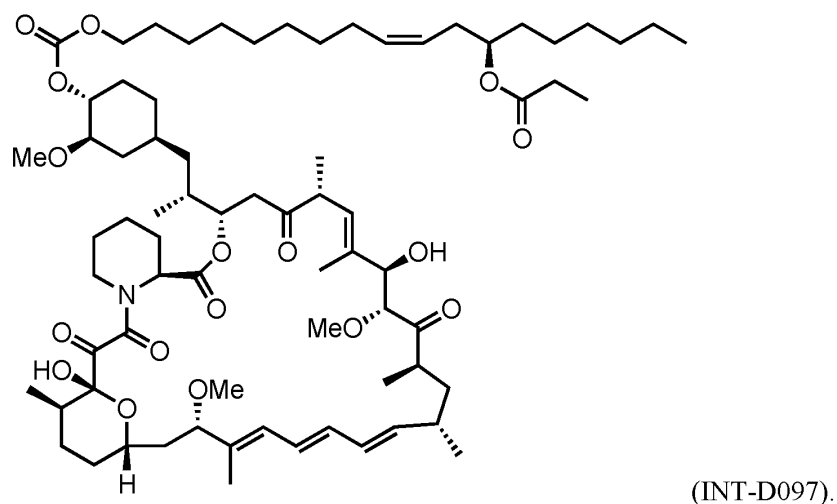
(INT-D034).

[0091] In some embodiments, dexamethasone in Formula XV or INT-D034 is replaced with a different corticosteroid, optionally selected from alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, diflorasone, difluocortolone, difluprednate, fluclorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortal, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol.

[0092] In some embodiments, the second lipid conjugate comprises sirolimus and has the structure of Formula XVI, or a pharmaceutically acceptable salt thereof, wherein X1 (i.e. the second cleavable linkage or the second cleavable linkage) is as defined herein (optionally carbonate), and L is a lipophilic moiety, optionally wherein L has Formula I:



[0093] In some embodiments, the first lipid conjugate has the structure of INT-D097 or is a pharmaceutically acceptable salt thereof:



[0094] In some embodiments, sirolimus in Formula XVI or INT-D097 is replaced with a different mTOR/mTORC inhibitor, optionally selected from everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-0812, Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-

1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1, Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxelisib (XL765), VS-5585 (SB2343), W922, WAY-600, WYE-125132 (WYE-132), WYE-354, or WYE-687, optionally everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or tacrolimus.

[0095] Nucleic acid(s) encoding epitopes

[0096] The one or more nucleic acids encode one or more epitopes (e.g. a plurality of epitopes). Each epitope in the one or more epitopes is an epitope targeted by autoreactive T cells in an autoimmune disease or condition (e.g. T1D, MS, vitiligo, Hashimoto's thyroiditis, celiac disease, IBD). In some embodiments, the one or more epitopes comprises epitopes from a plurality of autoantigens targeted by autoreactive T cells in the autoimmune disease or condition and/or antigens that trigger autoreactive T cells in the autoimmune disease or condition. In other embodiments, the one or more epitopes comprises epitopes from a single antigen/autoantigen, optionally a full length antigen/autoantigen.

[0097] In some embodiments, each epitope in the plurality of epitopes is an epitope of an autoantigen targeted by autoreactive T cells in T1D. In some embodiments, each epitope is from an insulin-producing beta cell.

[0098] In some embodiments, the plurality of epitopes comprises full-length and/or epitopic fragments from one or more, optionally two or more, of the following: preproinsulin, mature insulin components (A chain, B chain, and/or C-peptide), glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs).

[0099] In some embodiments, the plurality of epitopes comprises epitope(s) contained in the preproinsulin signal peptide. In some embodiments, the plurality of epitopes comprises at least one class I epitope of insulin B chain. In some embodiments, the plurality of epitopes comprises at least one class I epitope of GAD65. In some embodiments, the plurality of epitopes comprises at least one class I epitope of IGRP. In some embodiments, the plurality of epitopes comprises at

least one class I epitope of ZNT8. In some embodiments, the plurality of epitopes comprises at least one class II epitope of insulin B chain. In some embodiments, the plurality of epitopes comprises at least one class II epitope of GAD65. In some embodiments, the plurality of epitopes comprises at least one class II epitope of HSP60. In some embodiments, the plurality of epitopes comprises at least one class II epitope of IA-2 or IA-2beta. In some embodiments, the plurality of epitopes comprises at least one class II epitope of ZNT8. In some embodiments, the plurality of epitopes comprises at least one class II epitope of a hybrid insulin peptide. In some embodiments, the plurality of epitopes comprises a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 of the foregoing.

[0100] In some embodiments, the autoimmune disease or conditions is Multiple Sclerosis (MS). In some such embodiments, the one or more nucleic acids comprise full-length and/or epitopic fragments from one or more autoantigens selected from the group consisting of: myelin oligodendrocyte glycoprotein (MOG), proteolipid protein 1 (PLP1), myelin basic protein (MBP), myelin basic protein (MBP, and/or α B-crystallin (CRYAB). Amino acid sequences for these autoantigens are shown in Table 1A.

[0101] In some embodiments, the autoimmune disease or conditions is celiac disease (CD). In some such embodiments, the one or more nucleic acids comprise full-length and/or epitopic fragments from one or more antigens/autoantigens selected from gliadin (e.g. alpha gliadin, gamma gliadin, omega gliadin) and/or other CD antigens/autoantigens (e.g. including self-antigen tissue transglutaminase). Amino acid sequences for these antigens are shown in Table 1B.

[0102] In some embodiments, the autoimmune disease or conditions is thyroiditis. In some embodiments, the thyroiditis is Hashimoto's thyroiditis. In some such embodiments, the one or more nucleic acids comprise full-length and/or epitopic fragments from one or more autoantigens selected from the group consisting of: thyroid peroxidase (TPO), thyroglobulin (THYG), and/or other thyroiditis autoantigens. Amino acid sequences for these autoantigens are shown in Table 1A.

[0103] In some embodiments, the autoimmune disease or conditions is vitiligo. In some such embodiments, the one or more nucleic acids comprise full-length and/or epitopic fragments from one or more autoantigens selected from the group consisting of: Pmel17 (PMEL), Melanin-Concentrating Hormone Receptor 1 (MCHR1), Tyrosinase (TYR), and/or other vitiligo autoantigens. Amino acid sequences for these autoantigens are shown in Table 1A. mRNA

sequences are known, or can be generated by reverse translation and codon optimization using ubiquitously available software.

[0104] In some embodiments, the autoimmune disease or conditions is inflammatory bowel disease (IBD). In some such embodiments, the one or more nucleic acids comprise full-length and/or epitopic fragments from one or more antigens/autoantigens selected from the group consisting of: flagellin and/or other IBD antigens/autoantigens. Amino acid sequences for these antigens are shown in Table 1B. mRNA sequences are known, or can be generated by reverse translation and codon optimization using ubiquitously available software.

[0105] Table 1A: Human and Mouse Autoantigens

Disease	Antigen	Human UniProt ID	Sequence	Isoform	Mouse UniProt ID	Sequence
MS	Myelin oligodendrocyte glycoprotein (MOG)	Q16653	MASLSRPSLPSCCLCSFLL LLLLQVSSSYAGQFRVIG PRHPIRALVGDEVELPCR ISPGKNATGMEVGWYRPP FSRVVHLYRNGKDQDGDQ APEYRGRTELLKDAIGEG KVTLRIRNVRFSDGGFT CFFRDHSYQEEAAMELKV EDPFYWVSPGVLVLLAVL PVLLQITVGLIFLCLQY RLRGKLRAEIENLHRTFD PHFLRVPCWKITLFVIVP VLGPLVALI ICYNWLHRR LAGQFLEELRNPF (SEQ ID NO: 1)	Q16653- 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	Q61885	MACLWSFSWSPSCFLSL LLLLQLSCSYAGQFR VIGPGYPIRALVGDEA ELPCRISPGKNATGME VGWYRSPFSRVVHLYR NGKDQDAEQAPEYRGR TELLKETISEGKVTLR IQNVRFSDGGYTCCFF RDHSYQEEAAMELKVE DPFYWVNPGLVTLIAL VPTILLQVPVGLVFLF LQHRLRGKLRAEVENL HRTFDPHFLRVPCWKI TLFVIVPVLGPLVALI ICYNWLHRRLAGQFLE ELRNPF (SEQ ID NO: 2)
MS	myelin basic protein (MBP)	P02686	MGNHAGKRELNAEKASTN SETNRGESEKRNLGELS RTTSEDNEVFGEADANQN NGTSSQDTAVTDSKRTAD PKNAWQDAHPADPGSRPH LIRLFSRDAPGREDNTFK DRPSEDELQTIQEDSAA TSESLDVMAEQKRPSQRH GSKYLATASTMDHARHGF LPRHRDTGILDSIGRFFG GDRGAPKRGSGKDSHHPA RTAHYGS LPQKSHGRTQD ENPVVHFFKNI VTPRTPP PSQGKGRGLSLSRFSWGA EGQRPFGYGGRASDYKS AHKGFKGVDAGGTLSKIF	P02686- 1, 2, 3, 4, 5, 6	P04370	MGNHSGKRELSAEKAS KDGEIHRGEAGKKRSV GKLSQTASEDSDFVGE ADAIQNNGTSAEDTAV TDSKHTADPKNNWQGA HPADPGNRPHLIRLFS RDAPGREDNTFKDRPS EDELQTIQEDPTAAS GGLDVMAEQKRPSQRS KYLATASTMDHARHGF LPRHRDTGILDSIGRF FSGDRGAPKRGSGKDS HTRTTHYGS LPQKSQH GRTQDENPVVHFFKNI VTPRTPPPSQGKGRD

			KLGGDRSRSGSPMARR (SEQ ID NO: 3)			SRSGSPMARR (SEQ ID NO: 4)
MS	proteolipid protein 1 (PLP1)	P60201	MGLLECCARCLVGAPFAS LVATGLCFFGVALFCGCG HEALTGTEKLIETYFSKN YQDYEYLINVIHAFQYVI YGTASFFFLYGALLLAEG FYTTGAVRQIFGDYKTTI CGKGLSATVTGGQKGRGS RGQHQAHSLEERVCHCLGK WLGHPDKFVGITYALT WLLVFACSAVPVYIYFNT WTTTQSIAPSKTSASIG SLCADARMYGVLWNAFP GKVCNLLSICKTAEFQ MTFHLFIAAFVGAAATLV SLLTFMIAATYNFAVLKL MGRGTKF (SEQ ID NO: 5)	P60201- 1,2	P60202	MGLLECCARCLVGAPF ASIVATGLCFFGVALF CGCGHEALTGTEKLIE TYFSKNYQDYEYLINV IHAFQYVIYGTASFFF LYGALLLAEGFYTTGA VRQIFGDYKTTICGKG LSATVTGGQKGRGSRG QHQAHSLEERVCHCLGK WLGHPDKFVGITYALT VWLLVFACSAVPVYI YFNTWTTTQSIAPSK TSASIGSLCADARMYG VLPWNAFPKVCNLL LSICKTAEFQMTFHLF IAAFVGAAATLVSLLT FMIAATYNFAVLKLMG RGTKF (SEQ ID NO: 6)
MS	Alpha beta crystallin (CRYAB)	P02511	MDIAIHPWIRRPFFPH SPSRFLDQFFGEHLLED LFPTSTSLSPFYLRPPSF LRAPSWFDTGLSEMRLEK DRFSVNLVDVHFSPEELK VKVLGDVIEVHGKHEERQ DEHGFISREFHRKYRIPA DVDPLTITSSLSDGVL VNGPRKQVSGPERTIPIT REEKPAVTAAPKK (SEQ ID NO: 7)		P23927	MDIAIHPWIRRPFFPH FHSPSRFLDQFFGEHL LESDFSTATSLSPFY LRPPSFLRAPSWIDTG LSEMRLEKDRFSVNL VKHFSPEELKVKVLGD VIEVHGKHEERQDEHG FISREFHRKYRIPADV DPLTITSSLSDGVL VNGPRKQVSGPERTIP ITREEKPAVAAAAPKK (SEQ ID NO: 8)
Hashimoto's thyroiditis	Thyroglobulin (THYG)	P01266	MALVLEIFTLLASICWVS ANIFEYQVDAQPLRCEL QRETAFLKQADYVPPQAE DGSFQTVQCQNDGRSCWC VGANGSEVLGSRQGRPV ACLSFCQLQKQIILSGY INSTDTSYLPQCQDSGDY APVQCDVQVQCWCVD GMEVYGRQLGRPKRCPR SCEIRNRLLHGVGDKSP PQCSAEGEFMPVQCKFVN TTDMMIFDLVHSYNRFPD AFVTFSSFQRRFPEVSGY CHCADSQGRELAETGLEL LLDEIYDTIFAGLDLPST FTETTLRYILQRRFLAVQ SVISGRFRCPTKCEVERF	P01266- 1,2,	008710	MTALVLWVSTLLSSVC LVAANIFEYQVDAQPL RCELQREKAFLKQAE YVPQCSEDSFQTVQC QNDGQSCWCVDSDGRE VPGSRQLGRPTVCLSF CQLHKQRILLGSYINS TDALYLPQCQDSGNYA PVQCDLQRVQCWCVD EGMEVYGRQGRPTR CPRSCAIRNRLLHGV GDRSPQCTADGEFMP VQCKFVNTTMMIFDL IHNYNRFPDAFVTFSS FRGRFPEVSGYCYCAD SQGRELAETGLELLLD EIYDTIFAGLDQASTF

			TATSFCHPYVPSCRNGD YQAVQCQTEGPCWCVDAQ GKEMHGTRQQGEPPSCAE GQSCASERQQALSRLYFG TSGYFSQHDLFSSPEKRW ASPRVARFATSCPPTIKE LFVDSGLLRPMVEGQSQQ FSVSENLLKEAIRAIFPS RGLARLALQFTTNPKRLQ QNLFGGKFLVNVGQFNLS GALGTRGTFFNFSQFFQQL GLASFLNGGRQEDLAKPL SVGLDSNSSTGTPEAAKK DGTMNKPTVGSFGFEINL QENQNALKFLASLLELPE FLLFLQHAI SVPEDVARD LGDVMETVLSSQTCEQTP ERLFVPSCTTEGSYEDVQ CFSGECWCVNSWGKELPG SRVRGGQPRCPTDCEKQR ARMQSLMGSQPAGSTLFV PACTSEGHFLPVQCFNSE CYCVDAEGQAI PGTRSAI GKPKKCPTPCQLQSEQAF LRTVQALLSNSSMLPTLS DTYIPQCSTDGQWRQVQC NGPPEQVFELYQRWEAQN KGQDLTPAKLLVKIMSYR EAASGNFSLFIQSLYEAG QQDVFPVLSQYPSLQDVP LAALEGKRPQPRENILLE PYLFWQILNGQLSQYPGS YSDFSTPLAHFDLNCWC VDEAGQELEGMRSEPSKL PTCPGSCCEEAKLRVLQFI RETEEIVSASNSSRFPLG ESFLVAKGIRLRNEDLGL PPLFPREAFAEQFLRGS DYAIRLAAQSTLSFYQRR RFSPDDSA GASALLRSGP YMPQCDAFGSWEVPVQCHA GTGHCWCVDEKGGFIPGS LTARSLQIPQCPTTCEKS RTSGLLSWKQARSQENP SPKDLFVPACLETGEYAR LQASGAGTWCVPASGEE LRPGSSSSAQCPSLCNVL KSGVLSRRVSPGYVPACR AEDGGFSPVQCDQAQSC WCVMDSGEEVPGTRVTGG QPACESPRCPLPFNASEV VGGTILCETISGPTGSAM QQCQLLCRQGSWSVFPPG PLICSLESGRWESQLPQP		TQSTMYRILQRRFLAI QLVISGRFRCPTKCEV EQFAATRFHGHSYIPRC HRDGHYQTVQCQTEGM CWCVDAQGREVPGTRQ QGQPPSCAADQSCALE RQQALSRLFYFETPDYF SPQDLLSSEDRLAPVS GVRSDTSCPPRIKELF VDSGLLRSIAVEHYQR LSESRSLREAIRAVF PSRELAGLALQFTTNP KRLQQNLFGGTFLANA AQFNLSGALGTRSTFN FSQFFQQFGLPGFLNR DRVTTILAKLLPVRLDS SSTPETLRVSEKTVAM NKRNVGNFGFKVNLQE NQDALKFLVLSLELPE FLVFLQRAVSVPEIDIA RDLGDVMEMVFSAQAC KQMPGKFFVPSCTAGG SYEDIQCYAGECWCVD SRGKELDGSRRVGRGP RCPTKCEKQRAQMOSL ASAQPAGSSFFVPTCT REGYFLPVQCFNSECY CVDTEGQVIPGTQSTV GEAKQCPSVCQLQAEQ AFLGVGVLLSNSSMV PSISNVYIPQCSASGQ WRHVQCDGPHEQVFEW YERWKTQNGDGQELTP AALLMKIVSYREVASR NFSLFLQSLYDAGQQR IFPVLAQYPSLQDVPQ VVLEGATTPPGENIFL DPYIFWQILNGQLSQY PGPYSDFNMPLEHFNL RSCWCVD EAGQKLDGT QTKPGEIPACPGPCEE VKLRVLKFIKETEEIV SASNASSFPLGESFLV AKGIQLTSEELDLPQ FPSRDAFSEKFLRGGE YAIRLAAQSTLTIFYQS LRASLGKSDGAASLLW SGPYMPQCNMIGGWEP VQCHAGTGQCWCVDGR GEFIPGSLMSRSSQMP QCPTNCELSRASGLIS AWKQAGPQRNPGPGDL FIPVCLQTGEYVRKQT SGTGTWCVPASGEGM
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			RACQRPQLWQTIQTQGHF QLQLPPGKMCSADYADLL QTFQVFILDELTARGFCQ IQVKTFGTLVSI PVCNNS SVQVGCLTRERLGVNVTW KSRLEDIPVASLPDLHDI ERALVGKDLLGRFTDLIQ SGSFQLHLDSKTFPAETI RFLQGDHFGTSPRTWFGC SEGFYQVLTSEASQDGLG CVKCPGEGSYSQDEECIPC PVGFYQEQAGSLACVPCP VGRTTISAGAFSQTHCVT DCQRNEAGLQCDQNGQYR ASQKDRGSGKAFCDVDEG RRLPWWETEAPLEDSQCL MMQKFEKVPESKVI FDAN APVAVRSKVPDSEFPVMQ CLTDCTEDEACSFFTVST TEPEISCDFYAWTSDNVA CMTSDQKRDALGNSKATS FGSLRCQVKVRSHGQDSP AVYLKKGQGSTTTLQKRF EPTGFQNMLSGLYNPIVF SASGANLTD AHLFCLLAC DRDLCCDGFVLTQVQGGA IICGLLSSPSVLLCNVKD WMDPSEAWANATCPGVTY DQESHQVILRLGDQEFIK SLTPLEGTQDTFTNFQVQ YLWKDSMDGSRPESMGCR KDTVPRPAS PTEAGLTTE LFSPVDLNQVIVNGNQS SSQKHWLFKHLFSAQQAN LWCLSRCVQEHSCQLAE ITESASLYFTCTLYPEAQ VCDDIMESNAQGCRILIP QMPKALFRKKVILEDKVK NFYTRLPFQKLMGISIRN KVPMSEKSI SNGFFECER RCDADPCCTGFGFLNVSQ LKGGEVTCCLTLNSLGIQM CSEENGGAWRILDCGSPD IEVHTYPPFGWYQKPIAQN NAPSFCLVVLPSLTEKV SLDSWQSLALSSVVVDPS IRHFDVAHVSTAATSNFS AVRDLCLSECSQHEACLI TTLQTQPGAVRCMFYADT QSCTHSLQGQNCRLLLRE EATHIYRKPGISLLSYEA SVPSVPISTHGRLGRSQ AIQVGTSWKQVDQFLGVP YAAPPLAERRFQAPEPLN		PVNTNGSAQCPLGCDV LKSRALSRKVGLGYSP VCEALDGAFSPVQCDL AQGSCWCVLGSGEEVP GTRVVGTPPACESPQC PLPFSGSDVADGVIFC ETASSSGVTTVQQCQL LCRQGLRSAFSPGPLI CSLESQHWVTLP PPRA CQRPQLWQTMQTQAHF QLLLPPGKMCSVDYSG LLQAFQVFILDELIAR GFCQIQVKTFGTLVSS TVCDNSSIQVGC LTAE RLGVNVTWKLQLEDIS VGSLPDLYSIERAVTG QDLLGREFADLIQSGRF QLHLDSKTF SADTTLY FLNGDSFVTSPTQLG CMEGFYRVPTTRQDAL GCVKCPGEGSFSDGRC TPCPAGTYQEQAGSSA CIPCPRGRTTITTGAF SKTHCVTDCQKNEAGL QCDQNGQYQASQKNRD SGEVFCVDSEGRKLQW LQTEAGLSESQCLMIR KFDKAPESKVI FDANS PVIVKSSVPSADSPLV QCLTDCANDEACSF LT VSTMESEVSCDFYSWT RDNFACVTS DQEQDAM GSLKATSFGLRCQVK VRNSGKDSLAVYVKKG YESTAAGQKSFEPTGF QNVLSGLYSPVVFSA GANLTDHTYCLLACD NDSCCDGFIITQVKGG PTICGLLSSPDILLCH INDWRDTSATQANATC AGVTYDQGS RQMTLSL GGQEF LQGLALLEGTQ DSFTSFQQVYLWKDSD MGSRPESMGCEGMVP RSDFP GDMATELFSVP DITQVIVNTSHSLPSQ QYWLFTHLFSAEQANL WCLSRCAQEPIFCQLA DITKSSSLYFTCFLYP EAQVCDNMESNAKNC SQILPHQPTALFRRKV VLNDRVKNFYTRLPFQ KLTGISIRDKVPMMSGK LISNGFFECERLCDRD
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			WTGSWDASKPRASCWQPG TRTSTSPGVSEDCLYLNV FIPQNVAPNASVLVFFHN TMDREESEGWPAGDGSFL AAVGNLIVVTASYRVGVF GFLSSSGSEVSGNWGLLD QVAALTWVQTHIRFGGD PRRVSLAADRGADVASI HLLTARATNSQLFRRAVL MGGSALS PAAVISHERAQ QQAIALAKEVSCPMSSSQ EVVSCLRQKPANVLNDAQ TKLLAVSGPFHYWGVID GHFLREPPARALKRSLWV EVDLLIGSSQDDGLINRA KAVKQFEESRGRTSSKTA FYQALQNSLGGEDSDARV EAAATWYYSLEHSTDDYA SFSRALENATRDYFIICP IIDMASAWAKRARGNVFM YHAPENYGHGSLELLADV QFALGLPFYPAYEGQFSL EEKSLSLKIMQYFSHFIR SGNPNYPYEFSTRKVPTFA TPWPDFVPRAGGENYKEF SELLPNRQGLKKADCSFW SKYISSLKTSADGAKGGQ SAEEEEELTAGSGLRED LLSLQEPGSKTYSK (SEQ ID NO: 9)			PCCTGFGFLNVSQQLQG GEVTCITLNSMGIQTC NEESGATWRIIDCGSE DTEVHTYYPFGWYQKPA VWSDTPSFCPSAALQS LTEEKVTSDSWQTLAL SSVIVDPSIKHFDVAH ISTAATSNFMAQDFC LQQCSRHQDCIVTTLQ IQPGVVRVCFYYPDIQN CIHSLSRHTCWLLLHE EATYIYRKSGIPLVQS DVTSTPSVRIDSFGQL QGGSQVIKVGTAWKQV YRFLGVPYAAPPLADN RFRAPVNLNWTGSWDA TKPRASCWQPGTRTPT PPQINEDCLYLVNFVP ENLVSNASVLVFFHNT MEMEGSGGQLTIDGSI LAAVGNFIVVTANYRL GVFGFLSSGSDEVAGN WGLLDQVAALTWVQSH IGAFGGDPQRVTLAAD RSGADVASHLLISRP TRQLFRKALLMGGSA LSPAAIISPERAQQA AALAKEVGCPTSSIQE VVSCLRQKPANILNDA QTKLLAVSGPFHYWGP VVDGQYLRELPSRRLK RPLPVKVDLLIGGSQD DGLINRAKAVKQFEES QGRTNSKTAFYQALQN SLGGEDSDARILAAAV WYYSLEHSTDDYASFS RALENATRDYFIICPM VNMASLWARRTRGNVF MYHVPESYGHGSLELL ADVQYAFGLPFYSAYQ GQFSTEEQSLSLKVMQ YFSNFIRSGNPNYPHE FSRKAAEFATPWPDFI PGAGGESYKELSAQLP NRQGLKQADCSFWSKY IQTLKDADGAKDAQLT KSEEDLEVGPGLEED LSGSLEPVPKSYSK (SEQ ID NO: 10)
Hashimoto's thyroiditis	Thyroid peroxidase (TPO)	P07202	MRALAVLSVTLVMACTEA FFPFISRGKELLWGKPEE SRVSVLEESKRLVDTAM YATMQRNLLKRGILSPAQ LLSFSKLPEPTSGVIARA		P35419	MRTLGAMAIMLVVMGT VIFLSFILRSRDILCG KTMKSHVISAVETSQSL MVDHAVYNTMKRNLKK REVLSPAQLLSFFKLP

			AEIMETSIQAMKRKVNLIK TQQSQHPTDALSEDLLSI IANMSGCLPYMLPPKCPN TCLANKYRPITGACNNRD HPRWGASNTALARWLPPV YEDGFSQPRGWNPGFLYN GFPLPPVREVTRHVIQVS NEVVTDDDRYSDLLMAWG QYIDHDIAFTPQSTSKAA FGGGADCQMTCENQNPCF PIQLPEEARPAAGTACLP FYRSSAACGTGQQGALFG NLSTANPRQQMNGLTSFL DASTVYGSSPALERQLRN WTSAEGLLRVHARLRDSG RAYLPFVPPRAPAACAPE PGIPGETRGPCFLAGDGR ASEVPSLTALHTLWLREH NRLAALKALNAHWASADA VYQEARKVVGALHQIITL RDYIPRILGPEAFQQYVG PYEGYDSTANPTVSNVFS TAAFRFGHATIHPLVRRL DASFQEHDPDLPGLWLHQA FFSPWTLRLRGGLDPLIR GLLARPAKLQVQDQLMNE ELTERLFVLSNSSTLDA SINLQGRDHGLPGYNEW REFCGLPRLETPADLSTA IASRSVADKILDLYKHPD NIDVWLGGLAENFLPRAR TGPLFACLIQKQMKALRD GDWFWWENSHVFTDAQRR ELEKHSLSRVIDNTGLT RVPMDAFQVGKFPEDFES CDSITGMNLEAWRETFPQ DDKCGFPESVENGDVHC EESGRVLVYSCRHGIEL QGREQLTCTQEGWDFQPP LCKDVNECADGAHPCHA SARCRNTKGGFQCLCADP YELGDDGRTCVDSGRLP VTWISMSLAALLIGGFAG LTSTVICRWTRTGKSTL PISETGGTPELRCGKHQ AVGTSPQRAAQDSEQES AGMEGRDTHRLPRAL (SEQ ID NO: 11)			ESTSGAISRAAEIMET SIQVMKREQSQFSTDA LSADILGTIANLSGCL PFMLPPRCPDTCCLANK YRPITGACNNRDHPRW GASNTALARWLPPVYE DGFSQPKGWNPNFLYH GFPLPPVREVTRHLIQ VSNEAVTEDDQYSDFL PVWGQYIDHDIALTPQ STSTAAGWGGVDCQLT CENQNPCFPPIQLPSNS SGTTACLPFYRSSAAC GTGDQGALFGNLSAAN PRQQMNGLTSFLDAST VYGSSPGVEKQLRNWS SSAGLLRVNTLHLDAG RAYLPFATAACAPEPG TPRTNRTPCFLAGDGR ASEVPALAAVHTLWLRL EHNRLASAFKAINKHW SANTAYQEARKVVGAL HQIITMRDYIPKILGP DAFRQYVGPYEGYNPT VNPTVSNIFSTAAFRF GHATVHPLVRRLNTDF QEHTELPRLQLRDVFF RPWRLIQEGGLDPIVR GLLARAALQVQGQLM NEELTERLFVLSNVGT LDLASINLQGRDHGL PDYNEWREFCGLSRLE TPAELNKAIANRSMVN KIMDLKXHADNIDVWL GGLAEKFLPGARTGPL FACIIGKQMKALRDGD RFFWENTNVFTDAQRQ ELEKHSLSRVIDCNTG LTRVPVDAFRIGKFPQ DFESCEDIPSMDLELW RETFPQDDKCVFPPEEV DNGNFVHCEESGKLV VYSCFHGYKLQGEQV TCTQKGWDSEPPVCKD VNECADLTHPPCHPSA QCKNTKGSFQCVCTDP YVLGEDEKTCIDSGRL PRASWVSIALGALLIG GLASLTWIVICRWTHA DKKATLPITERVTTQS GCRKSQGRGISPHKAA AQDTGQEPASGSRVLL CE (SEQ ID NO: 12)
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vitiligo	Melanin-concentrating hormone receptor 1 (MCHR1)	Q99705	MDLEASLLPTGPNASNTS DGPDLNLTSAAGSPPTGSI SYINIIMPSVFGTICLLG IIGNSTVIFAVVKSKLH WCNNVPDIFIINLSVVDL LFLLGMPFMIHQLMGNGV WHFGETMCTLITAMDANS QFTSTYILTAMADRYLA TVHPISSTKFRKPSVATL VICLLWALSFISITPVWL YARLIPFPGGAVGCGIRL PNPDTDLYWFTLYQFFLA FALPFVVITAAYVRILQR MTSSVAPASQRSIRLRTK RVTRTAIAICLVFFVCWA PYYVLQTLQLSISRPTLT FVYLYNAAISLGYANSCL NPFVYIVLCETFRKRLVL SVKPAAQQLRAVSNAQT ADEERTESKGT (SEQ ID NO: 13)	N/A	Q8JZL2	MDLQASLLSTGPNASN ISDGQDNFTLAGPPPR TRSVSYINIIMPSVFG TICLLGIVGNSTVIFA VVKSKLHWCSNVPDI FIINLSVVDLLFLLGM PFMIHQLMGNGVWHFG ETMCTLITAMDANSQF TSTYILTAMADRYLA TVHPISSTKFRKPSMA TLVICLLWALSFISIT PVWLYARLIPFPGGAV GCGIRLPNPDTDLYWF TLYQFFLAFAFPFVVI TAAYVKILQRMTSVA PASQRSIRLRTKRVR TAIAICLVFFVCWAPY YVLQTLQLSISRPTLT FVYLYNAAISLGYANS CLNPFVYIVLCETFRK RLVLSVKPAAQQLRT VSNAQTAEERTESKGT (SEQ ID NO: 14)
vitiligo	Tyrosinase (TYR)	P14679	MLLAVLYCLLWSFQTSAG HFPRACVSSKNLMEKECC PPWSGDRSPCGQLSGRGS CQNILLSNAPLGPQFPFT GVDDRESWPSVFNRTCQ CSGNFMGFNCNCKFGFW GPNCTERRLLVRNIFDL SAPEKDKFFAYLTAKHT ISSDYVPIGTYGQMKNG STPMFNDINIYDLFVVMH YYVSM DALLGGSEIWRDI DFAHEAPAFLPWHRFL RWEQEIQKLTGDNFTIP YWDWRDAEKCDICTDEYM GGQHPTNPNLSPASFSS SWQIVCSRLEEYNSHQSL CNGTPEGPLRRNPGNHDK SRTPLRPSSADVEFCLSL TQYESGSM DKAANFSFRN TLEGFASPLTG IADASQS SMHNALHIYMG TMSQVQ GSANDPIFLLHHAFVDSI FEQWLRHRPLQEVYPEA NAPIGHNRESYMPFIPL YRNGDFFISSKDLGYDYS YLQSDPD SFQDYIKSYL EQASRIWSWLLGAAMVGA VLTALLAGLVSLLCRHKR KQLPEEKQPLLMEKEDYH		P11344	MFLAVLYCLLWSFQIS DGHFPRACASSKNLLA KECCPPWMGDGSPCGQ LSGRGSCQDILLSSAP SGPQFPFKGVDDRESW PSVFNRTCQC SGNFM GFNCNCKFGFGGPN TEKRVLIRRNIFDL SEKNKFYSYLTAKHT ISSVYVIPTGTGQGMN NGSTPMFNDINIYDLF VWMHYVSRDTLLGGS EIWRDIDFAHEAPGFL PWHRLFLLWEQEIRE LTGDNFTVPYWDWRD AENCDICTDEYLGGRH PENPNLLSPASFSSW QIICSRSEYNSHQVL CDGTPEGPLLRNPGNH DKAKTPLRPSSADVEF CLSLTQYESGSM DRTA NFSFRNTLEGFASPLT GIADPSQSSMHNALHI FMNGTMSQVQGSANDP IFLLHHAFVDSIFEQW LRRHRPLLEVYPEANA PIGHN RDSYMPFIPL YRNGDFFITSKDLGYD YSYLQESDPGFYRNYI EPYLEQASRIWPWLLG

			SLYQSHL (SEQ ID NO: 15)			AALVGAVIAAALSGLS SRLCLQKKKKKKQPQE ERQPLLMDDKDDYHSL YQSHL (SEQ ID NO: 16)
vitiligo	Melanocyte protein Pmel 17 (PMEL)	P40967	MDLVLRCLLHLAVIGAL LAVGATKVPNRNQDWLGV RQLRTKAWNRQLYPEWTE AQRDCWRGGQVSLKVS DGPTLIGANASFSIALNF PGSQKVLDPGQVIWVNT IINGSQVWGGQPVYPQET DDACIFPDGGPCPSGSWS QKRSFVYVWKTWGWQV LGGPVSGLSIGTGRAMLG THTMEVTVYHRRGSRYS PLAHSSAFTITDQVPFS VSVSQLRALDGGNKHFLR NQPLTFALQLHDPGSGYLA EADLSYTWDFGSSGTLI SRALVVTHTYLEPGPVTA QVVLQAAIPLTSCGSSPV PGTTDGHRTAEAPNTTA GQVPTTEVVGTTPGQAPT AEPGTTTSVQVPTTEVIS TAPVQMPTAESTGMTPEK VPVSEVMGTTLAEMSTPE ATGMTPAEVSIVVLSGTT AAQVTTTEWVETTARELP IPEPEGPDASSIMSTESI TGSLGPLLDGTATLRLVK RQVPLDCVLYRYGSFSVT LDIVQGIESAEILQAVPS GEGDAFELTVSCQGGLPK EACMEISSPGCQPPAQR CQPVLPSPACQLVLHQIL KGGSGTYCLNVSLADTNS LAVVSTQLIMPQGEAGLG QVPLIVGILLVMAVVLA SLIYRRRLMKQDFSVPL PHSSSHWLRLPRIFCSCP IGENSPLLSGQQV (SEQ ID NO: 17)		Q60696	MGVQRRSFLPVLVLSA LLAVGALEGSRNQDWL GVPRQLVTKTWNRQLY PEWTEVQGSNCWRGGQ VSLRVINDGPTLVGAN ASFSIALHFPQSQKVL PDGQVIWANTTIINGS QVWGGQPVYPQEPDDA CVFPDGGPCPSGPKPP KRSFVYVWKTWGWQV VLGGPVSRSLIATGHA KLGTHTMEVTVYHRRG SQSYVPLAHASSTFTI TDQVPFSVSVSQLQAL DGETKHFLRNHPLIFA LQLHDPGSGYLAEADLS YTWDFGDTGTILISRA LDVTHTYLES GS VTAQ VVLQAAIPLVSCGSSP VPGTTDGYMPTAEAPG TTSRQGT TTKVGTTP GQMPTTQPSGTTVVQM PTEVTATTSEQMLTS AVIDTTAEVSTTEGT GTTPTRPSTTVAQAT TTEGPDASPLPTQSS TGSISPLDDTDTIML VQRQVPLDCVLYRYGS FSLALDIVQGIESAEI LQAVPFSEGDFAELTV SCQGGLPKEACMDISS PGCQPPAQRCLCQSVPP SPDCQLVLHQLVKGS GTYCLNVSLADANSLA VASTQLVVPQG DGLG QAPLLVGILLVLVAVV LASLIHRRLKKQGSV SQMPHGSTHWLRLPPV FRARGLGENSPLLSGQ QV (SEQ ID NO: 18)

[0106] Table 1B. Autoimmune triggering foreign antigens

Disease	Antigen	Antigen source (species example)	UniProt ID	Sequence
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Celiac disease	Alpha gliadin	<i>Triticum aestivum</i>	P02863	MKTFLILVLLAIVATTATTAVRFPVPQLQPQ NPSQQQPQEQVPLVQQQFLGQQQPFPQPQ YPQPQPFPSQLPYLQLQFPQPQLPYSQPQP FRPQQPYQPQPQYSQPQQPISQQQQQQQQ QQQQQQQQQILQQILQQQLIPCMDVVLQQHN IAHGRSQVLQQSTYQLLQELCCQHLWQIPEQ SQCQAIHNVVHAIILHQQQKQQQPSSQVSF QQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQ LPQFEEIRNLALQTLPMCENVYIPPYCTIAP FGIFGTN (SEQ ID NO: 19)
Celiac disease	Gamma gliadin	<i>Triticum aestivum</i>	P08079	MKTLLILITILAMAITIGTANMQVDPSSQVQW PQQQPVPQPHQPFSSQQPQQTFPQPQQTFPHQ PQQQFPQPQQPQQQFLQPQQPFPQPQQPYP QQPQQPFPQTQQPQQLFPSQQPQQQFSQPQ QQFPQPQQPQQSFQQQPPFIQPSLQQQVNP CKNFLQQCKPVSLVSSLWSMIWPQSDCQVM RQCCQQLAQIPQQLQCAAHTIIHSIIMQQ EQEQQQGMHILLPLYQQQQVGQGTLVQGG IIQ (SEQ ID NO: 20)
Celiac disease	Omega gliadin	<i>Triticum aestivum</i>	U5U6N3	MKTFLIFVLLAMAMKIATAARELNPSNKELO SPQQSFSHQQQPFPQQPYQPYPSSQQPYPS QQPFTPQPQFPQQSQPFTQPQQPTPLQPQ QFPQPQQPQQPFPQPQQPFPWQPQQPFPQ TQQSFPLQPQQPFPQPQQPFPQPQLQFPQQ PEQIIPQQPQQPFLLESQQPFPQPQQPFPQ PQQLIPMQPQQPFPQQSQSQPFPQGPQQLF PELQQPIPQQPRQPFLQFPQQPFPQQSQPFP PQQPHQPQQPYPQQQPYGSSLTSIGGQ (SEQ ID NO: 21)
IBD	Flagellin	<i>Clostridioides difficile</i>	Q18CX7	MRVNTNVSALIANNQMGRNVNGQSKSMEKLS SGVRIKRAADDAAGLAISEKMRAQIKGLDQA GRNVQDGISVVQTAEGSLEETGNILQRMRTL SLQANEINNTEEREKIADELTLQKDEIERI SSSTEFNGKKLLDGTSSSTIRLQVGASYGTNV SGTSNNNNEIKIQLVNTASIMASAGITTASI GSMKAGGTTGTDAAKTMVSSLDAAKSLNSS RAKLGAQQNRLESTQNNLNNTLENVTAAESR IRDTDVASEMVNLSKMNILVQASQSMQAQAN QQPQGVLLG (SEQ ID NO: 22)

[0107] Epitopic fragments of an antigen can be any size that is bound by major histocompatibility complex (MHC) class I (class I epitopes or HLA-I epitopes) or MHC class II (class II epitopes or HLA-II epitopes). Class I epitopes are typically 8-11 amino acids long (9-10 amino acids being most typical), although longer peptides (e.g. up to 15 amino acids) are bound less frequently. Class

II epitopes are typically 11-25 amino acids long, but shorter or longer fragments are possible. Each epitope (class I or class II) may optionally be flanked on either or both sides by flanking amino acids, which are residues from the antigen that naturally flank the epitope. In some embodiments, the flanking amino acids are 0-25 amino acids on each end of the epitope. In some embodiments, epitopes are flanked on each side by 5-8 amino acids, optionally 6-7 amino acids, except: (1) when an epitope is less than 5 amino acids from an N- or C-terminus, the flanking amino acids at that end will only be as long as the number of flanking amino acids found between the epitope and the terminus (e.g. 0, 1, 2, 3, or 4 amino acids); and (2) longer than 8 flanking amino acids may be used where epitopes are clustered closely together (e.g. when epitopes are 9-25 amino acids apart). Where epitopes are overlapping, a multi-epitopic region may be included rather than separate epitopes separated by flanking amino acids.

[0108] In some embodiments, the plurality of epitopes comprises one or more class I epitopes. In some embodiments, the plurality of epitopes comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes. In some embodiments, each class I epitope (and any flanking amino acids present on either end) is separated by a cleavable or noncleavable linker peptide, e.g. a class I linker peptide. A class I linker peptide (or class I linker) is a short peptide sequence rich in aliphatic (particularly small aliphatic, e.g. Ala), aromatic and/or basic residues, with a C-terminal tyrosine or basic residue (Lys or Arg) that enables proteosomal cleavage (e.g. chymotrypsin-like or trypsin-like protease cleavage) at the linker's C terminus. The linker assists with cleavage between epitopes and also prevents junctional epitopes (discussed below). A non-limiting example of a class I linker peptide used in multi-epitope designs is AAY (SEQ ID NO:23; Bergmann et al., 1996, *J. Immunol.* 157 (8): 3242–3249), which has been additionally suggested to promote binding to TAP transporter for enhanced epitope presentation (Dong et al., 2020, *Front. Immunol.* 11:1784). In some embodiments, the class I linker peptide is 3-5 amino acids long, and consists of non-acidic amino acids and a C-terminal tyrosine, lysine, or arginine. Where epitopes are overlapping, a multi-epitopic region may be included rather than separate epitopes separated by flanking amino acids and linkers; optionally with flanking amino acids on either end of the multi-epitopic region.

[0109] In some embodiments, the plurality of epitopes comprises one or more class II epitopes. In some embodiments, the plurality of epitopes comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes. In some embodiments, each class II epitope (and any flanking amino acids present on

either end) is separated by a linker peptide, e.g. a class II linker peptide. A class II linker peptide (or class II linker) is a sequence that disrupts the formation of junctional epitopes by low Class II anchor position binding. A junctional epitope is formed by the C-terminal segment from the first epitope and an N-terminal segment from the second epitope. The emergence of a junctional epitope may lead to unintended immunogenic effects, eliciting an immune response towards irrelevant junctional epitopes and hindering the responses to the intended epitopes. A class II linker placed in between class II epitopes prevents junctional epitopes by hindering binding of the linker to the class II groove. Without the class II linker, the junction between two intended epitopes may have very high binding to the Class II groove, resulting in presentation of that junctional epitope. Class II linkers are not bound in the class II groove because they are rich in G and P residues which are not found at main anchor positions in MHC class II. More broadly, sequences that contain amino acid residues that are rarely found in anchor positions of MHC Class II molecules will serve as good Class II linkers (e.g. short Gly and Pro rich sequences; Livingston et al., 2002, *J. Immunol.* 168 (11): 5499–5506). Sequences rich in glycine and proline also provides structural flexibility between adjacent epitopes. Non-limiting examples of class II linker peptides used in multi-epitope designs are GGGSGGG (SEQ ID NO: 24), GGGGS (SEQ ID NO: 25), and GPGPG (SEQ ID NO: 26). In some embodiments, the class II linker peptide is 4-10 amino acids long, wherein at least 50% of the amino acids are glycine, optionally containing at least one proline, optionally wherein non-glycine or non-proline residues are serine or alanine. Where epitopes are overlapping, a multi-epitopic region may be included rather than separate epitopes separated by flanking amino acids and linkers.

[0110] In some embodiments, a class I epitope is separated from a class II epitopes by a cleavable or non-cleavable linker. The peptide linker can be any peptide linker. In some embodiments, the linker is cleavable. In some embodiments, the linker is non-cleavable. In some embodiments, the cleavable peptide linker provides a cleavage target for both proteasomal and lysosomal degradation systems (Dolenc et al., 1998, *FEBS Letters* 434:357-361; Nezafat et al., 2014, *J. Theor. Biol.* 349:121–134). A non-limiting example used in multi-epitope designs is GPGPG (SEQ ID NO: 26). In some embodiments, the linker is a class II linker (as defined above). A class I linker, or the sequence HEYGAEALERAG (SEQ ID NO: 65) may also be used.

[0111] In some embodiments, each of the one or more nucleic acids is a messenger RNA (mRNA) molecule. Each mRNA molecule comprises a 5' untranslated region (5'UTR), a signal sequence encoding a signal peptide, a coding region (encoding the epitopes), a stop codon, a 3' untranslated region (3'UTR), and a polyadenine (polyA) tail. Each mRNA also comprises a 5' cap. In some embodiments, each mRNA comprises a cap1 structure (e.g., m7G(5')ppp(5')Am), which has a role in facilitating translation and providing resistance to nucleases. The polyA tail serves to protect the RNA from exonuclease degradation. Each of the above mRNA components is well known to the skilled person, and any functional versions of the foregoing would be effective.

[0112] The 5'UTR and 3'UTR regulate mRNA half-life and protein translation. The 5'UTR spans the sequence from the transcription start site to the start codon, but does not include the start codon. A 5'UTR has a strong Kozak sequence and a secondary structure which stabilizes the mRNA and also enables scanning by small ribosome subunit to localize the start codon. The 5'UTR may be synthetic or derived/related from the 5'UTR of an abundantly expressed gene, for example human α -globin genes (HBA1 and HBA2), or the native 5'UTR of the antigen mRNA in question. A non-limiting example of a synthetic 5'UTR is

GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGAGCCACC (SEQ ID NO:27),

with the last 9 nucleotides forming the Kozak sequence. The 3'UTR starts immediately after the stop codon and continues until the transcriptional termination signal. The poly A tail follows the 3'UTR. Sequences derived from and/or related to the 3'UTRs from α - and β -globins, which are abundantly expressed in erythrocytes, are commonly used for the 3'UTRs in mRNA therapeutics. 3'UTRs from other abundantly expressed genes of specific cell types would be expected to be favourable for stability and translation as well. The 3'UTR from the native antigen mRNA would also work. A non-limiting example of a 3'UTR is

GCUGCCUUCUGCGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCU
UUGAAUAAAGCCUGAGUAGGAAGGCGGCCGCGCUCGAGCAUGCAUCUAGA (SEQ ID NO:28).

PolyA tails may be 50-250 nt long, typically around 80-200 nt.

[0113] A non-limiting example of a signal sequence is the preproinsulin signal peptide (see Table 2A for sequences). Another non-limiting example is human tissue plasminogen activator (tPA)

signal peptide (see Table 2A for sequences). In some embodiments, the signal sequence is the preproinsulin signal sequence, which has the additional benefit of including epitopes.

[0114] RNA sequences may or may not be codon-optimized for expression in a particular subject (e.g. human or mouse). In some embodiments, the one or more nucleic acids are codon-optimized for expression in humans. In some embodiments, the one or more nucleic acids are codon-optimized for expression in mice.

[0115] Antigens/epitopes can be fused to sequences derived from cytoplasmic domains of proteins containing endolysosomal trafficking sequence motifs. The two main classes of sorting signals are tyrosine- and leucine-based signals (reviewed in Bonehill *et al.* 2005 *The Journal of Gene Medicine* 7(6): 686-695). Tyrosine based signals are cytoplasmic sequences with the consensus motif YXXØ (SEQ ID NO: 159), where X is any amino acid and Ø is an amino acid with a bulky hydrophobic side chain (e.g. Ala, Val, Leu, Ile, Phe, Met, Trp), e.g. YXXA in the case of MHC Class I trafficking domain (MITD; Lizée *et al.* 2003 *Nat Immunol* 4: 1065-1073). Leucine based signals contain an important leucine residue in the cytoplasmic domain. Clathrin-associated adaptor protein (AP) complexes interact with the aforementioned motifs to mediate localization to the organelles of the endolysosomal system. As such, fusion of antigens (single or multi-epitope or full-length antigens) with domains of these proteins improves MHC Class II presentation of antigens and CD4 T cell responses. Non-limiting examples of proteins/domains containing endolysosomal trafficking sequence motifs include: MITD, invariant chain (CD74) (Sanderson *et al.*, 1995 *Proc. Natl. Acad. Sci. USA* 92:7217–7221; Diebold *et al.*, 2001 *Gene Ther.* 8:487–493), lysosome-associated membrane protein (LAMP) 1 (Bonehill *et al.*, 2004 *J. Immunol.* 172: 6649 – 6657; Rowell *et al.*, 1995 *J. Immunol.* 155: 1818 –1828; Wu *et al.*, 1995 *Proc. Natl. Acad. Sci. USA* 92: 11671–11675; Bonini *et al.*, 2001 *J. Immunol.* 166: 5250–5257), LAMP2 (Lin *et al.*, 1993 *FASEB J.* 7: 1070–1080), and DC-LAMP (Bonehill *et al.*, 2004 *J. Immunol.* 172: 6649 – 6657). The MITD is a MHC Class I trafficking signal used to enhance targeting of the encoded protein to endosomal antigen-processing compartments in order to enhance antigen loading (to both Class I and Class II loading) (Kreiter *et al* 2008, *J. Immunol.* 180(1):309–318). MHC Class I molecules are found in all the compartments where MHC Class II processing takes place. Similar targeting strategies to promote Class II presentation involve coupling antigens to other trafficking proteins that reside in endolysosomal compartments (e.g. invariant chain) or traffic to late

endosomal antigen-processing compartments upon DC activation (e.g. cathepsins) or complexes that participate in MHC class II processing (e.g. H2-M; Lappazio et al., 2021, *Vaccines* 9, 1053). In some embodiments, the at least one nucleic acid encodes a polypeptide comprising an endolysosomal trafficking sequence motif. In some embodiments, the endolysosomal trafficking sequence motif is from MITD, invariant chain, LAMP1, LAMP2, or DC-LAMP. In some embodiments, the polypeptide comprising the endolysosomal trafficking sequence motif comprises MITD, invariant chain, LAMP1, LAMP2, or DC-LAMP. In some embodiments, the polypeptide comprising the endolysosomal trafficking sequence motif is MITD. In some embodiments, the MITD sequence is human MITD (SEQ ID NO:55). Each of the references in this paragraph are incorporated by reference in their entirety.

[0116] Table 2A provides RNA and amino acid sequences for a list of exemplary (non-limiting) epitopes, linkers and MITD. In some embodiments, the epitopes comprise the full length or a shorter epitopic fragment of any one or more of those epitopes listed in Table 2A. Portions of the sequences flanking the epitopes (i.e. flanking residues) are shown underlined.

[0117] Table 2A: Sequence list for exemplary epitopes, linkers, and MITD.

Category	Name	Amino acid sequence	RNA sequence
Signal peptide (mouse)	PPI2 signal peptide	MALWMRFLPLLALLF LWESHPTQA (SEQ ID NO:29)	AUGGCCUGUGGAUGAGAUUCCUGCCUCUGCUGGC UCUGCUGUCCUGUGGGAGUCUCACCCUACACAGG CU (SEQ ID NO:30)
Signal peptide (human)	tPA signal peptide	MDAMKRGLCCVLLLC GAVFVSA (SEQ ID NO:31)	AUGGACGCUAUGAAGCGGGGCCUCUGCUGUGUUCU GCUUCUGUGCGUGUGUGUUUUUGUGAGUGCU (SEQ ID NO:32)
Class I epitope (mouse)	InsB 8-30	<u>GSHLVEALYLVCGER</u> <u>GFFYTPMS</u> (SEQ ID NO:33)	<u>GGCUCUCACCUGGUGGAAGCUCUGUAUCUCGUGUG</u> <u>CGGCGAGAGAGGCUUCUUCUACACACCUAUGAGC</u> (SEQ ID NO:34)
Class I epitope (mouse)	IGRP 199-221	<u>VHMASLSVYLKTNVF</u> <u>LFLFALGF</u> (SEQ ID NO:35)	<u>GUGCACAUGGCUAGCCUGUCUGUGUACCUCAGAC</u> <u>CAACGUGUCCUGUUUCUGUUCGCCCUGGGCUUC</u> (SEQ ID NO:36)
Class I epitope (mouse)	GAD65 539-561	<u>EYGTMTVSYQPLGDK</u> <u>VNFFRMVI</u> (SEQ ID NO:37)	<u>GAGUACGGCACAACCAUGGUGUCCUACCAGCCUCU</u> <u>GGGCGACAAAGUGAACUUCUUCAGAAUGGUCAUC</u> (SEQ ID NO:38)
Class I epitope (mouse)	ZnT8 179-201	<u>ITVSGCAVAANIVLT</u> <u>MILHQRF</u> (SEQ ID NO:39)	<u>AUAACAGUUUCCGGCUGCGCUGUUGCUGCCAACAU</u> <u>CGUCUUGACAAUGAUCUUGCAUCAGCGAAAUUUU</u> (SEQ ID NO:40)
Class II epitope (mouse)	2.5HIP	<u>GGPGAGDLQTLALWS</u> <u>RMDQLAKELT</u> (SEQ ID NO:41)	<u>GGAGGACCAGGUGCUGGCGAUCUUCAGACUCUGGC</u> <u>UCUGUGGUCCAGAAUGGACCAGCUGGCCAAAGAGC</u> <u>UGACA</u> (SEQ ID NO:42)

Class II epitope (mouse)	InsB 2-30	<u>VKQHL</u> CGSHLVEALY LVCGERGFFYTPMS (SEQ ID NO:43)	GUGAAGCAGCACCUGUGUGGAUCCCAUCUGGUCGA GGCCCUUGUACCUUGUGUGUGGCGAACGGGGAUUCU <u>UUUACACCCCAAUGUCU</u> (SEQ ID NO:44)
Class II epitope (mouse)	GAD65 199-227	TANTNMFTYEIAPVF <u>VLLEYVTLK</u> KMRE (SEQ ID NO:45)	ACCGCCAACACCAACAUGUUCACCUACGAGAUCGC CCCUGUGUUCGUGCUGCUGGAAUACGUG <u>ACCCUGA</u> <u>AGAAGAUGAGAGAA</u> (SEQ ID NO:46)
Class II epitope (mouse)	GAD65 279-307	<u>EHS</u> HFSLLKKGAAALG IGTDSVILIKDER (SEQ ID NO:47)	GAGCACUCUCACUUCAGUCUGAAAAAGGGCGCUGC CGCUCUCGGCAUCGGCACAGAUUCUGUGAUCCUGA <u>UCAAGUGCGACGAAAGA</u> (SEQ ID NO:48)
Class II epitope (mouse)	HSP60 430-467	AAVEEGIVLGGGICAL LRCIPALDSLKPANE <u>DQKIGIEI</u> (SEQ ID NO:49)	GCUGCUGUGGAAGAGGGAAUUGUGCUUGGAGGCGG CUGCGCCUGCUGAGGUGUAUCCUGCUCUGGACU CUCUGAAGCCCGCCAACGAGGAUCAGAAGAUCGGC AUCGAGAU (SEQ ID NO:50)
Class II epitope (mouse)	IA-2b 748-784	<u>PNSSLVAQ</u> REENAPK NRSLAVLTYDHSRI <u>LLKSQNS</u> (SEQ ID NO:51)	CCUAAUAGCUCUCUGGUGGCCAGAGAGAGGAAAA CGCCCUAAGAACAGAUCCUGGCCGUGCUGACAU ACGACCACGCCUCUAGAAUCCUGCUGAAGUCCAG AACUCC (SEQ ID NO:52)
Class II epitope (mouse)	ZnT8338-366	<u>SSF</u> DLHSLTIQIESA ADQDPSCLLCEDPQ (SEQ ID NO:53)	AGCAGUUUUGACCUGCAUUCCUCACAAUCCAAAU UGAGAGCGCCGCCGACCAAGAUCCAGUUGCCUGC <u>UGUGCGAAGAUCCUCAA</u> (SEQ ID NO:54)
MITD (human)	MITD	<u>IVGIVAGLAVLAVVV</u> IGAVVATVMCRKSS GGKGGSYSQAASSDS <u>AQGS</u> DSVSLTA (SEQ ID NO:55)	AUCGUGGGCAUCGUGGCUGGACUGGCUGUGCUGGC AGUGGUGGUUAUUGGAGCCGUGGUGGCCACAGUGA UGUGCAGAAGAAAGAGCAGCGCGGCAAAGGCGGC AGCUAUUCUCAGGCCGCCUCUAGCGAUUCUGCCCA GGGCUCUGACGUGUCUCUGACAGCU (SEQ ID NO:56)
Class I Linker (synthetic)	Class I Linker	AAY (SEQ ID NO:23)	GCCGCCUAC (SEQ ID NO:57) or GCCGCUUAC (SEQ ID NO:58)
Class II Linker (synthetic)	Class II Linker	GPGPG (SEQ ID NO:26)	GGCCCUGGACCUGGC (SEQ ID NO:59) or GGACCUGGACCAGGC (SEQ ID NO:60) or GGCCCAGGACCAGGC (SEQ ID NO:61) or GGACCCGGACCUGGG (SEQ ID NO:62) or GGCCCUGGACCAGGG (SEQ ID NO:63) or GGUCCCGGACCAGGA (SEQ ID NO:64)
Alternative Linker (synthetic)	Alternative Linker	HEYGAELERAG (SEQ ID NO:65)	CACGAUACGGUGCCGAAGCACUCGAACGCGCAGG G (SEQ ID NO:66)

[0118] Table 2B provides counterpart sequences for mouse and human. In some embodiments, the epitopes comprise the full length or a shorter epitopic fragment of any one or more of those human epitopes listed in Table 2B. Portions of the sequences flanking the epitopes (i.e. flanking residues) are shown underlined.

[0119] Table 2B: Mouse and corresponding human sequences.

Category (Antigen)	Name (Mouse)	Amino acid sequence (Mouse)	Name (Human epitope of same antigen)	Human sequence
Signal Peptide (Preproinsulin)	PPI2 signal peptide	<u>MALWMRFLPLLALL</u> <u>FLWESHPTQA</u> (SEQ ID NO:29)	PPI signal peptide	<u>MALWMRLLPLLALLAL</u> <u>WGPDPAAA</u> (SEQ ID NO:67)
Class I epitope (Insulin B chain)	InsB 8-30	<u>GSHLVEALYLVCGE</u> <u>RGFFYTPMS</u> (SEQ ID NO:33)	B3-B25	<u>NQHLCGSHLVEALYL</u> <u>VCGERGFF</u> (SEQ ID NO:68)
Class I epitope (IGRP)	IGRP 199-221	<u>VHMASLSVYLKTNV</u> <u>FLFLFALGF</u> (SEQ ID NO:35)	IGRP257-280	<u>GLVRNLGVLFGLGFAINSE</u> <u>MFL</u> (SEQ ID NO:69)
Class I epitope (GAD65)	GAD65 539-561	<u>EYGTMTVSYQPLGD</u> <u>KVNFFRMVI</u> (SEQ ID NO:37)	GAD530-552	<u>APVIKARMMEYGTMTVS</u> <u>YQPLGD</u> (SEQ ID NO:70)
Class I epitope (ZnT8)	ZnT8 179-201	<u>ITVSGCAVAANIVL</u> <u>TMILHQRNF</u> (SEQ ID NO:39)	ZnT8 177-201	<u>MIIVSS(L/C)AVAANIVLT</u> <u>VVLHQRCL</u> (SEQ ID NO:71)
Class II epitope (Hybrid Insulin Peptide)	HIP2.5	<u>GGPGAGDLQTLALW</u> <u>SRMDQLAKELT</u> (SEQ ID NO:41)	An Ins hybrid epitope for example: C-pep:InsA C-pep:IAPP2 C-pep:NP-Y C-pep:IAPP1	<u>EAEDLQVGQVELGGG</u> <u>IVEQCCTSICSLY</u> (SEQ ID NO:72) <u>EAEDLQVGQVELGGGN</u> <u>AVEVLKREPLNYL</u> (SEQ ID NO:73) <u>EAEDLQVGQVELGGGS</u> <u>SPETLISDLLMRE</u> (SEQ ID NO:74) <u>EAEDLQVGQVELGGGT</u> <u>PIESHQVEKRRCN</u> (SEQ ID NO:75)
Class II epitope (Insulin B chain)	InsB 2-30	<u>VKQHLCGSHLVEAL</u> <u>YLVCGERGFYTPM</u> <u>S</u> (SEQ ID NO:43)	Ins B1-30	<u>FVNQHLCGSHLVEALY</u> <u>LVCGERGFYTPKT</u> (SEQ ID NO:76)
Class II epitope (GAD65)	GAD65 199-227	<u>TANTNMFTYEIAPV</u> <u>FVLLEYVTLKKMRE</u> (SEQ ID NO:45)	GAD65 194-226	<u>DWLTSTANTNMFTYEIAP</u> <u>VFVLEYVTLKKMRE</u> (SEQ ID NO:77)
Class II epitope (GAD65)	GAD65 279-307	<u>EHSFSLKKGAAAL</u> <u>GIGTDSVILIKCDE</u> <u>R</u> (SEQ ID NO:47)	GAD274-310	<u>IAFTSEHSFSLKKGAAAL</u> <u>GIGTDSVILIKCDE</u> <u>R</u> (SEQ ID NO:78)

Class II epitope (HSP60)	HSP60 430-467	<u>AAVEEGIVLGGGCA</u> <u>LLRCIPALDSLKPA</u> <u>NEDQKIGIEI</u> (SEQ ID NO:49)	HSP60 429-462	<u>RAAVEEGIVLGGGCALLR</u> <u>CIPALDSLTPANEDQK</u> (SEQ ID NO:79)
Class II epitope (option of IA-2 or IA-2beta)	IA-2b 748-784 or IA-2 700-730	<u>PNSSLVAQREENAP</u> <u>KNRSLAVLTYDHAS</u> <u>RILLKSQNS</u> (SEQ ID NO:51) or <u>EDHLNRDRDLAKEW</u> <u>QALCAYQAEPNTCA</u> <u>AAQ</u> (SEQ ID NO:80)	IA-2 (PTPRN)703-738	<u>HLRNRDRDLAKEWQALC</u> <u>AYQAEPNTCATAQGEG</u> <u>NIKKN</u> (SEQ ID NO:81)
Class II epitope (ZnT8)	ZnT8 338-366	<u>SSFDLHSLTIQIES</u> <u>AADQDPSCLLCEDP</u> <u>Q</u> (SEQ ID NO:53)	ZnT8 330-369	<u>VRREIAKALSKSFTMH</u> <u>SLTIQMESPVDQDPDC</u> <u>LFCEDPDC</u> (SEQ ID NO:82)
Epitope (insulin component of insulin hybrid peptide)	PPI2 1-82	<u>MALWMRFLPLLALL</u> <u>FLWESHPTQAFVKQ</u> <u>HLCGSHLVEALYLV</u> <u>CGERGFFYTPMSRR</u> <u>EVEDPQVAQLELGG</u> <u>GPGAGDLQTLAL</u> (SEQ ID NO:83)	INS(PPI)1-71	<u>MALWMRLLPLLALLAL</u> <u>WGPDPAAAFVNQHL</u> <u>CGSHLVEALYLVCGER</u> <u>GFYTPKTRREAEDLQV</u> <u>GQVELGGG</u> (SEQ ID NO:84)
Epitope (other component of insulin hybrid peptide)	CHGA 357-370	<u>WSRMDQLAKELTA</u> (SEQ ID NO:85)	IAPP2 fragment	<u>NAVEVLKREPLNYLPL</u> (SEQ ID NO:86)
			Neuropeptide Y fragment	<u>SSPETLISDLLMRE</u> (SEQ ID NO:87)
			Insulin A fragment	<u>GIVEQCCTSI</u> <u>CSLYQLENYCN</u> (SEQ ID NO:88)
			IAPP1 fragment	<u>TPIESHQVEKRCNTA</u> <u>TCAT</u> (SEQ ID NO:89)

[0120] In some embodiments, the epitopes are selected from the epitopes listed in James et al., T-Cell Epitopes and Neo-epitopes in Type 1 Diabetes: A Comprehensive Update and Reappraisal, *Diabetes* 2020;69(7):1311–1335, which is incorporated by reference, and/or from the epitopes listed in Table 3A, identified by their Immune Epitope Database (IEDB) ID number, an identifier that links to a public database of epitopes available online at https://www.iedb.org/home_v3.php. In some embodiments, the one or more nucleic acids encodes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of those listed in Table 3A; in some embodiments, the epitopes are from at least 2 different T1D autoantigens. Table 3B lists human epitopes in several other autoimmune diseases, identified by IEDB ID. In some embodiments, the one or more nucleic acids encodes 2, 3, 4, 5, 6,

7, 8, 9, 10, 11, 12, 13, 14, or 15 of those listed for a single autoimmune disease in Table 3B; in some embodiments, the epitopes are from at least 2 different antigens/autoantigens.

[0121] Table 3A: Human epitopes in T1D, identified by IEDB ID

1817,4395,4919,5947,6101,7560,7692,9070,9144,10091,10460,10461,10462,11360,12318,12787,13740,13741,14375,14733,15450,17277,17319,18247,19534,19535,21591,21626,22141,24314,25247,25275,28221,34554,38521,38563,38564,40453,41153,41154,41699,42921,45043,46426,50826,54857,54943,55556,56857,58388,60025,60974,61551,65621,65623,66549,67328,67925,67926,67927,67928,70902,74043,74105,74595,75980,76008,76171,76496,78105,78106,78120,78124,78125,78128,78129,78130,78131,78132,78133,78167,78168,78319,78320,78970,79004,79540,79553,79579,79582,79612,79901,95972,99134,100311,100725,100843,100844,100845,100882,100920,100981,101025,101026,101054,101067,101199,101237,101241,101242,101243,101245,101248,101469,101509,101609,101931,102085,102216,102287,102289,102294,102301,102306,102372,102379,102382,102433,102451,102454,102480,102491,102515,102517,102522,102537,102548,102549,102553,102579,102589,102599,102617,102619,102620,102642,102650,102655,102657,102675,102684,102687,102707,102713,102717,102728,102736,102750,102758,102765,102769,102775,102782,102794,102811,102842,102844,102846,102872,102877,102878,102889,102897,102899,102908,102911,102914,102918,102974,103035,103041,103164,103167,103168,103191,103219,103306,103321,103335,103368,103379,103380,103394,103406,103455,103556,103557,103560,103682,103705,103706,103728,103791,103803,103806,103808,103818,103823,103824,103829,103867,103868,103869,103885,103893,103908,103913,103917,103921,103922,103926,103939,103948,103952,103953,103968,103971,103978,104029,104030,104062,104085,104114,104118,104140,104141,104150,104163,104177,104189,104195,104204,104218,104234,104248,104316,104317,104325,104326,104336,104352,104361,104403,104413,104437,104441,104447,104452,104457,104481,104493,104502,104510,104511,104522,104523,104546,104547,104552,104566,104575,104578,104589,104590,104602,104603,104618,104620,104626,104629,104630,104649,104662,104666,104677,104695,104759,104767,104808,104844,104862,104895,104945,105004,105059,105120,105133,105161,105169,105172,105173,105191,105216,105261,105271,105275,105281,105302,105409,105471,105488,105563,105617,105674,105736,105766,105799,105816,105936,105950,105961,106039,106063,106096,106187,106206,106399,106593,106631,106637,107579,108307,108318,108319,108320,108321,108324,108326,108368,108369,108370,108490,108495,108511,108530,108531,108537,108677,108678,114753,114829,115182,115337,115471,115871,115919,116345,118268,118749,119442,119539,119541,119755,119774,119800,119807,119808,119819,119840,119848,119919,119931,119963,119966,119986,120079,120080,120088,120106,120111,120114,120115,120139,120160,120203,120222,120337,124997,125107,129349,129586,129587,130153,131210,131428,131748,134060,134118,134126,134229,134232,134238,134453,138762,138770,138777,138779,138780,138782,138808,139210,140054,140694,142006,142421,142482,142554,142591,142674,142717,142728,142730,142807,142811,142953,143023,143044,143056,143126,143155,143224,143226,143788,144204,144237,144239,144244,144253,144371,144419,144454,144472,144473,144498,144516,144546,146163,146837,146851,146855,146875,146893,147016,147076,147316,147339,150802,150806,152412,152449,152512,152515,152635,152653,152659,152681,152862,152951,152978,153060,153093,153133,153150,153845,153877,156616,156690,156760,156805,157610,158453,159292,159293,161850,161920,161952,162001,162014,162015,162016,162022,162023,162024,162026,162027,162043,162044,162060,162062,162064,162066,162069,162072,162073,162077,162078,162079,162080,162084,162085,162086,162087,162088,162089,162091,162093,162094,162108,162110,162121,162125,162126,162144,162146,162153,162157,162158,162159,162162,162163,162164,162166,162167,162168,162169,162171,162174,162178,162182,162195,162196,162199,162205,162206,162207,162208,162209,162246,162256,162257,162258,162262,162263,162264,162265,162266,162269,162270,162275,162378,162389,162392,162393,162394,162395,162396,162399,162402,162422,162423,162438,162451,162455,162457,162458,162459,162460,162475,162481,162482,162520,162546,162556,162564,162572,162573,162574,162576
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,162577,162583,162584,162589,162668,162704,162713,162765,162767,162768,162769,162778,162780,162781,162782,162783,162784,162785,162823,162824,162831,162853,162863,162864,162865,162866,162867,162868,162869,162881,162890,162892,162900,162928,162929,162930,162931,162932,162933,162940,162941,162943,162944,162945,162946,162947,162948,162949,162950,162951,162952,162953,162954,162955,162956,162957,162958,162960,162969,162997,163003,163005,163018,163020,163029,163030,163041,163054,163059,163060,163061,163062,163063,163076,163077,163078,163079,163080,163118,163119,163120,163146,163149,163150,163158,163239,163306,163373,163375,163392,163408,163411,163432,163439,163481,163528,163529,163531,163532,163541,163542,163544,163546,163554,163563,163580,163609,163630,163646,163648,163690,163691,163693,163694,163695,163696,163697,163698,163701,163702,163703,163704,163706,163707,163721,163766,163767,163781,163835,163841,163844,163845,163846,163847,163848,163849,163850,163851,163852,163853,163854,163855,163861,163866,163878,163884,163885,163888,163919,163922,163923,163926,163932,163934,163935,163936,163937,163941,163956,163957,163958,163959,163968,163976,163980,163981,163982,163983,163984,163985,163986,163987,163988,163989,163990,163994,163996,163999,164002,164003,164004,164006,164007,164008,164011,164012,164014,164020,164021,164024,167105,167110,167159,167160,167166,167181,168159,168235,168356,168451,168578,168764,168865,168867,168874,168880,168881,169149,169153,169725,169744,169790,170038,171037,171556,171844,171933,172015,172188,173061,173491,173562,174364,174366,174367,174376,174377,174379,174381,174385,174388,174399,174412,174440,174456,174484,174486,174496,174503,174513,174521,174524,174539,174542,174585,174610,174632,174637,174649,174655,174661,174667,174677,174694,174702,174706,174707,174710,174730,174732,174742,174743,174745,174759,174771,174788,174789,174790,174824,175775,175791,175831,175877,175878,175881,176355,176696,176769,177006,177012,177025,177035,177040,177106,177171,177189,177247,177259,177262,177265,177290,177322,177366,177585,177593,177606,177734,177779,177987,181328,186664,186680,186722,186725,186990,186992,186995,186999,187001,187007,187013,187017,187026,187030,187031,187032,187035,191238,191508,191512,191540,191546,191557,191663,191716,191717,191720,191728,191932,193721,193812,193944,193955,194255,194352,194404,194419,194483,194556,194603,194659,196400,196407,196419,196420,196439,196445,196459,196523,196701,196706,196713,196731,196810,196914,196915,196945,196952,197661,199660,200461,200960,200965,200969,200997,201137,201402,201529,201616,201721,201723,201862,202970,203088,203351,203376,203410,203411,203438,203916,204259,204292,204308,204311,204312,204322,204568,204577,204608,204609,204647,204716,204719,204720,204729,204766,204905,204944,205044,205132,205133,205145,205175,205296,205310,205320,205329,205331,205332,205335,205336,205340,205366,205379,205380,205399,205412,205423,205424,205489,205503,205559,205683,205802,205918,206149,206223,206229,206276,206277,206291,206300,206329,206453,206475,206477,206487,206518,206520,206606,206644,206647,206651,206658,206697,206712,206751,206834,206898,206926,206927,207031,207032,207034,207179,207203,207204,207206,207207,207331,207339,207343,207356,207358,207361,207364,207366,207371,207374,207386,207396,207412,207433,207437,207438,207504,207518,207654,207705,207750,207775,207856,207884,207978,207999,208085,208090,208110,208417,208423,208555,208626,208627,208665,208758,208904,208959,209080,209112,210189,211091,211145,211392,211405,211406,211441,211447,211448,211453,211455,211475,211488,211496,211515,211538,211547,211552,211587,211589,211592,211628,211780,211855,211926,211945,212031,212051,212173,212207,212236,212251,212263,212301,212354,212360,212387,212419,212554,212673,212845,212873,213093,213142,213274,213464,213520,213604,213706,213998,214033,214079,214284,214309,214321,214343,214409,214489,214519,214590,214592,214595,214721,215149,215311,215314,215326,215490,215498,215797,215831,215832,215836,215841,215845,215864,215867,215868,215871,215878,215889,215901,215925,215964,215967,215975,215980,215983,215984,215985,215988,215990,216018,216021,216044,216048,216058,216061,216086,216134,216140,216191,216204,216868,216877,216940,216941,216993,217009,217034,217091,217120,217141,217162,217168,217169,217191,217301,217312,217352,217370,217380,217879,217950,217985,218010,218199,218232,218301,218315,218323,218502,218688,218835,218840,218870,218886,218998,219034,219076,219128,219139,219190,219192,219193,219325,219328,219336,219396,21

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367,220401,220408,220414,220419,220431,220436,220700,220708,220721,220774,220775,220776,2
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25700,226392,226402,226405,226869,227039,228905,229882,229895,229910,230391,230397,230399
,230405,230433,231856,231872,231904,232106,233467,233610,233611,233892,234027,234028,2340
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4,234115,234138,234140,234141,234142,234143,234146,234147,234149,234150,234151,234160,234
162,234163,234164,234167,234212,234213,234282,234297,234298,234299,234301,234302,234305,2
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41,236242,236300,236691,236710,236737,236767,236768,236794,236796,236797,236798,236800,23
6802,236805,236811,236812,236816,236826,236828,236838,236841,236846,236860,236864,236875,
236887,236896,236897,236898,236900,236937,236947,236959,236960,237001,237003,237009,23701
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172,237190,237210,237218,237220,237223,237241,237242,237243,237247,237248,237249,237286,2
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,237477,237478,237480,237495,237497,237507,237514,237531,237535,237562,237563,237572,2375
88,237590,237591,237592,237593,237598,237599,237602,239478,239517,239518,239539,239540,23
9546,239547,239548,239549,239567,239569,239570,239571,239572,239608,239610,239613,239626,
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270,240277,240334,240336,240339,240344,240544,240545,240572,240602,240606,240607,240608,2
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,240669,240680,240684,240700,240702,240718,240724,240728,240732,240755,240756,240757,2407
60,240806,240816,241356,241358,241396,241473,241491,241542,241544,241670,242210,242328,24
2331,243078,243136,243236,243806,244101,244127,244260,244283,244307,418054,418057,418072,
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,418460,418474,418481,418506,418507,418508,418513,418515,418516,418517,418518,418519,4185
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8,419139,419145,419162,419195,419196,419214,419234,419278,419389,419544,419632,419731,419
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20287,420303,420329,420399,420406,420408,420615,421964,421974,422018,422020,422069,422072
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4,1855295,1855300,1855321,1855329,1855336,1855364,1855365,1855366,1855367,1855373,185537
6,1855377,1855384,1855385,1855386,1855393,1855394,1855401,1855403,1855404,1855407,185540
8,1855411,1855412,1855413,1855414,1855417,1855420,1855425,1855430,1855431,1855432,185543
4,1855441,1855442,1855443,1855444,1855445,1855446,1855447,1855450,1855451,1855452,185545
3,1855454,1855455,1855456,1855457,1855458,1855459,1855460,1855461,1855464,1855465,185546
6,1855467,1855469,1855470,1855473,1855474,1855475,1855476,1855477,1855478,1855489,185549
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6,1855607,1855610,1855611,1855612,1855615,1855616,1855617,1855618,1855619,1855631,185563
7,1855644,1855646,1855651,1855652,1855664,1855677,1855678,1855679,1855704,1855708,185572
0,1855725,1855726,1855727,1855733,1855735,1855737,1855740,1855744,1855746,1855747,185574
9,1855794,1855795,1855797,1855806,1855815,1855816,1855817,1855818,1855819,1855820,185582
1,1855822,1855823,1855824,1855825,1855826,1855832,1855838,1855875,1855876,1855878,185587
9,1855881,1855887,1855888,1855890,1855891,1855892,1855906,1855935,1855951,1855958,185595
9,1855960,1855961,1855962,1855963,1855971,1855972,1855973,1855974,1855977,1855978,185599
6,1855999,1856001,1856002,1856004,1856005,1856006,1856014,1856015,1856018,1856019,185602
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7,1856078,1856079,1856081,1856082,1856083,1856084,1856089,1856095,1856097,1856098,185614
3,1856171,1856172,1856179,1856180,1856187,1856188,1856216,1856223,1856225,1856232,185623
9,1856240,1856249,1856284,1856285,1856296,1856297,1856300,1856301,1856303,1856304,185630
5,1856306,1856307,1856309,1856310,1856313,1856314,1856317,1856325,1856328,1856330,185633
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4,1856606,1856608,1856609,1856610,1856619,1856620,1856624,1856627,1856628,1856629,185663
7,1856638,1856639,1856641,1856644,1856646,1856649,1856650,1856653,1856660,1856664,185667
2,1856673,1856674,1856677,1856679,1856680,1856684,1856688,1856691,1856698,1856710,185671
1,1856737,1856753,1856754,1856781,1856791,1856794,1856809,1856812,1856813,1856815,185681
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7,1856848,1856849,1856850,1856851,1856852,1856857,1856863,1856864,1856866,1856867,185686

8,1856869,1856870,1856874,1856875,1856876,1856879,1856880,1856886,1856887,1856888,185689
3,1856894,1856895,1856896,1856897,1856901,1856902,1856903,1856904,1856906,1856907,185690
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2,1857006,1857021,1857023,1857024,1857025,1857026,1857027,1857029,1857030,1857031,185703
2,1857039,1857043,1857044,1857045,1857050,1857051,1857052,1857058,1857086,1857087,185708
8,1857089,1857091,1857100,1857116,1857120,1857121,1857124,1857126,1857129,1857130,185713
1,1857133,1857134,1857135,1857136,1857137,1857139,1857140,1857141,1857142,1857143,185714
4,1857145,1857148,1857149,1857150,1857151,1857157,1857167,1857169,1857175,1857176,185718
1,1857229,1857239,1857255,1857267,1857268,1857271,1857273,1857274,1857276,1857277,185728
2,1857294,1857296,1857297,1857298,1857300,1857301,1857302,1857304,1857305,185730
6,1857307,1857308,1857311,1857313,1857314,1857315,1857316,1857317,1857318,1857319,185732
0,1857321,1857322,1857324,1857325,1857326,1857327,1857328,1857329,1857330,1857331,185733
3,1857334,1857335,1857336,1857337,1857338,1857339,1857340,1857341,1857343,1857344,185734
5,1857346,1857347,1857348,1857349,1857351,1857352,1857354,1857355,1857356,1857357,185735
8,1857359,1857360,1857362,1857370,1857371,1857379,1857412,1857429,1857431,1857438,185744
0,1857453,1857475,1857479,1857481,1857494,1857515,1857516,1857525,1857527,1857528,185753
1,1857532,1857537,1857539,1857558,1857570,1857571,1857572,1857573,1857574,1857575,185757
6,1857580,1857589,1857601,1857602,1857603,1857604,1857611,1857612,1857613,1857614,185761
5,1857617,1857620,1857622,1857631,1857633,1857635,1857636,1857637,1857644,1857645,185764
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6,1857687,1857688,1857689,1857690,1857695,1857705,1857719,1857723,1857739,1857743,185774
4,1857747,1857757,1857759,1857765,1857775,1857783,1857785,1857791,1857798,1857806,185781
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9,1857850,1857855,1857873,1857877,1857878,1857882,1857920,1857930,1857931,1857932,185793
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5,1858136,1858139,1858140,1858146,1858148,1858149,1858150,1858151,1858152,1858154,185815
5,1858157,1858158,1858159,1858160,1858161,1858162,1858163,1858164,1858165,1858166,185816
7,1858168,1858170,1858171,1858172,1858173,1858174,1858175,1858177,1858178,1858179,185818
1,1858183,1858184,1858185,1858187,1858188,1858189,1858190,1858191,1858225,1858226,185822
8,1858229,1858230,1858247,1858279,1858294,1858295,1858296,1858297,1858299,1858300,185830
1,1858302,1858303,1858321,1858337,1858370,1858371,1858372,1858376,1858377,1858378,185838
0,1858381,1858382,1858390,1858403,1858404,1858416,1858417,1858425,1858427,1858435,185844
0,1858441,1858442,1858446,1858447,1858448,1858451,1858452,1858460,1858461,1858462,185847
1,1858473,1858477,1858478,1858479,1858483,1858488,1858490,1858491,1858492,1858493,185849
4,1858495,1858503,1858510,1858516,1858517,1858518,1858523,1858524,1858527,1858529,185853
1,1858534,1858535,1858546,1858548,1858549,1858550,1858553,1858557,1858559,1858564,185856
6,1858580,1858584,1858624,1858625,1858627,1858634,1858642,1858644,1858654,1858663,185867
3,1858680,1858682,1858684,1858686,1858688,1858689,1858690,1858700,1858702,1858706,185870
7,1858708,1858709,1858711,1858714,1858716,1858719,1858721,1858724,1858729,1858733,185873
4,1858740,1858741,1858744,1858745,1858746,1858747,1858750,1858753,1858768,1858769,185877
0,1858771,1858772,1858774,1858776,1858785,1858786,1858807,1858808,1858810,1858819,185882
1,1858822,1858831,1858833,1858834,1858837,1858844,1858845,1858846,1858847,1858848,185885
6,1858857,1858870,1858872,1858873,1858884,1858885,1858886,1858887,1858889,1858894,185889
5,1858896,1858898,1858905,1858907,1858908,1858909,1858910,1858913,1858915,1858922,185895
4,1858963,1858966,1858967,1858968,1858969,1858970,1858972,1858974,1858975,1858976,185897
9,1858985,1858990,1858992,1859012,1859017,1859050,1859059,1859061,1859071,1859092,185909
6,1859103,1859105,1859106,1859107,1859109,1859110,1859120,1859121,1859122,1859123,185912
4,1859125,1859126,1859127,1859129,1859131,1859140,1859141,1859142,1859143,1859144,185914

5,1859146,1859147,1859160,1859161,1859179,1859182,1859184,1859194,1859195,1859212,1859227,1859231,1859235,1859241,1859252,1859257,1859263,1859266,1859268,1859269,1859295,1859296,1859310,1859317,1859321,1859322,1859337,1859368,1859372,1859373,1859374,1859375,1859376,1859377,1859378,1859382,1859383,1859384,1859385,1859386,1859387,1859388,1859389,1859390,1859391,1859392,1859393,1859395,1859396,1859397,1859398,1859399,1859401,1859402,1859403,1859404,1859405,1859406,1859407,1859408,1859409,1859410,1859412,1859413,1859443,1859459,1859463,1859478,1859481,1859482,1859483,1859484,1859489,1859490,1859491,1859501,1859519,1859522,1859523,1859559,1859575,1859597,1859611,1859622,1859624,1859625,1859626,1859627,1859628,1859629,1859631,1859644,1859651,1859652,1859653,1859659,1859660,1859661,1859663,1859664,1859665,1859666,1859667,1859668,1859673,1859675,1859676,1859677,1859678,1859679,1859680,1859681,1859682,1859683,1859685,1859686,1859687,1859688,1859690,1859693,1859695,1859697,1859698,1859713,1859733,1859744,1859748,1859749,1859750,1859796,1859797,1859807,1859829,1859831,1859845,1859859,1859860,1859864,1859873,1859874,1859876,1859877,1859881,1859888,1859907,1859925,1859926,1859948,1859952,1859955,1859961,1859976,1859978,1859979,1859989,1859990,1859991,1859992,1859993,1859994,1859995,1859996,1859998,1859999,1860001,1860002,1860003,1860004,1860005,1860006,1860007,1860017,1860019,1860020,1860031,1860032,1860036,1860043,1860044,1860058,1860063,1860064,1860125,1860126,1860127,1860128,1860129,1860130,1860131,1860141,1860145,1860152,1860157,1860158,1860159,1860160,1860161,1860162,1860174,1860176,1860177,1860179,1860201,1860202,1860204,1860207,1860210,1860211,1860216,1860217,1860225,1860226,1860227,1860228,1860229,1860231,1860243,1860244,1860245,1860246,1860247,1860254,1860255,1860256,1860259,1860271,1860272,1860274,1860278,1860279,1860280,1860282,1860287,1860288,1860311,1860312,1860317,1860324,1860325,1860327,1860329,1860330,1860335,1860346,1860349,1860351,1860352,1860357,1860358,1860360,1860363,1860364,1860372,1860376,1860379,1860381,1860386,1860388,1860389,1860394,1860399,1860400,1860407,1860411,1860421,1860422,1860433,1860452,1860454,1860455,1860456,1860458,1860461,1860464,1860465,1860466,1860468,1860471,1860473,1860474,1860475,1860476,1860478,1860479,1860480,1860481,1860506,1860513,1860514,1860532,1860538,1860545,1860557,1860558,1860582,1860585,1860590,1860604,1860612,1860613,1860637,1860640,1860657,1860659,1860663,1860664,1860665,1860666,1860667,1860668,1860669,1860670,1860675,1860677,1860679,1860688,1860689,1860690,1860691,1860705,1860715,1860739,1860741,1860742,1860745,1860746,1860747,1860750,1860753,1860754,1860755,1860756,1860766,2028608,2031654,2033523,2045055,2058839,2144892,2145186,2157207,2157261,2157280,2165801,2185798,2185799,2185800,2185801,2185802,2185805,2186278,2186461,2232975

[0122] Table 3B: Human epitopes in various autoimmune diseases, identified by IEDB ID

Multiple Sclerosis
<i>Human MOG MHC Class I (17 entries)</i>
114291,114368,114944,115626,117289,117317,117321,117361,117367,117382,117397,174906,174912,174993,175055,175080,950277
<i>Human MBP MHC Class I (318 entries)</i>
4706,4707,4708,4710,4715,8112,10673,13572,16818,26092,45514,54050,58971,59472,65061,67907,68846,71460,71685,79827,111192,112152,112163,112191,112393,112454,112782,112844,112977,113151,113286,113430,113507,113527,113942,114113,114133,114210,114541,114571,114595,114689,114741,115061,115260,115647,115654,115655,115662,116109,116316,116320,116339,117754,117756,117758,117780,117822,117889,117890,117892,117893,117894,117896,117898,117903,117912,117915,117927,117971,117997,118006,118015,118065,118069,118084,118093,118110,118122,118125,118136,118149,118150,118152,118153,118159,118180,118181,118183,118233,118831,119027,119809,120169,120201,120238,159300,234127,234136,234168,234173,241205,243795,243797,243834,447992,492178,541961,541967,544528,565232,565664,657631,664280,684994,688203,701102,837483,838

258,849139,883833,884771,885656,934290,934350,934732,934972,935028,936279,936514,936581,936582,936763,936801,937113,937114,937185,937186,937187,937410,937411,937412,937413,937414,937463,937464,937609,937721,937722,937723,937724,938311,938402,938403,938453,938454,938564,938700,938800,938801,938802,938803,938804,938805,939918,941426,941497,941498,942078,942179,942180,942181,942182,942223,942600,942628,943809,943846,943847,943848,943849,1018611,1018612,1019173,1052185,1083857,1084378,1088449,1088450,1088451,1088452,1089066,1089067,1090231,1090232,1090713,1090778,1091605,1092031,1093432,1094737,1095292,1095293,1096718,1096719,1096864,1096865,1097679,1097680,1097996,1097997,1097998,1098575,1098576,1098577,1098578,1098585,1098586,1098592,1098792,1098793,1098794,1098973,1098974,1098975,1098976,1099775,1099832,1099840,1099935,1099936,1100096,1100389,1100778,1100987,1101742,1101898,1102293,1102294,1104516,1106524,1106525,1106526,1109236,1112630,1112631,1113279,1114348,1114455,1114978,1115372,1115373,1115374,1115636,1116612,1116613,1116700,1116701,1116863,1118621,1118622,1119244,1119245,1119246,1120815,1120816,1123570,1123571,1124225,1124329,1183441,1185433,1185552,1185554,1189875,1190962,1195626,1199748,1199765,1199766,1212837,1219383,1219384,1219413,1224450,1224451,1227761,1227762,1227764,1227884,1233188,1237270,1237271,1240069,1242793,1242794,1259449,1262014,1264075,1269993,1271566,1271567,1273081,1274170,1277704,1278262,1284625,1287960,1304161,1304162,1304932,1304933,1304934,2062707,2062708,2070203,2072163,2072405
<i>Human PLP MHC Class I (54 entries)</i>
2521,2946,2947,6638,15142,16989,16990,17042,20782,20984,24204,25235,33961,37893,40328,40591,51645,51646,54959,59025,59324,65138,68981,69712,73394,73395,112126,116338,116783,116854,117744,117764,117959,118229,541283,542569,563709,933826,936933,936934,936935,936936,938591,941166,941167,941168,941169,941170,942733,989461,1102608,1245067,1303783,2063429
<i>Human MOG MHC Class II (87 entries)</i>
112139,112226,112255,112779,112785,112793,112815,112828,112832,112960,112968,113088,113303,113394,113569,113911,113912,114024,114291,114319,114333,114368,114371,115036,115078,115086,115088,115097,115101,115104,115106,115118,115124,115133,115134,115783,115860,116701,116702,116725,116754,116779,116795,116840,116841,116847,116853,117361,118650,118892,132993,133023,133099,133106,133109,133226,133339,133353,134881,134947,134984,135103,135152,135250,135262,135337,135345,135349,135368,135384,142417,142432,142445,142450,142452,146240,188869,191437,233288,233307,420657,575360,575750,1125056,1125062,1224142,2014257
<i>Human MBP MHC Class II (892 entries)</i>
1806,4707,4710,4714,4715,8112,10673,13572,13573,22218,26092,29482,31246,31247,35325,38676,45513,45514,47754,48922,54050,58971,59472,66814,67907,68846,69927,71460,79823,79827,79852,79881,79950,79978,79979,80095,95959,99216,111125,111191,111192,111279,112037,112039,112045,112046,112152,112163,112185,112191,112214,112273,112289,112304,112319,112393,112409,112456,112458,112476,112498,112529,112542,112593,112635,112645,112752,112755,112765,112772,112773,112775,112778,112782,112783,112796,112820,112821,112839,112844,112851,112856,112916,112936,112946,112962,112976,112977,112991,112992,113006,113016,113023,113037,113047,113080,113108,113114,113119,113147,113148,113151,113169,113172,113267,113281,113285,113286,113320,113321,113400,113402,113430,113435,113493,113494,113507,113514,113515,113527,113577,113641,113662,113670,113702,113716,113810,113850,113859,113942,114048,114116,114121,114132,114171,114172,114197,114210,114211,114226,114265,114266,114308,114345,114366,114367,114376,114388,114389,114496,114519,114525,114541,114566,114571,114595,114689,114731,114744,114764,114765,114766,114767,114768,114773,114798,114806,114838,114846,114851,114879,114884,114908,114925,114964,114979,114990,115057,115061,115102,115125,115260,115592,115597,115616,115622,115625,115627,115629,115636,115641,115644,115647,115648,115654,115655,115660,115662,115663,115691,115821,115829,115903,115957,115960,115988,116071,116072,116074,116075,116079,116089,116090,116095,116096,116097,116104,116106,116109,116116,116117,116141,116145,116146,116154,116165,116170,116177,116180,116182,116256,116296,116314,116320,116339,11634

6,116351,116360,116384,116388,116414,116436,116439,116455,116460,116461,116479,116517,116683,116716,116722,116724,116797,116826,116924,116976,116993,116995,117009,117023,117063,117271,117754,117889,117903,117927,118036,118040,118159,118181,119027,119613,119809,119892,119927,119972,119973,120169,120170,120201,120238,120244,120301,132884,132916,142420,161181,161219,181080,181101,224405,234094,234127,234136,234168,234173,234210,234294,234330,241205,492178,514319,514320,535102,535126,535794,535862,535924,536174,536704,537291,537558,537795,541961,541967,565232,657631,664280,684994,688203,733902,838258,841969,841970,842265,884771,910440,910455,910456,910457,910475,910486,910488,910489,910491,910519,910520,910525,912831,935028,936581,936582,936763,937113,937114,937185,937187,937410,937609,937721,937722,937723,938311,938402,938403,938801,938802,939918,941426,942179,942180,942181,942182,942628,943809,1088450,1088451,1088452,1088453,1089066,1089068,1089069,1090232,1090233,1090234,1090235,1090236,1090237,1090238,1090239,1090410,1090453,1090712,1090713,1090714,1090715,1090716,1090717,1090718,1090719,1092031,1092335,1092336,1093307,1093428,1093432,1095292,1096619,1096620,1096621,1096622,1096623,1096719,1096864,1096866,1096878,1096879,1096880,1096887,1097679,1097680,1097996,1097997,1097998,1097999,1098000,1098001,1098080,1098272,1098273,1098575,1098577,1098578,1098579,1098580,1098581,1098585,1098586,1098587,1098588,1098592,1098593,1098668,1098791,1098973,1098974,1098975,1098976,1099049,1099050,1099775,1099819,1099820,1099832,1099833,1099840,1099935,1099936,1099937,1100026,1100097,1100098,1100099,1100100,1100101,1100282,1100283,1100389,1100390,1100391,1100577,1100578,1100648,1100649,1100756,1100757,1100758,1100778,1100779,1100782,1100783,1100784,1100827,1100988,1100989,1101124,1101125,1101126,1101127,1101128,1101742,1101743,1101744,1101745,1103515,1103516,1103517,1104057,1104580,1104581,1104582,1104969,1105227,1105228,1105229,1105230,1105231,1105682,1105683,1106526,1106527,1106528,1106529,1106530,1106531,1106532,1106533,1111528,1112630,1112631,1112757,1112924,1112925,1112961,1113279,1113521,1114045,1114175,1114348,1114381,1114382,1114454,1114455,1114456,1114457,1114458,1114482,1114483,1114484,1114494,1114978,1115203,1115298,1115299,1115300,1115363,1115372,1115373,1115374,1115636,1115637,1115638,1116421,1116422,1116612,1116613,1116614,1116615,1116700,1116701,1116702,1116862,1116863,1116864,1116865,1116866,1116867,1116868,1116869,1116870,1117151,1117152,1117153,1117154,1117155,1117683,1117705,1117901,1118274,1118621,1118622,1118623,1118624,1118726,1118727,1118728,1118729,1119244,1119245,1119246,1119247,1119248,1119249,1119619,1119620,1119621,1119622,1119623,1119660,1119661,1119860,1120815,1120816,1123079,1123080,1123570,1124201,1124202,1124226,1124227,1124228,1124330,1124331,1124332,1183441,1183442,1183443,1183444,1183445,1183446,1185434,1185553,1185554,1185555,1185556,1185557,1188597,1188598,1189330,1189331,1189332,1189333,1189334,1189335,1189336,1189337,1189875,1189876,1190300,1190301,1190963,1193149,1199748,1199749,1199750,1199751,1199752,1199753,1199765,1199767,1199768,1200218,1200219,1200220,1201527,1201528,1204404,1206687,1206688,1211732,1212811,1212837,1212838,1212839,1212840,1212841,1212842,1212843,1212844,1212845,1213434,1213435,1213436,1213437,1213438,1213439,1213440,1217209,1217210,1217211,1217212,1217213,1217517,1217518,1219385,1219386,1219387,1219414,1219415,1219416,1219636,1219637,1220136,1220137,1220847,1221235,1221694,1221695,1224450,1224452,1224453,1224591,1224592,1225000,1225001,1225326,1225327,1225328,1225440,1225462,1225463,1226051,1226052,1226053,1226333,1227074,1227323,1227324,1227761,1227762,1227763,1227764,1227765,1227766,1227767,1227768,1227769,1227770,1227868,1227884,1227885,1227886,1227887,1227888,1227889,1227890,1227891,1227892,1227969,1228116,1228117,1228517,1228518,1228929,1228930,1228931,1229453,1229454,1233188,1233189,1237272,1238623,1238624,1238625,1238626,1238627,1238628,1240070,1240071,1240072,1240576,1242022,1242023,1242024,1242794,1242795,1244476,1244477,1244596,1244597,1244598,1247050,1247051,1247052,1247053,1249343,1252951,1253011,1253012,1253013,1255090,1257342,1257343,1260714,1260715,1260716,1262014,1263668,1263669,1264409,1264969,1267520,1267521,1268290,1268674,1268675,1269466,1269467,1269468,1269469,1269579,1269840,1269841,1269842,1269843,1269939,1269940,1269946,1269947,1269948,1269949,1269950,1269951,1269952,1269994,1269995,1269996,1269997,1271955,1271956,1273082,1273279,1273280,1274171,12

74172,1276883,1276884,1276885,1276886,1276887,1276888,1277423,1277424,1277437,1277438,1277439,1277440,1277441,1277704,1277705,1277706,1277707,1277708,1277709,1278261,1278262,1278586,1278587,1279189,1281239,1281410,1281686,1281687,1281688,1281689,1281972,1283183,1283184,1284164,1284165,1284626,1284627,1284845,1284846,1286451,1286452,1287960,1287961,1287962,1287963,1288075,1288076,1288077,1288078,1288990,1288991,1292171,1292172,1292173,1304039,1304163,1304801,1304802,1304803,1304804,1304805,1304806,1304807,1304808,1304933,1304934,1304935,1304936,1304937,1347308,1867622,1867949,1867950,1893552,1908277,2224796,2230239
<i>Human PLP MHC Class II (119 entries)</i>
909,4970,5227,5516,5967,6572,15969,17762,18476,18488,20985,22429,23599,25220,30875,31008,35803,36651,40353,40592,40762,41007,41605,46317,47692,50042,50275,51191,58952,63975,69164,71892,73159,103126,106502,107951,111378,111688,112126,112246,112298,112320,112354,112462,112523,112561,112603,112661,112674,112690,112739,112756,113449,114177,114358,114391,114456,115112,115747,116006,116673,116686,116717,116723,116726,116746,116849,116860,116911,116928,116973,116994,117017,117032,117035,117039,117073,117075,117076,117117,117349,117767,117849,117861,117871,117881,117884,117897,117939,117950,117985,118033,118142,118207,118230,121729,121854,121943,121991,153256,153277,165608,165611,165628,165631,165641,165652,165689,541283,566648,736408,736759,736872,737144,737249,737253,738004,738036,1102608
<i>Human CRYAB MHC Class I (62 entries)</i>
565521,749455,751227,751419,751420,771383,771939,774322,774940,776061,776187,776188,776189,778867,779285,779579,918181,935433,936414,984250,1059312,1091954,1091955,1093210,1093211,1093712,1093737,1096339,1096340,1096600,1106863,1108814,1108815,1113398,1114227,1124422,1189046,1196971,1204585,1205196,1210928,1211027,1211900,1214392,1214393,1217005,1218611,1229129,1243060,1249111,1258681,1258682,1258683,1264043,1268243,1271521,1275908,1279335,1283802,1301703,2066498,2253323
<i>Human CRYAB MHC Class II (237 entries)</i>
29066,112083,112224,112767,112771,112784,112788,112789,112791,112800,112804,112806,112817,112825,112833,112840,112843,112858,112859,112969,112973,112974,113035,113069,113074,113077,113142,535403,535754,565521,751227,751419,759859,774322,984250,1093210,1093211,1093712,1093713,1093714,1093715,1093716,1093717,1095897,1095898,1096339,1096340,1096341,1096342,1096600,1098306,1098307,1098308,1098309,1098310,1098311,1106863,1107150,1107259,1107303,1108814,1108815,1108816,1108817,1108818,1113102,1113398,1115910,1115911,1120134,1121614,1124422,1185732,1185733,1185734,1185735,1185736,1189046,1189047,1189048,1189049,1195817,1195818,1195819,1196972,1196973,1196974,1198880,1199267,1204585,1204586,1205196,1208130,1208131,1210929,1210930,1210931,1210932,1210933,1211028,1211730,1211731,1211900,1211901,1211902,1211903,1211904,1211905,1211906,1211907,1214038,1214039,1214040,1214041,1214042,1214392,1214393,1214394,1214395,1214396,1214397,1214398,1217005,1218240,1218611,1223783,1227975,1227976,1227977,1227978,1227979,1227980,1227981,1228275,1228777,1229129,1229130,1229131,1229132,1229682,1229683,1229684,1229685,1231268,1232067,1232068,1232069,1232070,1232071,1232072,1232073,1235586,1235587,1235588,1235589,1236141,1243060,1248755,1248756,1248757,1248758,1249226,1249227,1249228,1249229,1251108,1254083,1254084,1254085,1254086,1254474,1255295,1258682,1258683,1258684,1260369,1263941,1264565,1264566,1264937,1265625,1265626,1266353,1268244,1270722,1271521,1271522,1271523,1271524,1271525,1271526,1271811,1271812,1271813,1272643,1274884,1275908,1279335,1279336,1279339,1279340,1280865,1280866,1280867,1280868,1282112,1282113,1282114,1282115,1282116,1282117,1282118,1282119,1282485,1283312,1283803,1287140,1290884,1290885,1290886,1295477,1296000,1296001,1296782,1301704,1301920,1301921,1305270,1379035,1380735,1380971,1382030,1382055,1387821,1390609,1391522,1577360
Hashimoto's Thyroiditis
<i>Human Thyroglobulin MHC Class I (206 entries)</i>

123164,125565,130441,130563,144353,144410,164764,164765,165257,506176,772121,935146,99352 9,1028146,1084886,1088591,1088592,1088593,1089189,1089190,1091210,1091574,1093375,109364 6,1095078,1097093,1097352,1102958,1105533,1108635,1109186,1112418,1113155,1114797,111716 5,1117166,1117167,1117599,1117805,1118097,1118208,1118722,1118723,1122292,1123874,118207 4,1182075,1182076,1182225,1183161,1183915,1184333,1185954,1187080,1187428,1187604,118762 2,1189634,1189768,1189956,1191258,1191452,1193158,1197194,1197313,1200207,1206589,120737 1,1207601,1210010,1213485,1214625,1214737,1214738,1214739,1214740,1214831,1215463,121581 4,1216063,1216802,1217022,1217091,1218472,1222225,1222226,1222528,1223632,1223932,122405 9,1224475,1226170,1227065,1227631,1228204,1231933,1233375,1235135,1235136,1235450,123730 7,1244204,1245150,1245787,1246421,1247144,1248463,1250585,1251022,1251180,1253550,125357 6,1253577,1253861,1255188,1255189,1256048,1257854,1260579,1261064,1261065,1263631,126367 7,1265123,1265504,1265956,1265957,1266517,1266770,1267282,1267824,1268140,1269464,126950 4,1269516,1269517,1271848,1271849,1272509,1273324,1273429,1273486,1274065,1274718,127524 2,1275490,1275990,1275991,1276745,1278562,1279099,1279223,1279224,1279318,1279355,128007 5,1281066,1281259,1281550,1281551,1281672,1281854,1282665,1282666,1283168,1285700,128832 4,1290206,1293887,1293888,1294387,1294966,1296219,1296322,1297149,1297686,1297687,129768 8,1299044,1300985,1301776,1301777,1302279,1302776,1302777,1303280,1303281,1306092,130636 2,1307134,1307630,1311321,1311398,1311401,1311498,1311511,1311518,1311526,1852747,186664 3,2013321,2036004,2037952,2052206,2054785,2143913
<i>Human Thyroglobulin MHC Class II (487 entries)</i>
2840,13514,22342,39688,63197,122378,122595,122885,125565,126792,126897,126900,127842,1305 63,133885,144201,144202,144221,144222,144353,144410,144436,144452,144453,144482,144515,15 0157,161231,161258,164764,226501,534682,534695,534706,534802,935146,1088591,1088592,10885 93,1088594,1088595,1089189,1089190,1089703,1092414,1097093,1102958,1103228,1106567,11086 36,1109186,1109267,1112418,1113155,1117165,1117166,1117167,1117432,1118097,1118543,11187 22,1118723,1120420,1120421,1121012,1121013,1123894,1181439,1181440,1181441,1181442,11820 74,1182075,1182076,1182077,1182078,1182079,1182080,1182081,1182082,1182083,1182084,11820 85,1182263,1183916,1183917,1184334,1184335,1185005,1185664,1185665,1185942,1185953,11859 54,1185955,1185956,1185957,1185958,1185959,1185960,1185961,1185962,1185963,1186296,11870 81,1187429,1187430,1187431,1187432,1187549,1187562,1187563,1187623,1187624,1189080,11896 34,1189635,1190024,1191773,1192258,1197025,1197026,1197194,1197314,1199097,1199098,12005 31,1201408,1201409,1202533,1202534,1202535,1202547,1203343,1203449,1204385,1205606,12056 07,1205608,1206313,1206589,1208194,1208195,1208196,1208197,1209822,1209823,1209824,12134 55,1213456,1213485,1213486,1213487,1213488,1213489,1213490,1213491,1213492,1214738,12151 71,1215463,1215542,1215815,1215816,1216193,1216194,1216321,1216322,1217092,1217093,12170 94,1217095,1219002,1219003,1219138,1219139,1219140,1219141,1220179,1222225,1222226,12222 27,1222228,1222229,1222230,1222315,1222343,1222344,1223631,1225145,1225146,1225203,12252 60,1225261,1225303,1225540,1226155,1230198,1231133,1231933,1231934,1231935,1231936,12319 37,1235134,1235137,1235138,1235139,1235140,1235141,1235142,1235158,1235159,1235194,12353 37,1235450,1235451,1235452,1235453,1235454,1235478,1235610,1235611,1235612,1235882,12358 83,1235921,1236300,1236301,1236302,1238687,1238688,1244472,1245960,1245961,1246813,12468 14,1246815,1246816,1247145,1247146,1247147,1247148,1247149,1247509,1248036,1249089,12490 90,1249091,1249092,1249490,1249495,1249496,1249497,1249498,1250034,1250035,1250036,12505 85,1250586,1250587,1250588,1250589,1250590,1250638,1251009,1251048,1251135,1251212,12512 13,1251214,1252416,1252417,1252418,1253176,1253510,1253540,1253541,1253542,1253543,12535 50,1253551,1253575,1253576,1253577,1253578,1253862,1253863,1254250,1254251,1254421,12544 22,1255161,1255162,1255163,1255164,1255165,1255189,1255272,1255273,1255459,1255535,12555 36,1255537,1255538,1255539,1255540,1255541,1255579,1256048,1256110,1256156,1256338,12563 39,1256358,1257752,1257753,1257754,1257755,1259151,1259771,1259772,1261064,1261208,12635 74,1263678,1263679,1263680,1263841,1264172,1264252,1264424,1264863,1264958,1265063,12650 64,1265112,1265113,1265505,1265506,1265507,1265508,1265509,1265510,1265957,1266382,12665

16,1266517,1267108,1267109,1269504,1269517,1269535,1269838,1270616,1270976,1270977,1271850,1273486,1275243,1275244,1275245,1275246,1275247,1275248,1276746,1276747,1276748,1277035,1278562,1278719,1278720,1278721,1278722,1278957,1278958,1278959,1278960,1278961,1279135,1279319,1279320,1279321,1279322,1279323,1279324,1279325,1279326,1279327,1279961,1279962,1279963,1279964,1280075,1281550,1281551,1281552,1281553,1281554,1281555,1281671,1281672,1281673,1281828,1281829,1281855,1282666,1282667,1282869,1282870,1282871,1283957,1284345,1284377,1285373,1285701,1288275,1289599,1290372,1290373,1290920,1291131,1291132,1291133,1291134,1291685,1291961,1292743,1293887,1293888,1293889,1293890,1294387,1294481,1297778,1297779,1297780,1298792,1298793,1298794,1299042,1299043,1299045,1299192,1300985,1300986,1300987,1300988,1301776,1301777,1301778,1301779,1302303,1302623,1302776,1302777,1302778,1302779,1302780,1302781,1302782,1302783,1302784,1303280,1303281,1303282,1303283,1303284,1303847,1305891,1306092,1306196,1306197,1306198,1306199,1306346,1306737,1307134,1307135,1609330,2004732,2008394,2013894,2015889,2031920,2042507,2042813
<i>Human Thyroperoxidase MHC Class I (36 entries)</i>
164581,164587,165028,456646,748009,907056,1012022,1092929,1093978,1118354,1191125,1214046,1214150,1220882,1224557,1228732,1229340,1233550,1242282,1243101,1261921,1266106,1273951,1291100,1294048,1299462,1304482,1311318,1311362,1311396,1357666,1990461,2005488,2031378,2036578,2068097
<i>Human Thyroperoxidase MHC Class II (103 entries)</i>
25701,122263,123046,123097,123145,123196,123289,123295,123301,123328,123360,123404,123431,123456,123536,123558,123690,123698,123768,125474,128222,128254,128398,128408,128532,128890,128941,130338,130436,130439,130442,130444,130451,130467,130468,130478,130481,130483,130494,130496,130498,130500,130503,130504,130507,130511,130512,130516,130520,130528,130535,130545,130552,130554,130556,130557,130560,130562,130573,130576,130584,130588,130589,130596,130609,130615,130617,130618,130628,130639,130640,134004,134009,134066,134080,134081,134083,134158,134168,134254,569484,569535,569697,569708,569765,1182464,1182465,1190364,1192437,1193312,1211793,1211794,1214220,1215664,1220882,1247214,1254924,1254925,1254926,1254927,1270668,1276501,1304482
Vitiligo
<i>Human Melanin-concentrating hormone receptor MHC Class I (9 entries)</i>
418203,454053,727393,942034,1199535,2061425,2068498,2068499,2071601
<i>Human Melanin-concentrating hormone receptor MHC Class II (1 entry)</i>
1284068
<i>Human Tyrosinase MHC Class I (96 entries)</i>
785,1353,2683,2684,3203,9617,9650,16749,16859,16860,27869,27952,30046,37088,37113,37954,38770,41989,52619,54313,57494,58495,59991,64772,65660,69794,72748,75043,75092,75117,162189,527190,541577,541732,541774,543799,543848,543849,544086,544215,563474,563874,564288,564432,565589,566158,566242,566252,566422,586007,841973,841974,842868,843074,843768,845458,845545,846860,846938,847012,847111,847267,849124,849327,850627,850760,986068,993194,993281,993513,1023258,1030055,1033597,1033598,1075406,1110135,1312980,1313326,1334627,2061689,2061704,2063262,2064066,2064770,2066280,2067953,2067954,2068059,2068279,2068280,2068541,2069060,2073798,2189010,2255987,2268611
<i>Human Tyrosinase MHC Class II (35 entries)</i>
2603,2604,10968,44281,51698,62642,74072,74875,75933,123964,124316,124704,124721,124841,132284,132285,132396,132488,841847,841848,841849,841850,843630,845045,845046,846540,846541,848431,848432,2268453,2268473,2268483,2268562,2268569,2268581
<i>Human Pmel17 MHC Class I (206 entries)</i>
2469,2688,2779,3147,5371,5603,6772,13654,16880,20891,21828,22607,22695,24177,24945,25344,28864,28923,33915,33916,36787,37110,37157,37995,38431,38526,38552,38716,40160,41953,51573,54500,54672,56091,58986,58987,59206,61345,64479,65498,69636,69706,69906,70354,71731,71732,74

546,74638,96518,232216,499746,506608,541458,541533,542085,544061,544394,544819,545022,545215,546155,546253,546667,546668,562125,562167,563499,563562,563820,563842,563894,563999,564276,564363,564513,564548,565170,565739,565803,566111,566227,566274,566275,566297,566380,566383,566414,566418,566460,708819,841780,842200,842430,842792,842908,843763,843835,844167,844168,844537,844583,844961,845017,845018,845019,845057,845322,846306,846958,847214,847312,847868,847869,847874,847881,847958,847959,848446,848559,848612,849194,849231,849521,849542,850208,850312,850368,850425,850734,850735,850752,993263,993375,993480,1000911,1028522,1031526,1032405,1032978,1075174,1075197,1075267,1075374,1075703,1075707,1075738,1075787,1075793,1075820,1084315,1084537,1084652,1085844,1086513,1086514,1086968,1086969,1086996,1087113,1125099,1125100,1125101,1596693,1596714,1596778,1596806,1865691,1865843,1866206,1866207,1866236,1866237,1866238,1866357,1867736,1867765,2048277,2061320,2061667,2061668,2061669,2063594,2064028,2064547,2065603,2065662,2066130,2066131,2066132,2066133,2067872,2067981,2068328,2068329,2068330,2073094,2073240,2073573,2073574,2073616,2073778,2232760,2232939,2259232,2265288,2265907
<i>Human Pmel17 MHC Class II (212 entries)</i>
2688,12141,12897,29672,33113,35090,42852,47728,70964,72906,123963,123990,124239,124413,124564,124595,124632,124726,124752,124791,194235,194306,194307,194308,194549,546155,563999,565803,566111,842068,842431,842498,842499,842792,842793,843522,843759,844163,844168,844537,844538,844539,844684,844685,844702,844980,844981,844982,845019,845057,845533,845534,845558,845606,847741,847742,847959,848160,848161,848162,848417,848418,848419,848420,848447,849310,849311,849493,849494,849542,850208,850415,850425,850515,889004,1075234,1075670,1075796,1334610,1334646,1864407,1864408,1864409,1864440,1864441,1864442,1864443,1864811,1864812,1865005,1865006,1865007,1865690,1865691,1866024,1866029,1866030,1866031,1866032,1866033,1866034,1866035,1866146,1866206,1866207,1866208,1866209,1866210,1866211,1866295,1866296,1866297,1866298,1866358,1866359,1866360,1866361,1866362,1866363,1866364,1866503,1866504,1866505,1866634,1866635,1866799,1866800,1867425,1867426,1867521,1867531,1867532,1867533,1867534,1867737,1867738,1867766,1867787,1867788,1867919,1867920,1868099,1868552,1868553,1868554,1868555,1868556,1868673,1868674,1868675,1868694,1868939,1868940,1869266,1869300,1869301,1869302,1869303,1869506,1869507,1869541,1869542,1869543,1869549,1869550,1869551,1869670,1869671,1869672,1869673,1869674,1869745,1869858,1869864,1869865,1869866,1869886,1869887,1869888,1869937,1870317,1870318,1870319,1870320,1870321,1870322,1870323,1870380,1870443,1870444,1870455,1870456,1870457,1870458,1870459,1870460,1870577,1909431,1909432,1909433,1918137,1920262,1920263,1927322,1927323,1936535,1941231,1941232,1942163,2041598,2163322,2232939
Celiac
<i>Gliadin MHC Class I (121 entries)</i>
1931,1947,3066,6889,12966,14468,17298,17363,17409,17410,23538,24750,27802,27803,27824,27826,27989,33490,36278,36288,38406,38407,38644,38784,38785,38786,38791,38930,38958,38965,41196,45459,46511,47551,48953,48954,50066,50135,50153,50154,50280,50750,50796,50810,50943,51375,51376,51813,51814,51817,51823,51825,51829,51830,51832,51875,51894,51895,51899,51909,51913,51918,51970,51992,51993,51995,51997,51998,52036,52133,52134,53020,53021,53035,57871,57872,57873,60398,60399,60537,60579,60581,61314,69726,70368,70369,70935,70936,71747,71749,72057,74343,75355,75389,75393,75397,75412,148663,540552,591422,591425,591442,591445,591447,591482,591484,591486,591489,591490,591493,591494,591500,591503,591505,591523,591524,591534,591556,591564,2258781,2258835
<i>Celiac disease MHC Class I epitopes (18)</i>
1931,36278,50943,51375,51376,60537,61314,69726,540552,591422,591425,591484,591490,591503,591523,591524,591534,591556
<i>Gliadin MHC Class II (446 entries)</i>

3067,17364,17367,17370,17372,17373,17374,17375,17630,17825,20305,22327,24601,26411,26648,26649,27988,36163,36278,36288,36291,36293,36294,36295,36414,37704,38893,38894,38897,38898,38901,38944,38951,38957,38968,39165,47541,47542,47547,47548,47561,47569,47570,47579,49052,49061,49063,49064,49065,49066,49067,49069,49083,49084,49088,49089,49090,49091,49096,49100,49101,49102,49103,49104,49111,49128,49134,49387,49515,50151,50152,50341,50751,50812,50813,50943,50963,51489,51490,51508,51513,51514,51516,51517,51518,51748,51824,51897,51900,51907,51910,51923,51926,51927,51928,51932,51934,51936,51996,52027,52035,52056,52065,52081,52087,52092,52095,52103,52112,52130,52165,52172,52179,52311,52318,52601,52761,52869,57865,58209,58210,60537,64420,65612,70503,70564,70849,72058,72960,74880,75390,75413,75537,77887,77921,77980,77983,77984,78013,78383,78385,78386,78408,78444,78448,78449,78491,78493,78494,78495,78496,78497,78557,78802,94177,95391,95569,98383,98401,99835,109223,109516,109654,109655,109720,109721,109722,109723,109724,109725,109726,109727,109753,109759,109760,109769,109770,109771,109773,137260,137265,137270,137272,137273,139538,139539,139547,139548,139573,139574,139575,139577,139578,139579,139580,139581,139582,139586,139587,139588,139589,139591,139592,140280,140281,140282,140283,140319,148091,150215,150216,150400,150492,150503,150517,150518,150562,150657,151894,152063,152068,152069,152115,161144,167139,167140,167141,167143,172755,175520,175524,175525,175526,178159,225964,225965,226589,226591,226592,226647,226652,226653,226654,226657,226658,226668,226669,226671,226868,226882,228624,228682,234035,234152,234153,234154,234155,234156,234157,234158,234169,234170,234171,234178,234179,234180,234181,234182,234183,234184,234190,234191,234192,234193,234194,234195,234196,234197,234198,234199,234200,234201,234202,234203,234205,234206,234207,234208,234209,234214,234215,234216,234217,234218,234219,234220,234224,234225,234226,234227,234228,234229,234230,234231,234232,234233,234234,234235,234236,234237,234238,234239,234240,234241,234243,234244,234245,234255,234256,234257,234258,234259,234260,234261,234262,234263,234264,234265,234266,234267,234274,234275,234276,234277,234278,234279,234280,234281,234303,234304,238832,238877,238926,244047,474381,474382,474383,474386,474387,474388,474390,474391,474392,474393,474396,474397,474398,474399,474400,474401,474402,474403,474405,474407,474408,474409,474411,474415,474418,474419,474420,474421,474423,474425,474426,474428,474429,474432,474433,474439,474449,524131,540435,540552,558770,697307,742400,742401,810542,859257,914477,914478,914479,914480,914481,914482,922508,922509,956631,956673,956674,985508,1065957,1065965,1066025,1329965,1338842,1338843,1338863,1338867,1338868,1338874,1338875,1338876,1338877,1338878,1338879,1338897,1338940,1338945,1338956,1338962,1338973,1338974,1347303,1347304,1397395,1868573,2186127,2186211,2186212,2186213,2186214,2186228,2186243,2186257,2186258,2186265,2186270,2186271,2238164,2238175,2249112,2265287,2265585,2265602
<i>Celiac disease MHC Class II epitopes (749)</i>
1932,2518,3067,6508,10776,12568,13943,17299,17367,17370,17372,17373,17374,17375,17820,17825,20304,20305,22327,24601,25091,26411,26582,26645,26648,26649,27268,27825,33763,36163,36278,36291,36294,36653,38645,38787,38882,38883,38884,38886,38888,38889,38893,38894,38897,38898,38900,38901,38944,38951,39165,39187,42401,42420,42989,46863,47392,47541,47542,47547,47548,47561,47569,47570,47579,49037,49047,49053,49061,49063,49064,49065,49066,49069,49083,49084,49088,49089,49090,49091,49096,49102,49103,49105,49111,49128,49134,49159,49387,49491,50151,50152,50341,50812,50813,50943,51029,51098,51116,51324,51489,51490,51508,51513,51514,51515,51517,51518,51818,51824,51897,51900,51907,51911,51916,51923,51926,51927,51928,51930,51931,51932,51934,51935,51936,51938,52014,52027,52035,52050,52065,52071,52081,52087,52092,52095,52100,52103,52109,52112,52119,52123,52126,52130,52165,52172,52311,52318,52601,52761,52788,52869,53182,57865,58209,58210,60533,64420,65612,67427,69727,70407,70450,70564,70626,70632,72056,72058,72960,73456,74085,74880,75390,75413,75537,77797,77887,77921,77980,77983,77984,78013,78383,78385,78386,78491,78493,78495,78557,78802,94177,95391,95569,95721,98383,98401,99835,108981,108982,108995,109223,109480,109516,109654,109655,109656,109720,109722,109723,109724,109725,109726,109727,109753,109760,109769,109771,109773,114227,133783,133844,137260,137265,137270,137272,137273,139537,139538,139539,139547,139548,139573,139574,139575,

139577,139578,139579,139580,139581,139582,139586,139587,139588,139589,139590,139591,139592,140319,148091,150215,150216,150400,150492,150503,150517,150518,150561,150562,150657,150697,161144,164705,165179,165184,165185,165197,165238,167134,167136,167138,167140,167141,167143,170369,171907,172620,172686,172732,172735,172737,172738,172739,172740,172741,172742,172743,172744,172745,172746,172747,172748,172749,172750,172751,172752,172755,172756,175520,175521,175522,175523,175524,175525,175526,175527,175528,175529,175530,175531,175532,178159,225964,225965,226589,226590,226591,226592,226647,226652,226653,226654,226657,226658,226668,226669,226670,226671,226868,226882,228624,228682,234171,234224,238832,238845,238877,238925,238926,244039,244043,244044,244047,244048,244050,244051,244072,420753,474381,474382,474383,474384,474385,474386,474387,474388,474390,474391,474392,474393,474395,474396,474397,474398,474399,474400,474401,474402,474403,474404,474405,474406,474407,474408,474409,474410,474411,474414,474415,474416,474417,474418,474419,474420,474421,474422,474423,474424,474425,474426,474427,474428,474429,474430,474431,474432,474433,474434,474435,474436,474437,474438,474439,474440,474441,474442,474443,474444,474445,474446,474447,474449,524131,534686,534743,534789,540435,540552,558548,558643,558769,558770,561910,697307,742400,742401,745559,745562,745625,745628,745629,745630,745631,745632,745633,745635,745636,745637,745638,745639,745640,745641,745642,810542,914477,914478,914479,914480,914481,914482,922508,922509,956631,956673,956674,984665,985508,985517,985518,985520,985590,985596,985597,985614,985615,985617,985618,985619,985620,985684,985693,1065957,1065965,1066025,1311344,1311345,1311346,1311347,1311348,1311349,1311350,1311352,1311353,1311380,1311381,1311382,1311383,1311402,1311411,1311417,1311418,1311420,1311421,1311422,1311423,1311424,1311425,1311426,1311427,1311428,1311429,1311430,1311432,1311433,1311434,1311435,1311436,1311437,1311438,1311439,1311440,1311441,1311445,1311446,1311447,1311448,1311449,1311450,1311451,1311452,1311453,1311454,1311455,1311456,1311457,1311459,1311460,1311462,1311463,1311464,1311465,1311466,1311467,1311468,1311469,1311470,1311471,1311472,1311473,1311474,1311475,1311476,1311477,1311478,1311479,1311480,1311481,1311482,1311483,1311484,1311485,1311486,1311487,1311488,1311505,1311506,1311508,1311515,1311516,1311530,1311531,1311532,1338811,1338812,1338813,1338814,1338815,1338816,1338817,1338818,1338820,1338821,1338823,1338824,1338825,1338826,1338827,1338828,1338829,1338830,1338831,1338832,1338833,1338834,1338835,1338836,1338840,1338842,1338843,1338844,1338845,1338846,1338847,1338848,1338849,1338850,1338851,1338852,1338853,1338854,1338855,1338857,1338858,1338859,1338860,1338861,1338862,1338863,1338864,1338865,1338866,1338867,1338868,1338869,1338870,1338873,1338874,1338875,1338876,1338877,1338878,1338879,1338880,1338881,1338882,1338883,1338884,1338885,1338886,1338887,1338888,1338889,1338890,1338891,1338892,1338893,1338894,1338895,1338896,1338897,1338898,1338899,1338900,1338901,1338902,1338903,1338904,1338905,1338906,1338907,1338908,1338909,1338910,1338911,1338912,1338913,1338914,1338915,1338916,1338917,1338918,1338919,1338920,1338921,1338922,1338923,1338924,1338925,1338926,1338927,1338928,1338929,1338930,1338931,1338932,1338933,1338934,1338935,1338936,1338937,1338938,1338939,1338940,1338941,1338942,1338943,1338944,1338945,1338946,1338947,1338948,1338949,1338950,1338951,1338952,1338953,1338956,1338957,1338958,1338959,1338960,1338961,1338962,1338963,1338964,1338965,1338966,1338967,1338968,1338969,1338970,1338973,1338974,1338975,1338976,1338977,1347303,1347304,1397395,1868573,2186127,2186211,2186212,2186213,2186214,2186228,2186229,2186230,2186243,2186257,2186258,2186265,2186270,2186271,2238160,2238164,2238175,2238233,2249112,2265286,2265298,2265303,2265304,2265305,2265306,2265307,2265308,2265309,2265585,2265602

[0123] Multi-epitope constructs may be designed using the following procedure:

- 1) **Selection of a leader sequence (aka signal peptide):** a leader sequence is selected from one disease-relevant antigen/autoantigen. If no leader sequences are present in the endogenous antigen/autoantigen sequence, alternate leader sequences from other proteins can be selected.
- 2) **Selection of epitopes:** one or more Class I epitopes and one or more Class II epitopes of a single antigen/autoantigen or >1 antigens/autoantigens are selected (e.g. from the IEDB database).
- 3) **Flanking amino acids:** the core epitope sequence is extended out on both sides (e.g. by 6 amino acids). The flanking regions can be extended further to gain greater epitope coverage (i.e. a multi-epitopic regions) for different HLA haplotypes.
- 4) **Addition of linker peptides:** each Class I epitope is separated by a class I linker. Each class II epitope is separated by a class II linker. A linker (e.g. a class II linker) is placed between the class I and class II epitope sets.
- 5) **Addition of a C-terminal MHC Class I trafficking domain (MITD) or another polypeptide comprising an endolysosomal trafficking sequence motif:** this sequence immediately follows the final epitope (no linker in between).
- 6) **Reverse translation to a nucleic acid ORF:** the resulting amino acid sequence after completion of step 5 is reverse translated into a nucleic acid sequence. This is done using software tools that are ubiquitous online or commercially (e.g. Codon Optimization Tool, by Integrated DNA Technologies, freely available at www.idtdna.com/pages/tools/codon-optimization-tool).
- 7) **Codon optimization:** The ORF is codon-optimized to improve expression in the host organism and remove start codons in alternate open reading frames. This is done using software tools that are ubiquitous online or commercially (e.g. Codon Optimization Tool, by Integrated DNA Technologies, freely available at www.idtdna.com/pages/tools/codon-optimization-tool).
- 8) **5' and 3'UTRs:** can be selected from any number of published or proprietary sequences following well established guidelines.

[0124] An example (i.e. non-limiting) coding sequence structure for a multi-epitope is shown below (note that each epitope also contains flanking amino acids on both sides)

Autoantigen/Antigen Leader Sequence – Class I epitope – AAY – Class I epitope – AAY
 - Class I epitope - GP GPG – Class II epitope – GP GPG – Class II epitope – GP GPG –
 Class II epitope – GP GPG – Class II epitope – GP GPG – Class II epitope – MITD (or other
 trafficking domain to improve retention in antigen processing pathway)

[0125] Full-length antigen constructs may be designed using the following procedure:

- 1) **Selection of a leader sequence (aka signal peptide):** a leader sequence is selected from one disease-relevant antigen/autoantigen. If no leader sequences are present in the endogenous antigen/autoantigen sequence, alternate leader sequences from other proteins can be selected.
- 2) **Reverse translation and codon optimization:** the protein coding sequence is reverse translated and codon-optimized to improve expression in the host organism and remove start codons in alternate open reading frames. This is done using software tools, which are ubiquitous online.
- 3) **5' and 3'UTRs:** can be selected from any number of published or proprietary sequences following well established guidelines.

[0126] In some embodiments, each of the one or more nucleic acids is an mRNA independently configured according to one of the following formulas:

A-B-C-L¹-D-L²-E-F (Formula IIa)

A-B-D-L¹-C-L²-E-F (Formula IIb)

A-B-C-L²-E-F (Formula IIc)

A-B-D-L²-E-F (Formula IId)

A-B-C-L¹-D-F (Formula IIe)

A-B-D-L¹-C-F (Formula II f)

A-G-F (Formula IIg)

A-B-C-F (Formula IIh)

A-G-L²-E-F (Formula IIi)

A-B-D-F (Formula IIj)

A-B-G-F (Formula IIk)

A-B-G-L²-E-F (Formula III)

wherein:

A is a 5' untranslated region (5'-UTR);

B is a signal peptide, optionally a signal peptide from the same antigen/autoantigen as one of the one or more epitopes;

L¹ is a cleavable linker, optionally a class II linker, optionally GPGPG (SEQ ID NO: 26);

C encodes one or more class I epitopes, each class I epitope separated by a class I linker peptide, and optionally flanked on both sides by 0-25 flanking amino acids;

L² is an optional linker (without limitation any linker defined herein, e.g. class I linker, class II linker, any linker cleavable by the proteasome, or any other linker, including non-cleavable linkers);

D encodes one or more class II epitopes, each class II epitope separated by a class II linker peptide, and optionally flanked on both sides by 0-25 flanking amino acids;

E encodes a polypeptide comprising an endolysosomal trafficking sequence motif (e.g. MITD, invariant chain (CD74), LAMP1, LAMP2, DC-LAMP, or a trafficking domain enhancing Class II antigen presentation);

F is a 3' untranslated region (3'UTR) further comprising a polyadenine tail; and

G is a full length antigen/autoantigen (which may or may not have an endogenous signal peptide).

[0127] In some embodiments, C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes. In some embodiments, D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes. In some

embodiments, the epitopes are from a plurality of antigens/autoantigens. In some embodiments, L¹ is GPGPG (SEQ ID NO: 26). In some embodiments, the class I linker peptide is AAY (SEQ ID NO: 23). In some embodiments, the class II linker peptide is GGGSGGG (SEQ ID NO: 24), GGGGS (SEQ ID NO: 25), or GPGPG (SEQ ID NO: 26). In some embodiments, L² is absent.

[0128] In some embodiments, C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes. In some embodiments, D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes. In some embodiments, the epitopes (C, D, or C and D) are selected from Table 3. In some embodiments, the epitopes are epitopic fragments from T1D autoantigens selected from the group consisting of: preproinsulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2β), zinc transporter 8 (ZnT8), and hybrid insulin peptides (HIPs). In some embodiments, G is GAD65. In some embodiments, G is preproinsulin. In some embodiments, G is IA-2 or IA-2beta. In some embodiments, G is IGRP. In some embodiments, G is ZnT8. In some embodiments, the epitopes are from a plurality of antigens/autoantigens. In some embodiments, L¹ is a class II linker, optionally GPGPG (SEQ ID NO: 26). In some embodiments, the class I linker peptide is AAY (SEQ ID NO: 23). In some embodiments, the class II linker peptide is GGGSGGG (SEQ ID NO: 24), GGGGS (SEQ ID NO: 25), or GPGPG (SEQ ID NO: 26).

[0129] In some embodiments, the one or more nucleic acids comprises a construct of Formula III (Multi), which encodes polypeptide of SEQ ID NO: 90 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - GPGPG - HIP2.5 - GPGPG - InsB 2-30 - GPGPG - GAD65 199-227 - GPGPG - GAD65 279-307 - GPGPG - HSP60 430-467 - GPGPG - IA-2b 748-784 - MITD – 3'UTR – Poly A tail (Formula III)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFA
LGFAAYEYGTMTVSYQPLGDKVNFFRMVIGPGPGGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLC
GSHLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLKKMREGPGPGGEHSHFSLKK
GAAALGIGTDSVILIKCDERGPGPAAVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPN
SSLVAQREENAPKNRSLAVLTYDHASRILLKSQNSIVGIVAGLAVLAVVVIGAVVATVMCRKSSGGKGG
SYSQAASSDSAQGSVDVSLTA (SEQ ID NO: 90)

[0130] In some embodiments, the one or more nucleic acids comprises a construct of Formula IV (Multi+Zn), which encodes polypeptide of SEQ ID NO: 91 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - ZnT8 179-201 - GPGPG - HIP2.5 - GPGPG - InsB 2-30 - GPGPG - GAD65 199-227 - GPGPG - GAD65 279-307 - GPGPG - HSP60 430-467 - GPGPG - IA-2b 748-784 - GPGPG - ZnT8 338-366 - MITD – 3'UTR – Poly A tail (Formula IV)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFALGFAAYEYGTMTMSYQPLGDKVNFFRMVIAAYITVSGCAVAANIVLTMILHQRNFGPGPGGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLGSHLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLLKMMREGPGPGGEHSHFSLKKGAAALGIGTDSVILIKCDERGPGPGA AVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPNSSLVAQREENAPKNRSLAVLTYDHASRILLKSQNSGPGPGSSFDLHSLTIQIESAADQDPSCLLCEDPQIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDS AQGSDVSLTA (SEQ ID NO: 91)

[0131] In some embodiments, the one or more nucleic acids comprises a construct of Formula V (Multi-reverse), which encodes polypeptide of SEQ ID NO: 92 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - HIP2.5 - GPGPG - InsB 2-30 - GPGPG - GAD65 199-227 - GPGPG - GAD65 279-307 - GPGPG - HSP60 430-467 - GPGPG - IA-2b 748-784 - GPGPG - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - MITD – 3'UTR – Poly A tail (Formula V)

MALWMRFLPLLALLFLWESHPTQAGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLGSHLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLLKMMREGPGPGGEHSHFSLKKGAAALGIGTDSVILIKCDERGPGPGA AVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPNSSLVAQREENAPKNRSLAVLTYDHASRILLKSQNSGPGPGGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFALGFAAYEYGTMTMSYQPLGDKVNFFRMVIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSDVSLTA (SEQ ID NO: 92)

[0132] In some embodiments, the one or more nucleic acids comprises a construct of Formula VI (Multi 1), which encodes polypeptide of SEQ ID NO: 93 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - MITD –
3'UTR – Poly A tail (Formula VI)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFA
LGFAAYEYGTMTMSYQPLGDKVNFFRMVIIIGVIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAA
SSDSAQGSVDVSLTA (SEQ ID NO: 93)

[0133] In some embodiments, the one or more nucleic acids comprises a construct of Formula VII (Multi 2), which encodes polypeptide of SEQ ID NO: 94 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - HIP2.5 - GPGPG - InsB 2-30 - GPGPG - GAD65 199-227 - GPGPG -
GAD65 279-307 - GPGPG - HSP60 430-467 - GPGPG - IA-2b 748-784 - MITD – 3'UTR –
Poly A tail (Formula VII)

MALWMRFLPLLALLFLWESHPTQAGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLCGSHLVEALYL
VCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLKKMREGPGPGGEHSHFSLKKGAAALGIGTD
SVILIKCDERGPGPAAVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPNSSLVAQREEN
APKNRSLAVLTYDHASRILLKSQNSIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDS
AQGSVDVSLTA (SEQ ID NO: 94)

[0134] In some embodiments, the one or more nucleic acids comprises a construct of Formula VIII (Multi no MITD), which encodes polypeptide of SEQ ID NO: 95 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - GPGPG -
HIP2.5 - GPGPG - InsB 2-30 - GPGPG - GAD65 199-227 - GPGPG - GAD65 279-307 -
GPGPG - HSP60 430-467 - GPGPG - IA-2b 748-784 – 3'UTR – Poly A tail (Formula VIII)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFA
LGFAAYEYGTMTMSYQPLGDKVNFFRMVIGPGPGGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLC
GSHLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLKKMREGPGPGGEHSHFSLKK
GAAALGIGTDSVILIKCDERGPGPAAVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPN
SSLVAQREENAPKNRSLAVLTYDHASRILLKSQNS (SEQ ID NO: 95)

[0135] In some embodiments, the one or more nucleic acids comprises a construct of Formula IX (Multi-mini), which encodes polypeptide of SEQ ID NO: 96 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - GP GPG - HIP2.5 - GP GPG - GAD65 199-227 - MITD – 3'UTR – Poly A tail (Formula IX)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFA
LGFGPGPGGGPGAGDLQTLALWSRMDQLAKELTGPGPGTANTNMFTYEIAPVFVLLLEYVTLKKMREIVGI
VAGLAVLAVVVIGAVVATVMCRRKSSGGKGSYSQAASSDSAQGSVDVSLTA (SEQ ID NO: 96)

[0136] In some embodiments, the one or more nucleic acids comprises a construct of Formula X (Multi-tPA), which encodes polypeptide of SEQ ID NO: 97 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – tPA SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - GP GPG - HIP2.5 - GP GPG - InsB 2-30 - GP GPG - GAD65 199-227 - GP GPG - GAD65 279-307 - GP GPG - HSP60 430-467 - GP GPG - IA-2b 748-784 - MITD – 3'UTR – Poly A tail (Formula X)

MDAMKRGCLCCVLLLCGAVFVSAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFALG
FAAYEYGTMTMSYQPLGDKVNFFRMVIGPGPGGGPGAGDLQTLALWSRMDQLAKELTGPGPGVKQHLGCS
HLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPVFVLLLEYVTLKKMREGPGPGGEHSHFSLKKGA
AALGIGTDSVILIKCDERGP GPGA AVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEIGPGPGPNSS
LVAQREENAPKNRSLAVLT YD HASRILLKSQNSIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGSY
SQAASSDSAQGSVDVSLTA (SEQ ID NO: 97)

[0137] In some embodiments, the one or more nucleic acids comprises a construct of Formula XI (Multi+alt linker), which encodes polypeptide of SEQ ID NO: 98 (see Table 2A for exemplary RNA sequences of components), or the human counterpart thereof:

5'UTR – PPI2 SP - InsB 8-30 - AAY - IGRP 199-221 - AAY - GAD65 539-561 - HEYGAEALERAG - HIP2.5 - GP GPG - InsB 2-30 - GP GPG - GAD65 199-227 - GP GPG - GAD65 279-307 - GP GPG - HSP60 430-467 - GP GPG - IA-2b 748-784 - MITD – 3'UTR – Poly A tail (Formula XI)

MALWMRFLPLLALLFLWESHPTQAGSHLVEALYLVCGERGFFYTPMSAAYVHMASLSVYLKTNVFLFLFA
 LGFAAYEYGTMTVSQPLGDKVNFFRMVIHEYGAELERAGGGPGAGDLQTLALWSRMDQLAKELTGPGP
 GVKQHLGSHLVEALYLVCGERGFFYTPMSGPGPGTANTNMFTYEIAPV FVLLEYVTLKKMREGPGPGEH
 SHFSLKKGAAALGIGTDSVILIKCDERGP GPGAAVEEGIVLGGGCALLRCIPALDSLKPANEDQKIGIEI
 GPGPGPNSSLVAQREENAPKNRSLAVLTYDHASRILLKSQNSIVGIVAGLAVLAVVVIGAVVATVMCRRK
 SSGGKGGSYSQAASSDSAQGSVDVSLTA- (SEQ ID NO: 98)

[0138] In some embodiments, the one or more nucleic acids comprise any one or more of the above, wherein mouse sequences are replaced with human sequences (see Table 2B for conversion from mouse to human sequences), wherein RNA sequences are optionally codon-optimized for human expression.

[0139] In some embodiments, the one or more nucleic acids comprise any 1, 2, 3, 4, or 5 of the following full length T1D autoantigens Preproinsulin, IA-2 (or IA-2beta), ZnT8, GAD65 and IGRP. In some of these embodiments, the sequences are mouse sequences. In some of these embodiments, the sequences are human sequences. In some embodiments, each mRNA sequences encodes a single T1D autoantigen (Preproinsulin, IA-2, IA-2beta, ZnT8, GAD65 or IGRP) and is configured according to Formula IIk or Formula III.

[0140] In some embodiments, the one or more nucleic acids comprises a construct of Formula XI or Formula XII (insulin:insulin HIP constructs), which encode polypeptides of SEQ ID NO: 99 or SEQ ID NO: 100 (see Table 2A for exemplary RNA sequences of components). CHGA refers to Chromogranin A. The junction formed between this protein and full length insulin is a hybrid epitope, which naturally forms in NOD mouse beta cells and is a target of autoreactive T cells in NOD mice.

5'UTR – PPI2 1-82 – CHGA 357-370 – MITD - 3'UTR – PolyA tail (Formula XI)

MALWMRFLPLLALLFLWESHPTQAFVKQHLGSHLVEALYLVCGERGFFYTPMSRREVEDPQVAQLELGG
 GPGAGDLQTLALWSRMDQLAKELTAIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDS
 AQGSVDVSLTA (SEQ ID NO: 99)

5'UTR – PPI2 1-82 – CHGA 357-370 – 3'UTR – PolyA tail (Formula XII)

MALWMRFLPLLALLFLWESHPTQAFVKQHLGSHLVEALYLVCGERGFFYTPMSRREVEDPQVAQLELGG
 GPGAGDLQTLALWSRMDQLAKELTA (SEQ ID NO: 100)

[0141] A non-limiting polypeptide sequence comprising multiple epitopes involved in MS is shown below. The components of this sequence are shown in Table 3C, in which flanking sequences are shown in smaller font to distinguish them from epitope sequences. In some embodiments, the one or more nucleic acids comprise a construct encoding the components shown in Table 3C. In some embodiments, the one or more nucleic acids comprises a construct encoding the following polypeptide sequence:

MASLSRPSLPSCLCFLLLLLLQVSSSYAWYRPPFSRVVHLYRNGKDQDQAAAYQGKGRGLSLSRFSWG
AEGQRPGF AAYLLTFMIAATYNFAVLKLMGRGTKGPGPGKGNATGMEVGWYRPPFSRVVHLYRNGKDQD
GDQAGPGPGHGRGTQDENPVVHFFKNIVTPRTPPPSQGGPGPGHSLERVCHCLGKWLGHDPKDFVGITYALT
VVIVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYQAASSDSAQGSVDVSLTA (SEQ ID NO:
101)

A non-limiting nucleotide sequence for the above polypeptide is shown below. In some embodiments, the one or more nucleic acids comprises a construct having the following sequence:

atggcgagcctgagtagaccaagtctgccaaagctgcctttgtagctttctcctggtgctgctggtgcaag
ttagcagttcctacgcttggtaccgccctccatttagccgcgtcggtcacctctataggaacggtaaaga
ccaggacggagatcaagcggcttatcaaggcaagggccgaggggtgtcattgtcccggttttcctggggg
gccgaggggacaacgcccaggatttgcggttatctgcttacgtttatgatagccgcgacctaacttcg
cagtccttgaaattgatgggacgaggtactaaggggccgggacccgggcccggaaagaacgcgaccgggat
ggaagtggggtggtataggccaccgttttagtcgcgtcggtgcatattgtatcggaacggtaaggatcaagac
ggagatcaagcgggtccaggccctggacacgggagacacaggacgagaatcccgtggtccattttttta
aaaacattgtaacgcctaggaacctccgcctagccagggcggaccaggaccaggggcactcactcgaaag
ggtgtgccattgcctcggaagtggcttgggcatcctgacaaattcgtgggaattacatacgcgctcaca
gtcgtcattggttggtattgtcgccggccttgccgttttggtgtggttagtaattggggctggttgggcca
cagttatgtgcagaaggaaatcctctggtggttaagggtggtagttattctcaggcagcatccagcgactc
tgcacaggggtcagacgtcagtccttacagcctaa (SEQ ID NO: 102)

[0142] Table 3C: Human multi-epitope design for treating MS

	(IEDB ID or epitope name) Sequence
MOG1-29 signal peptide	MASLSRPSLPSCLCFLLLLLLQVSSSYA (SEQ ID NO: 103)
MOG Class I epitope+flank	(114944) WYRPPFSRVVHLYRNGKDQDQ (SEQ ID NO: 104)
AAY	AAY (SEQ ID NO: 23)
MBP Class I epitope+flank	(59472) QGKGRGLSLSRFSWGAEGQRPGE (SEQ ID NO: 105)
AAY	AAY (SEQ ID NO: 23)

PLP Class I epitope+flank	(541283) LLTFMIAATYNFAVLKLMGRGTK (SEQ ID NO: 106)
GPGPG	GPGPG (SEQ ID NO: 26)
MOG Class II epitope+flank	(112226) PGKNATGMEVGWYRPPFSRVVHLYRNGK _{DQDGDQA} (SEQ ID NO: 107)
GPGPG	GPGPG (SEQ ID NO: 26)
MBP Class II epitope+flank	(13572) SHGRTQDENPVVHFFKNIVTPRTPPPSQG (SEQ ID NO: 142)
GPGPG	GPGPG (SEQ ID NO: 26)
PLP Class II epitope+flank	(106502) HSLEKRVCHCLGKWLGHDPDKFVGI _{TYALT} TVV (SEQ ID NO: 108)
MITD	IVGIVAGLAVLAVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSVDVSLTA (SEQ ID NO: 55)

[0143] A non-limiting polypeptide sequence comprising multiple epitopes involved in Hashimoto's thyroiditis is shown below. The components of this sequence are shown in Table 3D, in which flanking sequences are shown in smaller font to distinguish them from epitope sequences. In some embodiments, the one or more nucleic acids comprise a construct encoding the components shown in Table 3D. In some embodiments, the one or more nucleic acids comprises a construct encoding the following polypeptide sequence:

MALVLEIFTLLASICWVSAGLREDLLSLQEPGSKTYAAYGEVSGNWGLLDQVAALTWVQTHIAAYTAIAS
RSVADKILDLYKHPDNIDGPGPGDSWQSLALSSVVDPSPSRHFDVAHVSTAAGPGPGCLMMQKFEKVPES
KVI_{FDANAPVAVRSKVPDSEFPVMQCLTGPGPGRTG}TKSTLPISETGGGTPELRCGKHQAVGTSPQRIVG
IVAGLAVLAVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSVDVSLTA (SEQ ID NO: 109)

A non-limiting nucleotide sequence for the above polypeptide is shown below. In some embodiments, the one or more nucleic acids comprises a construct having the following sequence:

atggcactgggttctggagatatctactctgttgccgagatattgttggtctctgccggtctccgagagg
acctgctctctctccaggaaccgggcagtaagacgtacgcagcttacggagaggtgtccggtaactggg
ccttctcgatcaagtggccgcactgacttgggtacaaacgcattatgcagcgtacactgcgatagcatcc
cgaagcgtcgcagataagattctcgacctgtacaaacatcctgataatatcgacggccccgggtccagggg
actcttggcagtccttggcactttcctccgtggttagatcctagtagatttagacattttgacgttgcca
cgctcagcactgctgcagggccaggaccgggtgtctgatgatgcagaaatttgagaaggtcccgaggtca
aaagtaattttcgacgccaacgctccgggtcgccgtaaggagcaaggtcccagattcagaatttccgggtga
tgcagtgtctgacaggacctgggcccgggacgggaacaaagtccacactccccatcagtgagaccgg
cggtggaacgcctgaactgcggtgcggttaaacatcaagctgtgggaacgtccccacagcgaatagtgggg
atagtcgctggactcgcagttctggctgtcgtagtcacggagcggttagttgctacagtaattgtgtcgga

gaaagagcagcggtggcaaaggaggatcttatagtcaggctgcgtcctctgactcagctcaggggtcaga
cgtttctttgactgcttaa (SEQ ID NO: 110)

[0144] Table 3D: Human multi-epitope design for treating Hashimoto's thyroiditis

	(IEDB ID or epitope name) Sequence
THYG 1-19 signal peptide	MALVLEIFTLLASICWVSA (SEQ ID NO: 111)
THYG Class I epitope+flank	(1117166) GLREDLLSLQEPGSKTY (SEQ ID NO: 112)
AAV	AAV (SEQ ID NO: 23)
THYG Class I epitope+flank	(164764) GEVSGNWGLLDQVAALTWVQTHI (SEQ ID NO: 113)
AAV	AAV (SEQ ID NO: 23)
TPO Class I epitope+flank	(907056) TALAASRSVADKILDLYKHPDNID (SEQ ID NO: 114)
GPGPG	GPGPG (SEQ ID NO: 26)
THYG Class II epitope+flank	(39688) DSWQSLALSSVVDPISRHFDAHVSTAA (SEQ ID NO: 115)
GPGPG	GPGPG (SEQ ID NO: 26)
THYG Class II epitope+flank	(534695) CLMMQKFEKVPESKVIDANAPVAVRSKVPDSEFVVMQCLT (SEQ ID NO: 116)
GPGPG	GPGPG (SEQ ID NO: 26)
TPO Class II epitope+flank	(130545) RTGTKSTLPISETGGGTPELRCKHQA _{AVGTSPQR} (SEQ ID NO: 117)
MITD	IVGIVAGLAVLAVVVIGAVVATVMCRKSSGGKGGSYSQAASSDSAQGSVDLSLA (SEQ ID NO: 55)

[0145] Two non-limiting polypeptide sequences comprising multiple epitopes involved in vitiligo are shown below. The components of these sequences are shown in Table 3E, in which flanking sequences are shown in smaller font to distinguish them from epitope sequences. The amino acid N* in the first sequence is used to denote Asn that can be post-translationally deamidated *in vivo*. In the second sequence, this amino acid is replaced with Asp, so deamidation is unnecessary. In some embodiments, the one or more nucleic acids comprise a construct encoding the components shown in Table 3E, where N* is optionally substituted with D. In some embodiments, the one or more nucleic acids comprises a construct encoding one of the following polypeptide sequences:

MLLAVLYCLLWSFQTSAGHMHNALHIYM(N*)GTMSQVQGSANDPAAYALPFVVITAAYVRILQRMTSSV
AAYHSSSAFTITDQVPFVSVSQLRAGPGPGKDLGYDYSYLQSDPDSFQDYIKSYLEGPGPGDIFIINL
SVVDLLFLLGMPFMIHQLMGNGVWGPGPGRQLRTKAWNRLYPEWTEAQRLLDCWRGGQVIVGIVAGLAVL
AVVVIGAVVATVMCRKSSGGKGGSYSQAASSDSAQGSVDLSLA (SEQ ID NO: 118)

MLLAVLYCLLWSFQTSAGHMHNLHIYMDGTMSQVQGSANDPAAYALPFVVITAAYVRILQRMTSSVAAY
 HSSSAFTITDQVPFVSQVSVSQLRAGPGPGKDLGYDYSYLQSDPDSFQDYIKSYLEGPGPGDIFIINLSVV
 DLLFLLGMPFMIHQLMGNVWGPGPGRQLRTRKAWNRQLYPEWTEAQRLLDCWRGGQVIVGIVAGLAVLAVV
 VIGAVVATVMCRRKSSGGKGSYSQAASSDSAQGSVDVSLTA (SEQ ID NO: 119)

Non-limiting nucleotide sequences for the above two polypeptides are shown below. In some embodiments, the one or more nucleic acids comprises a construct having one of the following sequences:

atgctccttgctgtgctttactgtctgctgtggagtttccaaacgtcagcgggacacatgcacaacgctc
 ttcacatatatatg**aac**gaaccatgtcacaagtccaggggtcagcgaacgacccagccgcttacgcgct
 cccttttgtagtcattacagccgcatatgtgagaatcctgcagcggatgacgagcagcgtcgcggttat
 cacagctccttctgcctttacgataaccgaccaagtgccttcagcgtcagtgtagtcaacttcgcgctg
 ggccgggaccaggaaaggatttggggtacgactatagctatcttcaggatagtgacccggattcatttca
 ggattatattaagtcatatttgaaggaccgggtccaggggacattttcatcataaacctcagcgttgta
 gaccttctcttctgttggggatgccgttcatacatcaacttatggggaacggagctctggggccctg
 gacctggacgacaacttcgcactaaggcctggaacagacagctttatcccagtgtagcgaagctcagag
 attggactgctggagaggtggccaagtcattgtgggtatagttgcggggctcgagtagtgcagtagtt
 gttataggggccgttgtggccacggtgatgtgcaggaggaaaagctccgggggtaagggggggtcctata
 gtcaggcggcatcaagcgattctgtctcaaggttctgacgtttcactcacagcttaa (SEQ ID NO:
 120)

atgctccttgctgtgctttactgtctgctgtggagtttccaaacgtcagcgggacacatgcacaacgctc
 ttcacatatatatg**gac**gaaccatgtcacaagtccaggggtcagcgaacgacccagccgcttacgcgct
 cccttttgtagtcattacagccgcatatgtgagaatcctgcagcggatgacgagcagcgtcgcggttat
 cacagctccttctgcctttacgataaccgaccaagtgccttcagcgtcagtgtagtcaacttcgcgctg
 ggccgggaccaggaaaggatttggggtacgactatagctatcttcaggatagtgacccggattcatttca
 ggattatattaagtcatatttgaaggaccgggtccaggggacattttcatcataaacctcagcgttgta
 gaccttctcttctgttggggatgccgttcatacatcaacttatggggaacggagctctggggccctg
 gacctggacgacaacttcgcactaaggcctggaacagacagctttatcccagtgtagcgaagctcagag
 attggactgctggagaggtggccaagtcattgtgggtatagttgcggggctcgagtagtgcagtagtt
 gttataggggccgttgtggccacggtgatgtgcaggaggaaaagctccgggggtaagggggggtcctata
 gtcaggcggcatcaagcgattctgtctcaaggttctgacgtttcactcacagcttaa (SEQ ID NO:
 121)

[0146] Table 3E: Human multi-epitope design for treating vitiligo

	(IEDB ID or epitope name) Sequence
Tyrosinase 1-18 signal peptide	MLLAVLYCLLSFQTSAGH (SEQ ID NO: 122)
Tyrosinase Class I epitope+flank	(75043) MHNALHIYMN*GTMSQV _{QGS} ANDP (SEQ ID NO: 123)
AAV	AAV (SEQ ID NO: 23)
MCHR1 Class I epitope+flank	(727393) ALPFVVI _{TAAYVRILQ} RM _{TSSV} (SEQ ID NO: 124)
AAV	AAV (SEQ ID NO: 23)
PMEL Class I epitope+flank	(28864) HSSSAFTITDQVPFSV _{SVS} QLRA (SEQ ID NO: 125)
GPGPG	GPGPG (SEQ ID NO: 26)
Tyrosinase Class II epitope+flank	(62642) KDLGYDY _{SYLQSDPD} SFQD _{YIKS} YLE (SEQ ID NO: 126)
GPGPG	GPGPG (SEQ ID NO: 26)
MCHR1 Class II epitope+flank	(1284068) DIFIINLSVVDLLFLLGMPFMIHQ _{LMGNGVW} (SEQ ID NO: 127)
GPGPG	GPGPG (SEQ ID NO: 26)
PMEL Class II epitope+flank	(72906) RQLRTKAWNRQLYPEWTEAQR _{LD} CWRGGQV (SEQ ID NO: 128)
MITD	IVGIVAGLAVLAVVVIGAVVATVMCRKSSGGKGGSYSQAASSDSAQGS _{VD} SLTA (SEQ ID NO: 55)

[0147] Two non-limiting polypeptide sequences comprising multiple epitopes involved in celiac disease are shown below. The components of these sequences are shown in Table 3F, in which flanking sequences are shown in smaller font to distinguish them from epitope sequences. The amino acid Q* in the first sequence is used to denote Gln that can be post-translationally deamidated *in vivo*. In the second sequence, this amino acid is replaced with Glu, so deamidation is unnecessary. In some embodiments, the one or more nucleic acids comprise a construct encoding the components shown in Table 3CF, wherein Q* is optionally substituted with E. In some embodiments, the one or more nucleic acids comprises a construct encoding one of the following polypeptide sequences:

MKTFLILVLLAIVATTATTALQQILQQQLIPCMDVVLQQHNIAHGPGPGLPYLQLQFPFPQPLPYSQPQP
FRGPGPGQPQQPQQPFPQPQQPFPWQPQQPGPGPGYPQPQPQYSQPQQPISQQQQQGGPGPGQPQQPFP
QQPQQPFPQPQLQFPQGGPGGQFPQPQQPQQSFPQQQPFFIQPSIVGIVAGLAVLAVVVIGAVVATVMCR
RKSSGGKGGSYSQAASSDSAQGS_{VD}SLTA (SEQ ID NO: 129)

MKTFLILVLLAIVATTATTALQQILQQQLIPCMDVVLQQHNIAHGPGPGLPYLQLQFPFPQPELPYSQPQP
FRGPGPGQPQQPQQPFPQPEQPFPWQPQQPGPGPGYPQPQPQYSQPEQPISQQQQQGGPGPGQPQQPFP
EQPQQPFPQPQLQFPQGGPGGQFPQPQQPQQSFPEQEPPFIQPSIVGIVAGLAVLAVVVIGAVVATVMCR
RKSSGGKGGSYSQAASSDSAQGS_{VD}SLTA (SEQ ID NO: 130)

Non-limiting nucleotide sequences for the above two polypeptides are shown below. In some embodiments, the one or more nucleic acids comprises a construct having one of the following sequences:

atgaagactttttctgatcttgggtgttgctggcgatcgtggcgacgactgcgaccaccgcgctgcaacaaa
tccttcagcaacagctgattccttgcatggacgtagtcctgcaacaacataacattgcgacggacctgg
tcccggtgctgccttatttgcaacttcagcccttccccaaccccagctgccctattctcaaccgcaacct
ttcagagggtgctgggccaaccgcagcagccacaacaaccctttccacaacctcaacagccgtttc
cgtggcagcctcaacaaccggggcccgacctgggacctacccccagcctcagccacagtattctcaacc
tcagcagccgataagtcaacagcagcaacagcaaggccctggacctggtcagccgcagcagcctttcccg
caacagcctcaacaaccattttcccagccgcaacttcaatttccgcaagggtccggaccgggtcagttcc
cgcaaccgcaacagccgcagcagagcttccccagcaacaaccacctttcatccagcctagcatagtcgg
tatcgtcgccggtctggcgtgctggcgtggtcgtaataggtgccgtcgtcgcaactgttatgtgcaga
cgaaaaagttcaggcggttaaggcggttcttattcacaggcagccagcagtgacagcgccagggtcag
acgttagtttgaccgcttaa (SEQ ID NO: 131)

atgaagactttttcttatcttgggtccttttggctatagtcgacgacagcagaccaccgcttgcaacaaa
ttctgcaacagcaactcataccctgcatggacgttggtgctccaacaacacaatatagcccacggtccagg
gcttggtgctgcgtatcttcaactccaaccatttctcagcccgaaacttccgtatttccaaccacagcca
ttcagaggacctcggtccaggacagccgcagcaaccgcagcagccctttcctcagcccgagcagcctttcc
cttggcaaccccagcagccaggtcccggtcctggcccatatccgcaaccacagccacagtactcccagcc
tgaacaaccataagtacagcagcagcaacagcaaggccccgggcccgggcagccacaacaacccttccca
gagcaaccccaacaaccgttcccgagccacagctccaattcccacaagggtccgtgggaatttc
cgagccccaacaaccacaacagtcctttccgaacaggaaccgcatatttattcagccgtctatagtcgg
gattgttgaggactggcgttctggcagtagtcgttataggtgccgtcggtgacacggtaattgtgcaga
aggaaaagctctggtgggaaaggcggtcatactctcagccgcctcctccgatagcgctcaaggatccg
acgtttccttgacagcttaa (SEQ ID NO: 132)

[0148] Table 3F: Human gliadin multi-epitope sequence for treating celiac disease

	(IEDB ID or epitope name/identifier) Sequence
Alpha gliadin signal peptide	MKTFLILVLLAIVATTATTA (SEQ ID NO: 133)
gliadin Class I epitope+flank	(51376) LQQILQQQLIPCMDVVLQQHNIH (SEQ ID NO: 134)
GPGPG	GPGPG (SEQ ID NO: 26)
DQ2.5 restricted gliadin Class II epitope	(DQ2.5-glia- α 1a) LPYLQLQPFPPQPQ*LPYSQPQFPR (SEQ ID NO: 135)

GPGPG	GPGPG (SEQ ID NO: 26)
DQ2.5 restricted gliadin Class II epitope	(DQ2.5-glia- ω 1) <u>QPPQPPQ</u> PFPQPQ*QPF ^{PWQPPQ} (SEQ ID NO: 136)
GPGPG	GPGPG (SEQ ID NO: 26)
DQ2.2 restricted gliadin Class II epitope	(DQ2.2-glia- α 2) <u>PYPQPPQ</u> QYSQPQ*QPI ^{SQQQQQ} (SEQ ID NO: 137)
	GPGPG (SEQ ID NO: 26)
DQ8 restricted gliadin Class II epitope	(DQ8-glia- g1a) <u>QPPQPPF</u> Q*QPQQPFPQ* ^{PQLQFPQ} (SEQ ID NO: 138)
	GPGPG (SEQ ID NO: 26)
DQ8.5 restricted gliadin Class II epitope	(DQ8.5-glia-g1) <u>QFPQPPQ</u> PQSFQ*QQ* ^{PPFIQPS} (SEQ ID NO: 139)
MITD	IVGIVAGLAVLAVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSVDLSLT (SEQ ID NO: 55)

[0149] A non-limiting polypeptide sequence comprising multiple epitopes involved in T1D is shown below. The components of this sequence are shown in Table 3G, in which flanking sequences are shown underlined to distinguish them from epitope sequences. In some embodiments, the one or more nucleic acids comprise a construct encoding the components shown in Table 3G. In some embodiments, the one or more nucleic acids comprises a construct encoding the following polypeptide sequence:

MALWMRLPLLLALLALWGPDPAAAFVNQHLGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVEAA
YTLAFLQDVMNILLQYVVKSFDRSTAAAYGLVRNLGVLFGLGFAINSEMFLLGPGPGVNQHLGSHLVEALY
LVCGERGFFYTPKTGPGPGLGGPGAGSLQPLALEGSLQKRGIVEQCCTSI^{CSGPGPLAFLQDVMNILL}
QYVVKSFDRSTKVIDGPGPGSPGGAI^{SNMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLKKGAA}
AALGGPGPGMVFDGK^{PQHTNVCFWYIPPSLR}TLEDNEERMSRLSKVAPVIKARMEYGT^{TMVSYQPLGDK}
VNFFRMVISNPAATHQDIDFLIEEIERLGGPGPGPVILLEKKSPLGQSQPTVAGQPSARPAEEYGYIVTD
QNVVGPALTFRIRHNEQNGPGPGPSWCEEPAQANMDISTGHMILAYMEDHLRNRDLAIVGIVAGLAVLA
VVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSVDLSLT (SEQ ID NO: 140)

A non-limiting nucleotide sequence for the above polypeptide is shown below. In some embodiments, the one or more nucleic acids comprises a construct having the following sequence:

atggcgcttttgatgcatgattgctcccgctgcttgctctcctgcactctggggacctgacccggcagcag
cttttggttaatcagcacctttgctggttcacaccttgtagaggccctgtacctcgcttgctggagaaagagg
ctttttctacactcccaaaactagacgagaggcagaggatctgcaggtaggccaagtgaagccgcttat
acactgcctttttgcaggatgtgatgaacattcttctgcagtacgtcgtaaagtcctttgatcgtagta
cagctgcctacggttttggtacggaacctcgagtgctctttggccttggtttcgccatcaattccgagat

gtttctgttgggcccctgggtcctggcggttaaccaacatttgtgtgggtctcatcttgtcgaagcactttat
 ctctgtgtgaggagaacgggggttcttttacactccaaaaacggggcccgaccgggcttgggtggcgggc
 ccggagctgggtcccttcaacctcttgccctcgagggtccctccaaaaagaggaatagtgaggcagtg
 ttgcacgagtatctgttcaggaccgggacctggcctggcttttctgcaggatgtaataatattcttctc
 caatacgtcgtcaagtctttcgacagatccacaaaggtcatagacgggcccgggctgtagtcccggag
 gcgccataagtaatatgtacgctatgatgattgccaggttcaagatgttccccgaggtaaaagaaaagg
 tatggccgctttgcccagactgattgcatttaccagcgaacattcccattttagccttaaaaaagggtgca
 gccgcgcttgggtgggcccgggaccaggaatggtgtttgacggcaagcctcagcacacgaacgtgtgcttct
 ggtatatacccccgctcacttcggacgctcgaagacaacgaggagcgcatgtcccgaactttccaaggtagc
 accggttataaaggcacgaatgatggaatacgggaccactatggtcagttaccagcccctgggcgataaa
 gtgaactttttcagaatggttatatcaaactcctgcccgtacgcacatcaagatatagatttcctcattgagg
 agatcgaaagactcgggtggaccgggaccgggtcctgtgcttcttgagaagaagtcaccgctgggccaag
 ccaaccgacagtcgcccgggcaaccctcagctcgcccggccgagggaatacggctacatcgtcactgac
 cagaacgttgttggaccagcacttacgtttcgcacccgccaacgaacaaaacgggtccaggtcccggcc
 cctcctggtgtgaggagcccgtcaagctaacatggacattagtagcaggacacatgatactcgcttatat
 ggaggaccacctccgaaatcgagatcgacttgctattgtcggtattgttgcgggactggcggtcctggca
 gtagtagtgattggggctgtggttagcaactgtcatgtgtcgccgcaagtcctctggtggaaagggcgga
 gttattcacaagccgctcttcagactccgcgcaaggaagtgcgtgagccttaccgcgtaa (SEQ ID
 NO: 141)

[0150] Table 3G: Human multi-epitope design for treating T1D

	Sequence
INS signal peptide + additional extension to span more epitopes	(Insulin 1-67) MALWMRLRLPLLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAE DLQVGQVE (SEQ ID NO: 143)
AAY	AAY (SEQ ID NO: 23)
GAD65 Class I epitope+flank	(GAD65 107-130) TLAFLQDVMNILLQYVVKSFDRST (SEQ ID NO: 144)
AAY	AAY (SEQ ID NO: 23)
IGRP Class I epitope+flank	(IGRP 258-280) GLVRNLGVLFGLGFAINSEMFL (SEQ ID NO: 145)
GPGPG	GPGPG (SEQ ID NO: 26)
INS Class II epitope+flank	(Insulin 26-54) VNQHLCGSHLVEALYLVCGERGFFYTPKT (SEQ ID NO: 146)
GPGPG	GPGPG (SEQ ID NO: 26)
INS Class II epitope+flank	(Insulin 68-101) LGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICS (SEQ ID NO: 147)
GPGPG	GPGPG (SEQ ID NO: 26)
GAD65 Class II epitope+flank	(GAD65 108-134) LAFLQDVMNILLQYVVKSFDRSTKVID (SEQ ID NO: 148)

GPGPG	GPGPG (SEQ ID NO: 26)
GAD65 Class II epitope+flank	(GAD65 240-293) <u>SPGGALSNMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLKKGAAALG</u> (SEQ ID NO: 149)
GPGPG	GPGPG (SEQ ID NO: 26)
GAD65 Class II epitope+flank	(GAD65 493-582) <u>MVFDGKPKQHTNVCFWYIPPSLRTLEDNEERMSRLSKVAPVIKARMMEYGTMTMSYQPLG</u> <u>DKVNFFRMVISNPAATHQDIDFLIEEIERLG</u> (SEQ ID NO: 150)
GPGPG	GPGPG (SEQ ID NO: 26)
IA-2 Class II epitope+flank	(IA-2 442-495) <u>PVLLLEKKSPPLGQSQPTVAGQPSARPAEEYGYIVTDQNVVGPALTFRIRHNEQN</u> (SEQ ID NO: 151)
GPGPG	GPGPG (SEQ ID NO: 26)
IA-2 Class II epitope+flank	(IA-2 647-681) <u>PSWCEEPAQANMDISTGHMILAYMEDHLRNRDRLA</u> (SEQ ID NO: 152)
MITD	<u>IVGIVAGLAVLAVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSVDVSLTA</u> (SEQ ID NO: 55)

[0151] In some embodiments, the one or more nucleic acids encodes a human insulin hybrid peptide that consists of residues 1-71 of human insulin (INS(PPI)1-71), followed immediately by one of four protein fragments from IAPP2, Neuropeptide Y, Insulin A, or IAPP1 (see Table 2B for sequences of the components). The mRNA sequence is configured according to either Formula XIII or Formula XIV:

5'UTR – INS(PPI)1-71 – protein fragment – MITD - 3'UTR – PolyA tail (Formula XIII)

5'UTR – INS(PPI)1-71 – protein fragment – 3'UTR – PolyA tail (Formula XIV)

[0152] In another aspect, there is provided an mRNA construct comprising: 5'UTR sequence; a sequence encoding a signal peptide; a multi-epitope coding sequence encoding a multi-epitope polypeptide; 3'UTR sequence; and a polyadenine tail. The multi-epitope polypeptide comprises three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, Class II epitope of hybrid insulin peptide. Epitopes in the multi-epitope polypeptide are separated from each other by cleavable linkers, and wherein the epitopes comprise epitopes from a plurality of T1D autoantigens. In some embodiments, Class I epitopes are separated from other Class I epitopes by a class I linker peptide. In some embodiments, Class II epitopes are separated from other Class II epitopes by a class II linker peptide. In some embodiments, the mRNA construct further comprises

a sequence encoding a polypeptide comprising an endolysosomal trafficking sequence motif (e.g. MITD) positioned 5' to the 3'UTR sequence.

[0153] The nucleic acids used in the immunomodulatory combinations or mRNA constructs herein can be isolated from natural sources, obtained from such sources as ATCC or GenBank libraries or prepared by synthetic methods. Synthetic nucleic acids can be prepared by a variety of solution or solid phase methods, and are widely available commercially. RNA sequences encoding any amino acid sequence is obtainable from codon tables, including RNA sequences that are codon optimized for expression in mice or humans. 5'UTR and 3'UTR sequences for humans are well known. The RNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, or may be chemically synthesized. In certain embodiments, the RNA encoding the antigen encompasses both modified and unmodified RNA.

[0154] Synthesized RNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and/or backbone modifications. In some embodiments, an RNA is or comprises natural nucleosides (e.g., adenosine, guanosine, cytidine, uridine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methyl cytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methyl cytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, pseudouridine, 5-methoxyuridine, and 5-methylcytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages). In some embodiments, uridines are replaced with modified uridine, e.g. 5-methoxyuridine (5moU), pseudouridine (Ψ), N1-methyl-pseudouridine (me1Ψ), and the like (see above). In some embodiments, cytidines are replaced with modified cytidine, e.g. 5-methylcytidine (5meC) and the like (see above). In some embodiments, uridines and cytidines are replaced. In some embodiments, the mRNA is capped with a 5' Cap1 structure. modified bases and 5' caps are available using commercial synthesis services.

[0155] RNA may be synthesized according to any of a variety of known methods. For example, the RNA in certain embodiments may be synthesized via *in vitro* transcription (IVT). Briefly, IVT

is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor.

[0156] In some embodiments, *in vitro* synthesized RNA encoding the antigen may be purified before formulation and encapsulation to remove undesirable impurities including various enzymes and other reagents used during RNA synthesis.

[0157] While RNA provided from *in vitro* transcription reactions may be desirable in certain embodiments, other sources of RNA are contemplated, such as RNA produced from bacteria, fungi, plants, and/or animals.

[0158] *Pathway of mRNA-encoded antigen uptake and presentation.* Messenger RNA (mRNA) therapeutics delivered by LNPs (or liposomes) are taken up by cells through endocytosis (or phagocytosis). Antigen presenting cells including dendritic cells are particularly efficient at taking up LNPs/liposomes (both through endocytosis or phagocytosis). Upon uptake into endosomes or phagosomes, ionizable lipids within LNPs/liposomes disrupt the endosomes, releasing mRNA into the cytosol. Normally mRNA would be translated to protein in the cytosol, but when a signal peptide is encoded in the mRNA, it will direct translation of the mRNA into protein in the endoplasmic reticulum (ER). This serves two purposes:

- 1) Once in the ER, the signal peptide is cleaved off. The protein can follow cross-presentation to MHC Class I following the cytosolic pathway (Joffre et al., 2012, *Nat. Rev. Immunol.* 12:557-569). MHC Class II antigen processing takes place across the ER, Golgi, plasma membrane, early and late endosomes and lysosomes, and thus MHC II processing is promoted from the signal peptide. When present, the MITD sequence on the C terminus helps traffick the protein to MHC Class II antigen processing sites (Kreiter et al 2008, *J. Immunol.* 180(1):309–318).
- 2) In the ER, the signal peptide is cleaved off and the protein is targeted for secretion. Secretion of the protein by the original target cell also allows neighbouring cells, particularly antigen-presenting cells, to take up this protein and process it via natural antigen-presentation pathways, amplifying the antigen-presentation.

[0159] *Cross-presentation on MHC Class I.* The resulting protein translated from the mRNA therapeutic into the ER could be transported back to the cytosol via retrotranslocation of proteins from ER into cytosol by ERAD proteins. Any protein that ends up in endosomal compartments (particularly with the help of the MITD signal) can undergo antigen export to the cytosol through the normal cross-presentation pathway. Similarly, in neighbouring cells that take up the secreted protein from the extracellular space, antigen cross-presentation is enabled via antigen export from endocytic compartments to the cytosol.

[0160] Once in the cytosol, the protein can be presented along natural cross-presentation pathways via proteosomal cleavage into peptide epitopes and transport of peptides back into the ER via transporter associated with antigen processing 1 (TAP1) and TAP2 for loading on MHC Class I molecules (reviewed in Joffre et al., 2012, *Nat. Rev. Immunol.* 12:557-569). Inside the ER, MHC-I is loaded, trafficked through the golgi apparatus, and presented on the cell surface.

[0161] *Presentation on MHC Class II.* Any protein that is secreted by the initially targeted cell can be taken up by neighbouring cells and processed along natural MHC Class II presenting pathways, which occurs in lysosomal compartments.

[0162] In the direct target cell that takes up the mRNA, once the mRNA is translated into the ER with the help of the signal peptide, it can be loaded onto MHC Class II at any subcellular location involved in MHC Class II antigen processing (including ER, Golgi, plasma membrane and endosomal compartments). In addition, trafficking signals (e.g. the MITD) can help target it to key processing compartments for enhanced MHC Class II presentation.

[0163] Delivery vehicles

[0164] A variety of delivery vehicles can be used to prepare the immunomodulatory combinations (alternatively referred to as “vaccine formulations”). These include, but are not limited to, nanoparticles, including lipid nanoparticles (LNPs), liposomes, polymer nanoparticles comprising lipids, polymer-based nanoparticles, emulsions, and micelles.

[0165] The lipid conjugates of the present disclosure are particularly amenable to incorporation into nanoparticles (e.g. lipid nanoparticles, liposomes, or polymer-based systems) comprising lipids or other hydrophobic components. The lipid-like properties of the lipid conjugate in certain embodiments may facilitate its loading into these or other delivery vehicles. For example, in some

embodiments, the loading efficiency into a given nanoparticle is 75% to 100%, 80% to 100% or most advantageously 90% to 100%. In some embodiments, the delivery vehicle(s) are liposomes and/or lipid nanoparticles.

[0166] In some embodiments, the lipid conjugates and nucleic acids are loaded into lipid nanoparticles or liposomes, by mixing them with lipid formulation components, including vesicle forming lipids and optionally a sterol. As a result, lipid nanoparticles and/or liposomes incorporating the cargo can be prepared using a wide variety of well described formulation methodologies known to those of skill in the art, including but not limited to extrusion, ethanol injection and in-line mixing. Such methods are described in Maclachlan, I. and P. Cullis, “Diffusible-PEG-lipid Stabilized Plasmid Lipid Particles”, *Adv. Genet.*, 2005. 53PA:157-188; Jeffs, L.B., et al., “A Scalable, Extrusion-free Method for Efficient Liposomal Encapsulation of Plasmid DNA”, *Pharm Res*, 2005. 22(3):362-72; and Leung, A.K., et al., “Lipid Nanoparticles Containing siRNA Synthesized by Microfluidic Mixing Exhibit an Electron-Dense Nanostructured Core”, *The Journal of Physical Chemistry. C, Nanomaterials and Interfaces*, 2012, 116(34): 18440-18450, each of which is incorporated herein by reference in its entirety.

[0167] While liposomes comprise an aqueous internal solution surrounded by a phospholipid bilayer, a lipid nanoparticle may alternatively comprise a lipophilic core. Such lipophilic core can serve as a reservoir for the lipid conjugate and/or the nucleic acids. Solid and liquid lipid nanoparticles can be used for the delivery of the lipid conjugate(s) and/or nucleic acid(s) as described herein.

[0168] Provided in one embodiment is a liposome or lipid nanoparticle that comprises a phospholipid bilayer and wherein the lipid conjugate forms a hydrophobic oil phase within the bilayer. Such delivery vehicles are described in WO 2020/191477 (PCT/CA2020/000039), which is incorporated herein by reference. In another embodiment, the delivery vehicle is a liposome.

[0169] In some embodiments, at least one delivery vehicle is a lipid nanoparticle, optionally wherein at least one lipid conjugate is incorporated into a lipid compartment of the lipid nanoparticle. In some embodiments, at least one delivery vehicle is a liposome, optionally wherein at least one lipid conjugate is incorporated within the oily phase of the lipid bilayer of the liposome.

[0170] In some embodiments, the delivery vehicle(s) is a nanoparticle (e.g. liposome or LNP) that comprises a lipid core stabilized by a surfactant. Vesicle-forming lipids may be utilized as stabilizers. In another embodiment, a delivery vehicle is a polymer-lipid hybrid system that comprises a polymer nanoparticle core surrounded by stabilizing lipid.

[0171] Nanoparticles may alternatively be prepared from polymers without lipids. Such nanoparticles may comprise a concentrated core of drug that is surrounded by a polymeric shell or may have a solid or a liquid dispersed throughout a polymer matrix.

[0172] The lipid conjugates and/or nucleic acid(s) described herein can also be incorporated into emulsions, which are drug delivery vehicles that contain oil droplets or an oil core. An emulsion can be lipid-stabilized. For example, an emulsion may comprise an oil filled core stabilized by an emulsifying component such as a monolayer or bilayer of lipids.

[0173] Micelles are self-assembling particles composed of amphipathic lipids or polymeric components that are utilized for the delivery of agents present in the hydrophobic core. Conjugating a drug to a scaffold molecule L and with a hydrophobic group R as described herein may improve drug loading into a micelle.

[0174] A further class of drug delivery vehicles known to those of skill in the art that can be used to encapsulate the lipid conjugate herein is carbon nanotubes.

[0175] Various methods for the preparation of the foregoing delivery vehicles and the incorporation of lipid-conjugated immunomodulatory agents therein are available and may be carried out with ease by those skilled in the art.

[0176] Certain lipid conjugates encompassed by the disclosure may form part of a carrier-free system. In such embodiments, the lipid conjugate can self-assemble into particles. Without being limiting, if the immunomodulatory agent is hydrophilic, then the amphiphilic pro-drug may assemble into nanoparticles with or without a stabilizer.

[0177] LNPs can be made using a wide variety of well described formulation methodologies including high pressure extrusion, ethanol injection, microfluidic mixing and in-line mixing.

[0178] In some embodiments, the polydispersity index (PDI) of the drug delivery vehicle comprising the lipid conjugate and/or the nucleic acid(s) is less than 0.40, 0.35, 0.30, 0.25, 0.20 or 0.15.

[0179] The lipid conjugates described herein are particularly amenable to high encapsulation efficiency in drug delivery vehicles. In one embodiment, the encapsulation efficiency of the lipid conjugate is 10 to 99%, 15 to 99%, 20 to 99% or 25 to 99%.

[0180] In some embodiments, the lipid conjugates in lipid nanoparticles are 5-20%, 7-15%, 9-12%, or about 10% of the total lipids. In some embodiments, the ratio of the first lipid conjugate to the second lipid conjugate is 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, or 9:1.

[0181] The delivery vehicle comprising the lipid conjugate(s) may be co-formulated with the nucleic acid(s) or the lipid conjugate(s) and nucleic acid(s) may be formulated in separate delivery vehicles. In some embodiments, the lipid conjugate(s) is co-formulated in the same delivery vehicle as the one or more nucleic acids.

[0182] The nucleic acid(s) may be formulated in a delivery vehicle, such as a lipid nanoparticle, comprising an ionizable or permanently charged lipid to produce a positive net charge. The negative charge of nucleic acids can be used to entrap the nucleic acids within liposomes or LNPs using ionic interactions with positive charges of lipids in the liposomes or LNPs. The charged lipid may comprise a lipophilic moiety that comprises a scaffold and one or more hydrocarbon side chains linked thereto by biodegradable groups, for example as described in co-owned WO 2021/026647; Application No. PCT/CA2020/051098, which is incorporated herein by reference.

[0183] Water soluble or hydrophilic molecules (like nucleic acids) can be loaded passively into liposomes that contain an aqueous core. The nucleic acids are added directly to the aqueous buffer that is used to form the vesicles. Liposomes are made using established methods (e.g., extrusion, ethanol injection, in-line mixing, microfluidics, and the like). This method generally yields low entrapment, but the quantity of cargo can be controlled by the concentrations used.

[0184] Examples of formulations for liposome vaccine systems are provided in the following: Schewendener 2014 *Ther Adv Vaccines* 2:159-182; Schmidt et al., 2016 *Pharmaceutics* 8:7; and Kersten 1995 *Biochim Biophys Acta* 1241:117-138.

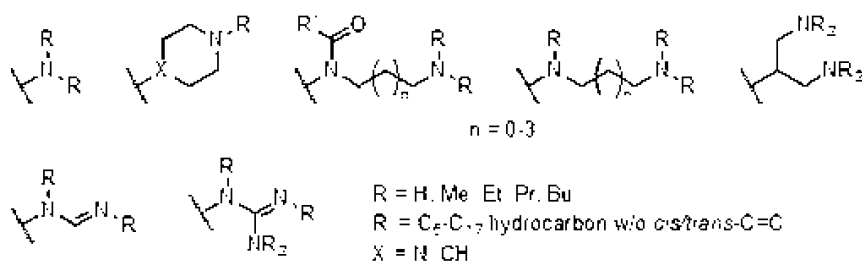
[0185] In certain embodiments, the permanently charged or ionizable lipid of LNP or liposome delivery vehicles comprises a head group and a linear or branched lipophilic moiety, e.g. having the structure of **Formula I** described above.

[0186] In one embodiment, the permanently charged or ionizable lipid comprises a scaffold moiety. The scaffold moiety in one embodiment is represented by L (L1 + L2 + L3 + L4 + L5) of **Formula I** and at least one R is present as a hydrocarbon side chain, wherein n + p is 1 or 1 to 8 or 1 to 7, or 1 to 6 or 1 to 5 or 1 to 4 or 1 to 3.

[0187] L1 of **Formula I** may be linked to a head group directly or via a linker. The linker may be linear, branched or a ring structure. Examples of suitable linker groups for ionizable or permanently charged lipids are well known in the art as provided in WO 2021/026647 (PCT/CA2020/051098), which is incorporated herein by reference.

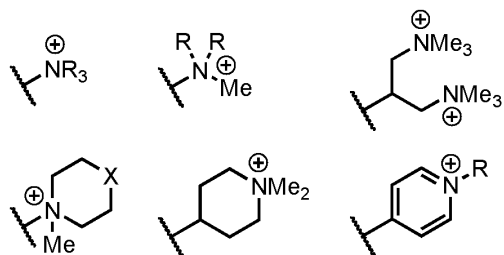
[0188] Examples of headgroups that may be linked directly or indirectly via a linker region to L1 include the following:

- (i) ionizable cationic moieties selected from the group consisting of:



;

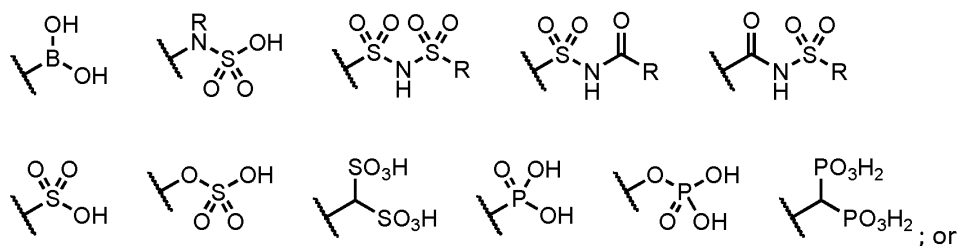
- (ii) permanently charged moieties selected from the group consisting of:



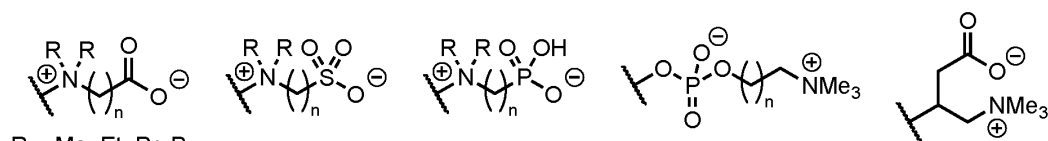
X = CH₂, NMe₂, O

R = Me, Et, Pr, Bu,

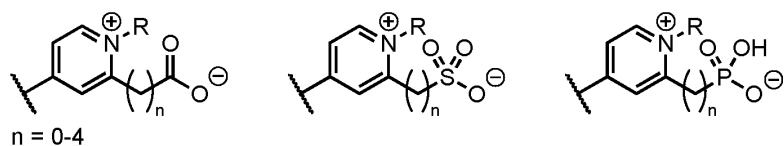
(iii) ionizable anionic moieties selected from the group consisting of:



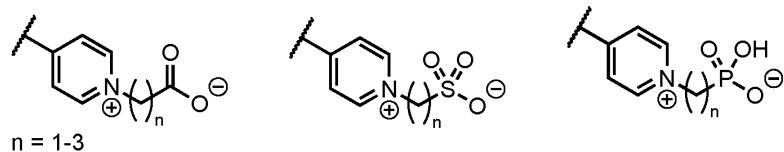
(iv) zwitterionic moieties selected from the group consisting of:



R = Me, Et, Pr, Bu
n = 1-3



n = 0-4



n = 1-3

[0189] Selective delivery to antigen presenting cells (APCs)

[0190] The compositions described herein may be used for delivery to APCs. By way of example and without being limiting, *in vivo* APC uptake of the delivery vehicles may be demonstrated in

the pancreatic islets (e.g., where APCs interact with beta cells to pick up beta-cell antigens) or in the pancreatic lymph nodes (e.g., where APCs interact and with T cells to present antigens and instruct the type of T cell response that will be initiated to that antigen). In one embodiment, the uptake of the delivery vehicle is selective for APCs, with limited uptake in non-APC immune cells. Determination of whether uptake is selective for APCs may be carried out using the methods of Example 1 herein. For example, there may be limited delivery of the delivery vehicles to endocrine cells in the islets, and non-APC immune cells (e.g., T cells) in the lymph node.

[0191] For example, the total population of delivery vehicles, such as lipid nanoparticles, in a formulation described herein that are delivered to non-APCs may be less than 20%, less than 15% or less than 10% as measured using the techniques of Example 1.

[0192] In some embodiments, the delivery vehicles are lipid nanoparticles which preferentially deliver to antigen presenting cells (APCs) in pancreatic islets and/or lymph nodes.

[0193] Methods/Uses

[0194] In some embodiments, the immunomodulatory combination may be used for treating an autoimmune disease or condition in a subject. In some embodiments, the autoimmune disease involves autoreactive T cells comprising both CD4⁺ T cells and CD8⁺ T cells. The delivery vehicle comprising the lipid conjugate and/or nucleic acids may delivered as a pharmaceutical formulation(s). The pharmaceutical formulation(s) may be administered at any suitable dosage, and may comprise a pharmaceutically acceptable excipient.

[0195] In some embodiments, the immunomodulatory combination may be used for treating an autoimmune disease involving autoreactive T cells comprising both CD4⁺ T cells and CD8⁺ T cells. These autoimmune diseases (such as T1D, MS, vitiligo, celiac, IBD) all have characteristic CD8⁺ T cell effector responses that cause tissue damage, which contributes to disease progression. Accordingly, there is provided a method of treating the autoimmune disease comprising administering the immunomodulatory combination to the subject.

[0196] In some embodiments, the immunomodulatory combination may be used for treating type 1 diabetes in a subject. Accordingly, there is provided a method of treating type 1 diabetes comprising administering the immunomodulatory combination to the subject. Latent autoimmune diabetes in adults (LADA) has similar autoimmune characteristics of type 1 diabetes. Accordingly,

there is provided a method of treating LADA comprising administering the immunomodulatory combination to the subject. Type 1 diabetes may be induced by immune checkpoint inhibitor treatment in cancer patients. Accordingly, there is provided a method of treating type 1 diabetes induced by immune checkpoint inhibitor treatment for cancer in a subject, comprising administering the immunomodulatory combination to the subject.

[0197] There is provided a method for treating Multiple Sclerosis (MS) in a subject, comprising administering the immunomodulatory combination as defined herein to the subject. Each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in MS. In some embodiments, the autoantigen(s) comprise one or more of myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin basic protein (MBP), and/or α B-crystallin.

[0198] There is provided a method for treating celiac disease in a subject, comprising administering the immunomodulatory combination as defined herein to the subject. Each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in celiac disease and/or antigens that trigger recognition of autoantigens by autoreactive T cells in celiac disease. In some embodiments, the antigen(s)/autoantigen(s) comprise gliadin.

[0199] There is provided a method for treating thyroiditis in a subject, comprising administering the immunomodulatory combination as defined herein to the subject. Each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in thyroiditis. In some embodiments, the autoantigen(s) comprises one or both of thyroid peroxidase and/or thyroglobulin.

[0200] There is provided a method for treating vitiligo in a subject, comprising administering the immunomodulatory combination as defined herein to the subject. Each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in vitiligo. In some embodiments, the autoantigen(s) comprising one or more of Pmel17, Melanin-Concentrating Hormone Receptor 1, and/or Tyrosinase.

[0201] There is provided a method for treating inflammatory bowel disease (IBD) in a subject, comprising administering the immunomodulatory combination as defined herein to the subject. Each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by

autoreactive T cells in IBD and/or antigens that trigger recognition of autoreactive T cells in IBD. In some embodiments, the autoantigen(s) comprise flagellin.

[0202] In those embodiments in which the lipid conjugate(s) and nucleic acid(s) are formulated in separate delivery vehicles, the separate delivery vehicles may be administered separately or together. If administered separately, the separate delivery vehicles may be administered sequentially to a subject. The time frame between administration of the separate delivery vehicles can be selected based on patient requirements. The delivery vehicles are typically each part of a pharmaceutical formulation comprising suitable excipients and pharmaceutically acceptable salts.

[0203] In some embodiments, the pharmaceutical formulation or formulations are administered intradermally or parentally, i.e., intra-arterially, intravenously, subcutaneously or intramuscularly. In some embodiments, the formulation(s) are administered intravenously, intramuscularly, subcutaneously, intraperitoneally, or intraarticularly. In another embodiment, the pharmaceutical compositions are administered intranasally, intravitreally, subretinally, intrathecally or via other local routes.

[0204] For example, but without limitation, the patient may receive a single intramuscular injection of LNP at a dose of 3-5 ug of mRNA, administered by a clinician, pharmacist or any other qualified professional, once every 1-3 months, as treatment of their autoimmune condition. Alternatively, a patient can self-administer LNP under the skin of their abdomen, thigh, or upper arm subcutaneously at a dose of 3-5 ug of mRNA with the use of a self-injector pen.

[0205] The compositions described herein may be administered to a subject, including a patient. This includes a human or a non-human subject. In some embodiments, the subject is a mouse. In some embodiments, the subject is human.

[0206] The following examples are given for the purpose of illustration only and not by way of limitation on the scope of the invention.

[0207] The following is a non-limiting list of exemplary embodiments:

Embodiment A1. An immunomodulatory combination comprising:

a first lipid conjugate comprising a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, wherein the first immunomodulatory agent is a corticosteroid;

a second lipid conjugate comprising a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, wherein the second immunomodulatory agent is an mTOR/mTORC inhibitor; and

one or more nucleic acids encoding one or more epitopes targeted by autoreactive T cells, wherein the one or more epitopes comprise one or more class II epitopes, optionally wherein the one or more epitopes are from a plurality of autoantigens and/or antigens that trigger recognition of autoantigens by autoreactive T cells in an autoimmune disease or condition;

wherein the first lipid conjugate, the second lipid conjugate, and the one or more nucleic acids are co-formulated together in a delivery vehicle, or are formulated in two or three separate delivery vehicles.

Embodiment A2. The immunomodulatory combination of embodiment A1, wherein:

the mTOR/mTORC inhibitor is sirolimus, everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-0812, Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1, Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxelisib (XL765), VS-5585 (SB2343), W922, WAY-600, WYE-125132 (WYE-132), WYE-354, or WYE-687, optionally sirolimus; and/or

the corticosteroid is alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, flucolorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortal, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol, optionally dexamethasone.

Embodiment A3. The immunomodulatory combination of embodiment A1 or 2, wherein the lipid conjugate has a cLogP value of 5-20, 6-18, 9-18, or 9-17.

Embodiment A4. The immunomodulatory combination of any one of embodiments A1 to A3, wherein the delivery vehicles are lipid nanoparticles and/or liposomes, optionally wherein the delivery vehicles are lipid nanoparticles, wherein the one or more nucleic acids are entrapped within a lipid nanoparticle or liposome that comprises a lipid that has or is ionizable to produce a positive net charge.

Embodiment A5. The immunomodulatory combination of embodiment A4, wherein the lipid conjugates in the lipid nanoparticles are 5-20%, 7-15%, 9-12%, or about 10% of the total lipids, optionally wherein the ratio of the first lipid conjugate to the second lipid conjugate is 1:9, 9:1, or 5:5.

Embodiment A6. The immunomodulatory combination of any one of embodiments A1 to A5, wherein the one or more epitopes further comprises one or more class I epitopes, optionally wherein the one or more class I epitopes comprise a plurality of class I epitopes, each class I epitope separated by a class I linker peptide, optionally wherein the class II epitopes are separated from the class I epitopes by a class II linker.

Embodiment A7. The immunomodulatory combination of any one of embodiments A1 to A6, wherein the one or more class II epitopes comprise a plurality of class II epitopes, each class II epitope separated by a class II linker peptide.

Embodiment A8. The immunomodulatory combination of any one of embodiments A1 to A7, wherein the plurality of epitopes comprises a full length antigen.

Embodiment A9. The immunomodulatory combination of any one of embodiments A1 to A8, wherein the one or more nucleic acids comprises a first nucleic acid encoding one or more class I epitopes, optionally wherein the class I epitopes are separated by class I linker peptides, and a second nucleic acid encoding one or more class II epitopes, optionally wherein the class II epitopes are separated by class II linker peptides.

Embodiment A10. The immunomodulatory combination of any one of embodiments A1 to A9, wherein each of the one or more nucleic acids is an mRNA independently configured according to one of the following formulas:

A-B-C-L¹-D-L²-E-F (Formula IIa)

A-B-D-L¹-C-L²-E-F (Formula IIb)

A-B-C-L²-E-F (Formula IIc)

A-B-D-L²-E-F (Formula IId)

A-B-C-L¹-D-F (Formula IIe)

A-B-D-L¹-C-F (Formula II f)

A-G-F (Formula IIg)

A-B-C-F (Formula IIh)

A-G-L²-E-F (Formula Iii)

A-B-D-F (Formula IIj)

A-B-G-F (Formula IIk)

A-B-G-L²-E-F (Formula III)

wherein:

A is a 5' untranslated region (5'-UTR);

B is a signal peptide, optionally a signal peptide from the same antigen/autoantigen as one of the one or more epitopes;

L¹ is a cleavable or non-cleavable linker, optionally a class II linker, optionally GPGPG (SEQ ID NO: 26);

C encodes one or more class I epitopes, each class I epitope separated by a class I linker peptide and optionally flanked on both sides by 0-25 flanking amino acids;

L² is an optional linker;

D encodes one or more class II epitopes, each class II epitope separated by a class II linker peptide and optionally flanked on both sides by 0-25 flanking amino acids;

E encodes a polypeptide comprising an endolysosomal trafficking sequence motif, optionally an MHC-I trafficking domain (MITD), invariant chain (CD74), LAMP1, LAMP2, or DC-LAMP;

F is a 3' untranslated region (3'UTR) further comprising a polyadenine tail; and

G is a full length antigen/autoantigen.

optionally wherein C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes, and D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes.

Embodiment A11. The immunomodulatory combination of any one of embodiments A1 to A10, wherein the one or more epitopes is a plurality of epitopes, and wherein each epitope in the plurality of epitopes is an epitope of an autoantigen targeted by autoreactive T cells in type 1 diabetes (T1D),

optionally wherein the plurality of epitopes comprises full-length and/or epitopic fragments from one or more, optionally two or more, of the following: preproinsulin, mature insulin components (A chain, B chain, and/or C-peptide), glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2β), Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs),

optionally wherein the epitopes are epitopic fragments from autoantigens selected from the group consisting of: preproinsulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), zinc transporter 8 (ZnT8), and hybrid insulin peptides (HIPs).

Embodiment A12. The immunomodulatory combination of any one of embodiments A1 to A9, wherein each of the one or more epitopes comprises an epitope of one or more antigens/autoantigens targeted by autoreactive T cells in one of the following:

Multiple Sclerosis (MS), optionally wherein the one or more antigens/autoantigens comprises one or more of myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin basic protein (MBP), and/or α B-crystallin;

celiac disease (CD), optionally wherein the one or more antigens/autoantigens comprises gliadin;

autoimmune thyroiditis, optionally Hashimoto's thyroiditis, optionally wherein the one or more antigens/autoantigens comprises one or both of thyroid peroxidase and/or thyroglobulin;

vitiligo, optionally wherein the one or more antigens/autoantigens comprises one or more of Pmel17, Melanin-Concentrating Hormone Receptor 1, and/or Tyrosinase; and

inflammatory bowel disease (IBD), optionally wherein the one or more antigens/autoantigens comprises flagellin.

Embodiment A13. A method for treating a subject having an autoimmune disease involving autoreactive T cells, the autoreactive T cells comprising both CD4⁺ T cells and CD8⁺ T cells, the method comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially.

Embodiment A14. A method for treating a subject having type 1 diabetes comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A11 to the subject, wherein the one or more nucleic acids encoding the plurality of epitopes, and the lipid conjugate(s) are administered together or sequentially.

Embodiment A15. A method for treating Multiple Sclerosis (MS) in a subject, comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A10 and A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in MS, optionally wherein the one or more autoantigens comprise one or more of myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin basic protein (MBP), and/or α B-crystallin.

Embodiment A16. A method for treating celiac disease in a subject, comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A10 and A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in celiac disease and/or antigens that trigger recognition of autoantigens by autoreactive T cells in celiac disease, optionally wherein the one or more autoantigens comprise gliadin.

Embodiment A17. A method for treating thyroiditis in a subject, comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A10 and A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in thyroiditis, optionally wherein the one or more autoantigens comprise one or both of thyroid peroxidase and/or thyroglobulin.

Embodiment A18. A method for treating vitiligo in a subject, comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A10 and A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in vitiligo, optionally wherein the one or more autoantigens comprise one or more of Pmel17, Melanin-Concentrating Hormone Receptor 1, and/or Tyrosinase.

Embodiment A19. A method for treating inflammatory bowel disease (IBD) in a subject, comprising administering the immunomodulatory combination as defined in any one of embodiments A1 to A10 and A12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in IBD and/or antigens that trigger recognition of autoantigens by autoreactive T cells in IBD, optionally wherein the one or more autoantigens comprise flagellin.

Embodiment A20. An mRNA construct comprising:

5'UTR sequence;

a sequence encoding a signal peptide;

a multi-epitope coding sequence encoding a multi-epitope polypeptide, the multi-epitope polypeptide comprising three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, wherein epitopes in the multi-epitope polypeptide are separated from each other by cleavable or noncleavable linkers, optionally wherein class I epitopes are separated by class I linker peptides and class II epitopes are separated by class II linker peptides, optionally wherein class I and class II linkers are separated by a class II linker, optionally wherein each epitope is flanked on both sides by 0-25 flanking amino acids, optionally wherein the multi-epitope comprises a signal peptide, and wherein the epitopes comprise epitopes from a plurality of antigens/autoantigens;

3'UTR sequence;

polyadenine tail; and

optionally, a sequence encoding a polypeptide comprising an endolysosomal trafficking sequence motif positioned 5' to the 3'UTR sequence.

Embodiment B1. An immunomodulatory combination comprising:

a first lipid conjugate comprising a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, wherein the first immunomodulatory agent is a corticosteroid;

a second lipid conjugate comprising a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, wherein the second immunomodulatory agent is an mTOR/mTORC inhibitor; and

one or more nucleic acids encoding a plurality of epitopes, wherein each epitope in the plurality of epitopes is an epitope of an autoantigen targeted by autoreactive T cells in type 1 diabetes (T1D);

wherein the first lipid conjugate, the second lipid conjugate, and the one or more nucleic acids are co-formulated together in a delivery vehicle, or are formulated in two or more separate delivery vehicles.

Embodiment B2. The immunomodulatory combination of embodiment B1, wherein the plurality of epitopes comprises full-length and/or epitopic fragments from one or more, optionally two or more, of the following: preproinsulin, mature insulin components (A chain, B chain, and/or C-peptide), glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs).

Embodiment B3. The immunomodulatory combination of embodiment B1 or B2, wherein the plurality of epitopes comprises one or more class I epitopes, optionally wherein the one or more class I epitopes comprise a plurality of class I epitopes, each class I epitope separated by a class I linker peptide.

Embodiment B4. The immunomodulatory combination of any one of embodiments B1 to B3, wherein the plurality of epitopes comprises one or more class II epitopes, optionally wherein the one or more class II epitopes comprise a plurality of class II epitopes, each class II epitope separated by a class II linker peptide.

Embodiment B5. The immunomodulatory combination of any one of embodiments B1 to B4, wherein the plurality of epitopes comprises a combination of one or more class I epitopes and one or more class II epitopes, optionally wherein the epitopes are separated by cleavable linkers.

Embodiment B6. The immunomodulatory combination of any one of embodiments B1 to B3, wherein the one or more nucleic acids comprises a first nucleic acid encoding one or more class I epitopes, optionally wherein the class I epitopes are separated by cleavable linkers, and a second nucleic acid encoding one or more class II epitopes, optionally wherein the class II epitopes are separated by cleavable linkers.

Embodiment B7. The immunomodulatory combination of any one of embodiments B1 to B6, wherein the one or more nucleic acids encodes one or more full length antigens, optionally selected from preproinsulin, mature insulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β) Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs).

Embodiment B8. The immunomodulatory combination of any one of embodiments B1 to B7, wherein each of the one or more nucleic acids is an mRNA independently configured according to one of the following formulas:

A-B-C-L¹-D-L²-E-F (Formula IIa)

A-B-D-L¹-C-L²-E-F (Formula IIb)

A-B-C-L²-E-F (Formula IIc)

A-B-D-L²-E-F (Formula IId)

A-B-C-L¹-D-F (Formula IIe)

A-B-D-L¹-C-F (Formula IIIf)

A-G-F (Formula IIg)

A-B-C-F (Formula IIh)

A-G-L²-E-F (Formula Iii)

A-B-D-F (Formula IIj)

A-B-G-F (Formula IIk)

A-B-G-L²-E-F (Formula III)

wherein:

A is a 5' untranslated region (5'-UTR);

B is a signal peptide, optionally a preproinsulin signal peptide;

L¹ is a cleavable linker, optionally HEYGAEALERAG (SEQ ID NO: 65);

C encodes one or more class I epitopes, each class I epitope separated by a class I linker peptide;

L² is an optional linker;

D encodes one or more class II epitopes, each class II epitope separated by a class II linker peptide;

E encodes an MHC-I trafficking domain (MITD);

F is a 3' untranslated region (3'UTR) further comprising a polyadenine tail; and

G is a full length autoantigen;

optionally wherein C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes, and D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes, optionally wherein the epitopes are epitopic fragments from autoantigens selected from the group consisting of: preproinsulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2β), zinc transporter 8 (ZnT8), and hybrid insulin peptides (HIPs).

Embodiment B9. The immunomodulatory combination of any one of embodiments B1 to B8, wherein:

the mTOR/mTORC inhibitor is sirolimus, everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-0812,

Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1, Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxtalib (XL765), VS-5585 (SB2343), W922, WAY-600, WYE-125132 (WYE-132), WYE-354, or WYE-687, optionally sirolimus; and/or

the corticosteroid is alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, fluclorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortal, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol, optionally dexamethasone.

Embodiment B10. The immunomodulatory combination of any one of embodiments B1 to B9, wherein the lipid conjugate has a cLogP value of 5-20, 6-18, 9-18, or 9-17.

Embodiment B11. The immunomodulatory combination of any one of embodiments B1 to B10, wherein the delivery vehicles are lipid nanoparticles and/or liposomes, optionally wherein the delivery vehicles are lipid nanoparticles, wherein the one or more nucleic acids are entrapped within a lipid nanoparticle or liposome that comprises a lipid that has or is ionizable to produce a positive net charge.

Embodiment B12. The immunomodulatory combination of embodiment B11, wherein the lipid conjugates in the lipid nanoparticles are 5-20%, 7-15%, 9-12%, or about 10% of the total lipids, optionally wherein the ratio of the first lipid conjugate to the second lipid conjugate is 1:9, 9:1, or 5:5.

Embodiment B13. The immunomodulatory combination of any one of embodiments B1 to B12, for use in treatment of type 1 diabetes in a subject.

Embodiment B14. A method for treating a subject having type 1 diabetes comprising administering the immunomodulatory combination as defined in any one of embodiments B1 to B12 to the subject, wherein the one or more nucleic acids encoding the plurality of epitopes, and the lipid conjugate(s) are administered together or sequentially.

Embodiment B15. An mRNA construct comprising:

5'UTR sequence;

a sequence encoding a signal peptide;

a multi-epitope coding sequence encoding a multi-epitope polypeptide, the multi-epitope polypeptide comprising three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, wherein epitopes in the multi-epitope polypeptide are separated from each other by cleavable linkers, and wherein the epitopes comprise epitopes from a plurality of autoantigens;

3'UTR sequence;

polyadenine tail; and

optionally, a sequence encoding an MHC-I trafficking domain (MITD) positioned 5' to the 3'UTR sequence.

Embodiment C1. An immunomodulatory combination comprising:

a first lipid conjugate comprising a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, wherein the first immunomodulatory agent is a corticosteroid;

a second lipid conjugate comprising a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, wherein the second immunomodulatory agent is an mTOR/mTORC inhibitor; and

one or more nucleic acids encoding a plurality of epitopes, wherein each epitope in the plurality of epitopes is an epitope of an autoantigen targeted by autoreactive T cells in type 1 diabetes (T1D);

wherein the first lipid conjugate, the second lipid conjugate, and the one or more nucleic acids are co-formulated together in a delivery vehicle, or are formulated in two or more separate delivery vehicles.

Embodiment C2. The immunomodulatory combination of embodiment C1, wherein the plurality of epitopes comprises full-length and/or epitopic fragments from one or more, optionally two or more, of the following: preproinsulin, mature insulin components (A chain, B chain, and/or C-peptide), glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs).

Embodiment C3. The immunomodulatory combination of embodiment C1 or C2, wherein the plurality of epitopes comprises one or more class I epitopes, optionally wherein the one or more class I epitopes comprise a plurality of class I epitopes, each class I epitope separated by a class I linker peptide.

Embodiment C4. The immunomodulatory combination of any one of embodiments C1 to C3, wherein the plurality of epitopes comprises one or more class II epitopes, optionally wherein the one or more class II epitopes comprise a plurality of class II epitopes, each class II epitope separated by a class II linker peptide.

Embodiment C5. The immunomodulatory combination of any one of embodiments C1 to C4, wherein the plurality of epitopes comprises a combination of one or more class I epitopes and one or more class II epitopes, optionally wherein the epitopes are separated by cleavable linkers.

Embodiment C6. The immunomodulatory combination of any one of embodiments C1 to C3, wherein the one or more nucleic acids comprises a first nucleic acid encoding one or more class I epitopes, optionally wherein the class I epitopes are separated by cleavable linkers,

and a second nucleic acid encoding on or more class II epitopes, optionally wherein the class II epitopes are separated by cleavable linkers.

Embodiment C7. The immunomodulatory combination of any one of embodiments C1 to C6, wherein the one or more nucleic acids encodes one or more full length antigens, optionally selected from preproinsulin, mature insulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β) Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs).

Embodiment C8. The immunomodulatory combination of any one of embodiments C1 to C7, wherein each of the one or more nucleic acids is an mRNA independently configured according to one of the following formulas:

A-B-C-L1-D-L2-E-F (Formula IIa)

A-B-D-L1-C-L2-E-F (Formula IIb)

A-B-C-L2-E-F (Formula IIc)

A-B-D-L2-E-F (Formula IId)

A-B-C-L1-D-F (Formula IIe)

A-B-D-L1-C-F (Formula IIff)

A-G-F (Formula IIg)

A-B-C-F (Formula IIh)

A-G-L2-E-F (Formula IIIi)

A-B-D-F (Formula IIj)

A-B-G-F (Formula IIk)

A-B-G-L2-E-F (Formula IIIl)

wherein:

A is a 5' untranslated region (5'-UTR);

B is a signal peptide, optionally a preproinsulin signal peptide;

L1 is a cleavable linker, optionally HEYGAEALERAG (SEQ ID NO: 65);

C encodes one or more class I epitopes, each class I epitope separated by a class I linker peptide;

L2 is an optional linker;

D encodes one or more class II epitopes, each class II epitope separated by a class II linker peptide;

E encodes an MHC-I trafficking domain (MITD);

F is a 3' untranslated region (3'UTR) further comprising a polyadenine tail; and

G is a full length autoantigen.

optionally wherein C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes, and D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes, optionally wherein the epitopes are epitopic fragments from autoantigens selected from the group consisting of: preproinsulin, glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), zinc transporter 8 (ZnT8), and hybrid insulin peptides (HIPs).

Embodiment C9. The immunomodulatory combination of any one of embodiments C1 to C8, wherein:

the mTOR/mTORC inhibitor is sirolimus, everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-0812, Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1,

Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxelotib (XL765), VS-5585 (SB2343), W922, WAY-600, WYE-125132 (WYE-132), WYE-354, or WYE-687, optionally sirolimus; and/or

the corticosteroid is alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, flucorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortal, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol, optionally dexamethasone.

Embodiment C10. The immunomodulatory combination of any one of embodiments C1 to C9, wherein the lipid conjugate has a cLogP value of 5-20, 6-18, 9-18, or 9-17.

Embodiment C11. The immunomodulatory combination of any one of embodiments C1 to C10, wherein the delivery vehicles are lipid nanoparticles and/or liposomes, optionally wherein the delivery vehicles are lipid nanoparticles, wherein the one or more nucleic acids are entrapped within a lipid nanoparticle or liposome that comprises a lipid that has or is ionizable to produce a positive net charge.

Embodiment C12. The immunomodulatory combination of embodiment C11, wherein the lipid conjugates in the lipid nanoparticles are 5-20%, 7-15%, 9-12%, or about 10% of the total lipids, optionally wherein the ratio of the first lipid conjugate to the second lipid conjugate is 1:9, 9:1, or 5:5.

Embodiment C13. The immunomodulatory combination of any one of embodiments C1 to C12, for use in treatment of type 1 diabetes in a subject.

Embodiment C14. A method for treating a subject having type 1 diabetes comprising administering the immunomodulatory combination as defined in any one of embodiments C1 to C12 to the subject, wherein the one or more nucleic acids encoding the plurality of epitopes, and the lipid conjugate(s) are administered together or sequentially.

Embodiment C15. An mRNA construct comprising:

5'UTR sequence;

a sequence encoding a signal peptide;

a multi-epitope coding sequence encoding a multi-epitope polypeptide, the multi-epitope polypeptide comprising three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, wherein epitopes in the multi-epitope polypeptide are separated from each other by cleavable linkers, and wherein the epitopes comprise epitopes from a plurality of autoantigens;

3'UTR sequence;

polyadenine tail; and

optionally, a sequence encoding an MHC-I trafficking domain (MITD) positioned 5' to the 3'UTR sequence.

EXAMPLES

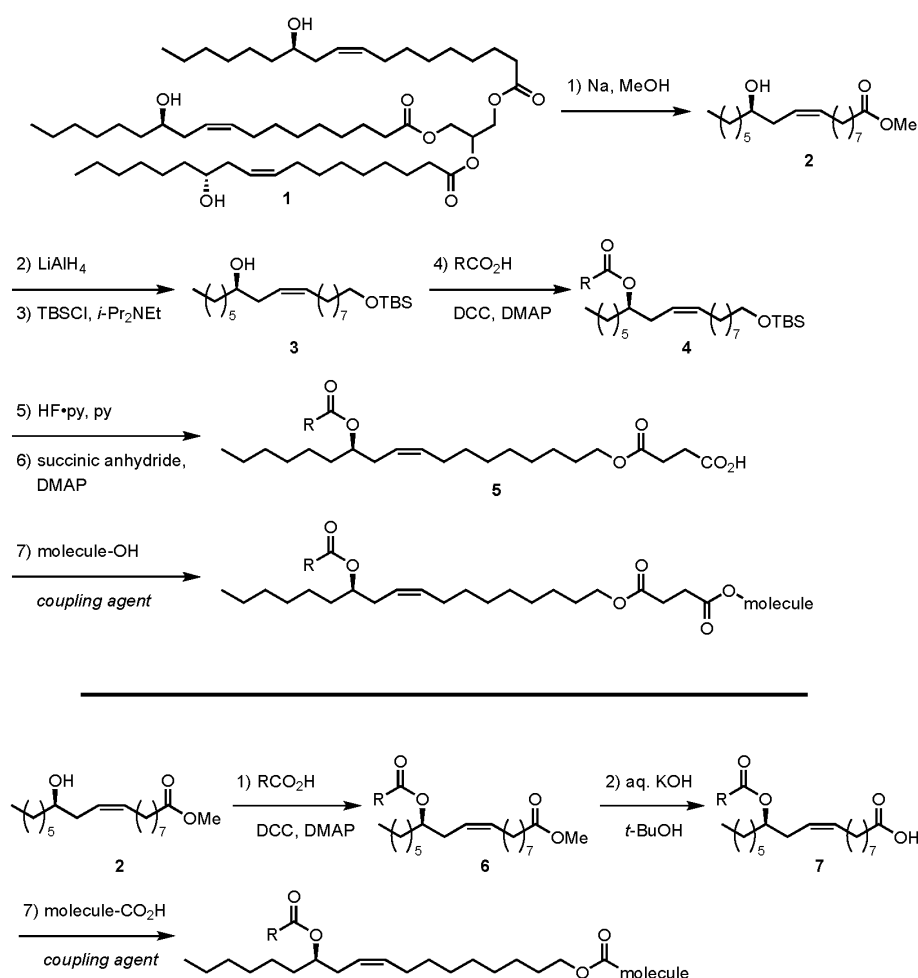
[0208] Synthesis of lipid conjugates

[0209] Various lipid conjugates were prepared using the synthesis procedures A-E set forth below.

[0210] All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated, except THF, (freshly distilled from Na/benzophenone under nitrogen), and Et₃N, DMF and CH₂Cl₂ (freshly distilled from CaH₂ under nitrogen). USP grade castor oil was purchased at a local pharmacy (Life™ Brand) and used as received. For NMR, chemical shifts are reported in parts per million (ppm) on the δ scale and coupling constants, J, are in hertz (Hz). Multiplicities are reported as “s” (singlet), “d” (doublet), “dd” (doublet of doublets), “dt” (doublet of triplets), “ddd” (doublet of doublets of doublets), “t” (triplet), “td” (triplet of doublets), “q” (quartet), “quin” (quintuplet), “sex” (sextet), “m” (multiplet), and further qualified as “app” (apparent) and “br” (broad).

[0211] The steps of the general synthesis of lipid conjugates based on hydroxy and carboxy derivatives of castor oil (ricinolein) are provided below in Scheme 1. This is followed by Scheme 1, referred to as general procedures A-E, describing the steps for producing the lipid conjugates of Examples E, S, T, and W below.

[0212] **SCHEME 1:** General synthesis of lipid conjugates based on hydroxy and carboxy derivatives of castor oil (ricinolein).

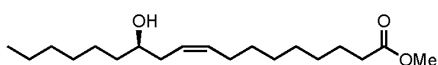


[0213] According to the synthesis reaction described above in Scheme I, castor oil, also known as ricinolein (a glyceride of ricinoleic acid) is the starting material.

[0214] In step 1) above, sodium methoxide (2.0 mL of 3.0 M solution in MeOH, 6.00 mmol, 0.20 equiv.) was added to a stirring, room temperature 1:1 THF/MeOH (30 mL) solution of the castor

oil (28.0 g, 30.0 mmol, 1.00 equiv.) in a round bottom flask under argon. After 14 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with Et_2O (3×150 mL). The combined organic layers were washed with water (1×150 mL), brine (1×150 mL), dried over Na_2SO_4 and concentrated to produce a clear, colourless oil of methyl (12*R*)-hydroxyoleate **1** (28.0 g, quantitative yield), which was used without further purification. The structure of methyl (12*R*)-hydroxyoleate and its physical properties are shown below:

[0215] Methyl (12*R*)-hydroxyoleate (**1**):



$R_f = 0.50$ (SiO_2 , 70:30 hexanes/ EtOAc);

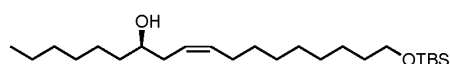
$^1\text{H NMR}$ (300 MHz, CDCl_3): δ 5.64-5.50 (m, 1H), 5.49-5.35 (m, 1H), 3.68 (s, 3H), 3.63 (quint., $J = 5.6$ Hz, 1H), 2.32 (t, $J = 7.6$ Hz, 2H), 2.23 (t, $J = 6.6$ Hz, 2H), 2.13-2.00 (m, 2H), 1.72-1.19 (m, 20H), 0.90 (t, $J = 6.4$ Hz, 3H).

[0216] According to 2) in the reaction scheme above, a room temperature THF (15 mL) solution of methyl (12*R*)-hydroxyoleate (9.37 g, 30.0 mmol) was added from an addition funnel over 20-30 min to a stirred, ice-cold THF (90 mL) suspension of LiAlH_4 (1.25 g, 33.0 mmol, 1.10 equiv.) in a round bottom flask under argon. After the addition was complete, the cold bath was removed. After 14 h, the reaction mixture was cooled in an ice bath, diluted with Et_2O (150 mL) and quenched with a quenching solution (1.25 mL H_2O , 1.25 mL aqueous 1 M NaOH , 3.75 mL H_2O), stirred for 1 h at room temperature and filtered through Celite, while washing thoroughly with Et_2O . The filtrate was concentrated on a rotary evaporator to yield the crude diol as a pale yellow oil (quantitative yield), which was used without further purification.

[0217] According to 3) of the above reaction scheme, a room temperature DMF (20 mL) solution of *tert*-butyldimethylsilyl chloride (3.96 g, 26.2 mmol, 1.00 equiv.) was added from an addition funnel over 30 min to a 10-15°C DMF (25 mL) solution of the above diol (8.21 g, 28.9 mmol, 1.10 equiv.) and *i*- Pr_2Net (5.73 mL, 32.8 mmol, 1.25 equiv.) in a round bottom flask under argon. The reaction mixture was allowed to warm up over 14 h, then quenched with saturated aqueous NH_4Cl and extracted with 1:1 Et_2O /hexanes (3×100 mL). The combined organic layers were washed with H_2O (3×100 mL), brine (1×100 mL), dried over Na_2SO_4 and concentrated on a rotary evaporator

to produce the crude primary silyl ether as a pale yellow oil. The crude was purified by filtration through a plug of silica gel (220 mL SiO₂, 99:1→95:5 hexanes/EtOAc) to yield a clear, colourless oil composed of the silyl ether **2** (8.38 g, 80% yield). The structure of the silyl ether **2** is shown below, as well as its physical properties:

[0218] I-1-(*tert*-Butyldimethylsilyl)-12-hydroxyoleyl alcohol (**2**):



$R_f = 0.16$ (SiO₂, 95:5 hexanes/EtOAc);

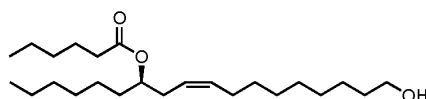
¹H NMR (300 MHz, CDCl₃): δ 5.64-5.50 (m, 1H), 5.49-5.35 (m, 1H), 3.68 (s, 3H), 3.63 (quint., $J = 5.6$ Hz, 1H), 2.32 (t, $J = 7.6$ Hz, 2H), 2.23 (t, $J = 6.6$ Hz, 2H), 2.13-2.00 (m, 2H), 1.72-1.19 (m, 20H), 0.90 (t, $J = 6.4$ Hz, 3H).

[0219] According to 4) of the above reaction scheme, N,N'-Dicyclohexylcarbodiimide (DCC) (495 mg, 2.40 mmol, 1.20 equiv.) was added to an ice-cold CH₂Cl₂ (6 mL) solution of RCO₂H (279 mg, 2.40 mmol, 1.20 equiv.) in a round bottom flask under argon, and the ice bath was subsequently removed and the resultant mixture stirred for 15 min. In this example, RCO₂H was hexanoic acid, although other acyl groups can be utilized to produce a desired hydrocarbon side chain S. The reaction mixture was cooled again in an ice bath, a CH₂Cl₂ (2 mL) solution of the silyl ether, I-1-(*tert*-Butyldimethylsilyl)-12-hydroxyoleyl alcohol **2** (797 mg, 2.00 mmol) was added, followed by DMAP (366 mg, 3.00 mmol, 1.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with Et₂O, stirred for 10 min, then filtered through Celite. The filtrate was concentrated on a rotary evaporator to yield the crude ester as a white semi-solid. The crude was purified by filtration through a plug of silica gel (20 mL SiO₂, 95:5 hexanes/EtOAc) to produce a clear, colourless oil as the intermediate ester (quantitative yield) having an $R_f = 0.53$ (SiO₂, 90:10 hexanes/EtOAc).

[0220] According to 5) of the reaction scheme above, neat HF•pyridine solution (0.74 mL of 70% HF in pyridine, 6.00 mmol, 3.00 equiv.) was added to a stirred, ice-cold THF (6 mL) solution of pyridine (0.48 mL, 6.00 mmol, 3.00 equiv.) and the above silyl ether (2.00 mmol) in a round bottom flask under argon. After 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃. The mixture was extracted with Et₂O (2×10 mL), then the combined organic extracts were washed with H₂O (1×10 mL), brine, dried over Na₂SO₄ and concentrated on a rotary

evaporator to afford the crude primary alcohol. The crude was purified by filtration through a plug of silica gel (20 mL, 90:10 hexanes/EtOAc) to produce a primary alcohol **3** (quantitative yield) as a clear, colourless oil having the structure and physical properties below:

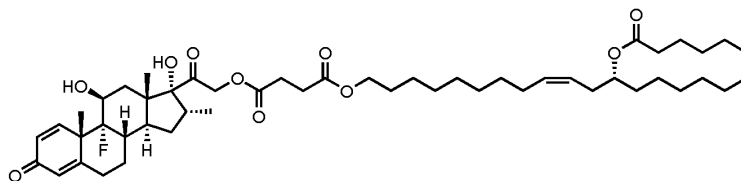
(12*R*)-Hexanoyloxyoleyl alcohol (**3**):



[0221] According to 6) of the reaction scheme above, solid succinic anhydride (400 mg, 4.00 mmol, 2.00 equiv.) and DMAP (611 mg, 5.00 mmol, 2.50 equiv.) were added to a stirring room temperature CH₂Cl₂ (6 mL) solution of the (12*R*)-Hexanoyloxyoleyl alcohol (**3**) (765 mg, 2.00 mmol, 1.00 equiv.) in a round bottom flask under argon. After 14 hours, the reaction was quenched with aqueous 1 M HCl and extracted with CH₂Cl₂ (2×15 mL). The combined organic extracts were then washed with aqueous 1 M HCl (1×15 mL), H₂O (2×15 mL), dried over Na₂SO₄ and concentrated on a rotary evaporator to afford the intermediate hemisuccinate (quantitative yield) as a pale yellow oil that was used without further purification. The intermediate had an *R*_f = 0.32 (SiO₂, 50:50 hexanes/EtOAc).

[0222] According to 7) in the reaction scheme, solid DCC (99 mg, 0.48 mmol, 1.20 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (2 mL) solution of the above hemisuccinate (232 mg, 0.48 mmol, 1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant mixture stirred for 15 min. The reaction mixture was cooled again in an ice bath and solid dexamethasone (157 mg, 0.40 mmol) and DMAP (73 mg, 0.60 mmol, 1.50 equiv.) were added. The reaction mixture was allowed to warm up over 14 h, diluted with Et₂O, stirred for 10 min, then filtered through Celite. The filtrate was concentrated to produce the crude, which was a pale yellow oil. The crude was purified by flash column chromatography (50 mL SiO₂, 80:20→50:50 hexanes/EtOAc) to yield a clear, colourless oil as desired pro-drug **4** (328 mg, 95% yield) having the structure and properties below:

2-((8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]118ctadic118rene-17-yl)-2-oxoethyl ((*R*,*Z*)-12-(hexanoyloxy)118ctadic-9-en-1-yl) succinate (**4**):



$R_f = 0.38$ (SiO_2 , 50:50 hexanes/EtOAc);

^1H NMR (300 MHz, CDCl_3): δ 7.22 (dd, $J = 10.2, 3.9$, 1H), 6.32 (dd, $J = 10.2, 1.7$, 1H), 6.1 (s, 1H), 5.44-5.17 (m, 9H), 5.00-4.81 (m, 2H), 4.43-4.22 (m, 4H), 4.21-4.06 (m, 2H), 3.16-3.01 (m, 1H), 2.84-2.51 (m, 11H), 2.50-2.23 (m, 9H), 2.21-1.48 (m, 25H), 1.45-1.15 (m, 34H), 1.14-1.00 (m, 1H), 1.03 (s, 3H), 0.95-0.81 (m, 10H).

[0223] The lipid conjugate is based on a ricinoleyl scaffold L with a hexanoyl ($\text{C}_6:0$) side chain conjugated to dexamethasone by a succinate linker (**INT-D034**).

In the above example, RCO_2H added in 4) of the above reaction was hexanoic acid to produce the hexanoyl side chain ($\text{C}_6:0$), although other fatty acids can be utilized to produce a desired hydrocarbon side chain R on the ricinoleyl scaffold.

[0224] **General Procedure A** - Acylation of (*R*)-1-(*tert*-Butyldimethylsilyl)-12-hydroxyoleyl alcohol **3** (**4a-h**):

[0225] DCC (1.20 equiv.) was added to a stirring, ice-cold CH_2Cl_2 solution of the desired carboxylic acid (1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath, a CH_2Cl_2 solution of alcohol **3** (1.00 equiv., 0.25 M in CH_2Cl_2) was added, followed by DMAP (1.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with Et_2O , stirred for 10 min, then filtered through Celite®. The filtrate was concentrated on a rotary evaporator to yield the crude ester as a white semi-solid. The crude was purified by filtration through a plug of silica gel (95:5 hexanes/EtOAc) to afford the pure ester.

[0226] **General Procedure B** - Desilylation-Succinylation of (12*R*)-Acyloxyoleyl alcohols **4a-h** (**5a-h**):

[0227] $\text{HF}\cdot\text{pyridine}$ solution (3.00 equiv. of 70% HF in pyridine) was added to a stirring, ice-cold THF (0.30 M relative to starting silyl ether) solution of pyridine (3.00 equiv.) and 12-acyl ricinoleyl alcohol silyl ether (1.00 equiv.) in a round bottom flask under argon. When TLC

indicated consumption of the starting material (2-8 h), the reaction mixture was quenched with saturated aqueous NaHCO₃. The mixture was extracted with Et₂O (2×10 mL), then the combined organic extracts were washed with H₂O (1×10 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator to afford the crude primary alcohol. The crude was purified by filtration through a plug of silica gel (90:10 hexanes/EtOAc), concentrated on a rotary evaporator and dried under high vacuum to afford the primary alcohol as a clear, colourless oil and used in the subsequent succinylation without further purification.

[0228] Solid succinic anhydride (2.00 equiv.) and DMAP (2.50 equiv.) were added to a stirring, room temperature CH₂Cl₂ (0.30 M relative to starting primary alcohol) solution of 12-acyl ricinoleyl alcohol (1.00 equiv.) in a round bottom flask under argon. After 14 hours, the reaction was quenched with aqueous 1 M HCl and extracted with CH₂Cl₂ (2×15 mL). The combined organic extracts were then washed with aqueous 1 M HCl (1×15 mL), H₂O (2×15 mL), dried over Na₂SO₄ and concentrated on a rotary evaporator. The residue was redissolved in hexanes, treated with activated carbon, filtered through Celite® and the filtrate concentrated to afford the intermediate hemisuccinate as a colourless to pale yellow oil that was used without further purification.

[0229] General Procedure C - Acylation of Methyl (12*R*)-Ricinoleate 2 (6a-c):

[0230] DCC (1.20 equiv.) was added to a stirring, ice-cold CH₂Cl₂ solution of the desired carboxylic acid (1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath, a CH₂Cl₂ solution of methyl (12*R*)-ricinoleate (1.00 equiv., 0.30 M in CH₂Cl₂) was added, followed by DMAP (1.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with hexanes, stirred for 10 min, then filtered through Celite®. The filtrate was concentrated on a rotary evaporator to yield the crude diester as a white semi-solid, which was purified by filtration through a plug of silica gel (95:5 hexanes/EtOAc) to afford the pure ester.

[0231] General Procedure D - Conjugation of Dexamethasone to Hemisuccinates 5a-h:

[0232] DCC (1.20 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (0.2 M in dexamethasone) solution of 12-acyl ricinoleyl hemisuccinate (1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled

again in an ice bath and solid dexamethasone (1.00 equiv.) and DMAP (1.50 equiv.) were added. The reaction mixture was allowed to warm up over 14 h, diluted with Et₂O, stirred for 10 min, then filtered through Celite®. The filtrate was concentrated to afford the crude as a pale yellow oil and subsequently purified by flash column chromatography (SiO₂, 80:20→50:50 hexanes/EtOAc) to afford a clear, colourless oil as the desired dexamethasone conjugate.

[0233] General Procedure E - Conjugation of Dexamethasone to Ricinoleic Acids 12a-b, 13:

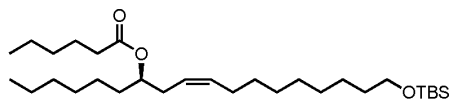
[0234] DCC (1.10 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (0.1 M in dexamethasone) solution of the acyloxystearic acid (1.10 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath and solid dexamethasone (1.00 equiv.) and DMAP (1.50 equiv.) were added. The reaction mixture was allowed to warm up over 14 h, diluted with Et₂O, stirred for 10 min, then filtered through Celite®. The filtrate was concentrated to afford the crude as a pale yellow oil and subsequently purified by flash column chromatography to afford a clear, colourless oil as the desired conjugate.

[0235] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl acetate (**4a**):

[0236] Acetyl chloride (0.43 mL, 6.00 mmol, 1.20 equiv.) was added dropwise to a stirring ice-cold CH₂Cl₂ (10 mL) solution of silyl ether **3** (2.00 g, 5.00 mmol, 1.00 equiv.), acetyl chloride (0.43 mL, 6.00 mmol, 1.20 equiv.), triethylamine (0.83 mL, 6.00 mmol, 1.2 equiv.) and DMAP (733 mg, 6.00 mmol, 1.20 equiv.) in a round bottom flask under argon, which was allowed to warm to room temperature. After 14 h, the reaction mixture was diluted with CH₂Cl₂, washed with saturated aqueous NH₄Cl (1×15 mL), water (2×15 mL) and dried over Na₂SO₄ and concentrated on a rotary evaporator. The residue was redissolved in eluent and passed through a plug of silica gel (30 mL SiO₂, 97:3 hexanes/EtOAc) to afford ester **4a** (1.83 g, 83%) as a pale yellow oil.

R_f = 0.45 (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.65-5.53 (m, 1H), 5.49-5.36 (m, 1H), 3.70-3.56 (m, 3H), 2.23 (t, J = 6.8 Hz, 2H), 2.13-2.00 (m, 2H), 1.58-1.23 (m, 22H), 0.97-0.86 (m, 12H), 0.07 (s, 6H).

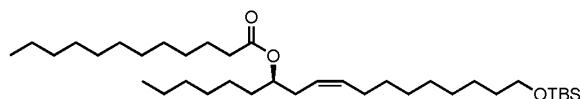


[0237] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl hexanoate (**4b**):

[0238] According to General Procedure A, silyl ether **3** (2.00 g, 5.00 mmol), hexanoic acid (697 mg, 6.00 mmol), DCC (1.24 g, 6.00 mmol) and DMAP (916 mg, 7.50 mmol) in CH₂Cl₂ (15 mL) provided 2.37 g of ester **4b** (2.39 g, quantitative yield) as a clear, colourless oil.

*R*_f = 0.43 (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.56-5.42 (m, 1H), 5.41-5.27 (m, 1H), 4.90 (quint., *J* = 6.3 Hz, 1H), 3.61 (t, *J* = 6.6 Hz, 2H), 2.37-2.22 (m, 4H), 2.10-1.96 (m, 2H), 1.71-1.45 (m, 6H), 1.43-1.19 (m, 22H), 0.91 (br s, 15H), 0.07 (s, 6H).

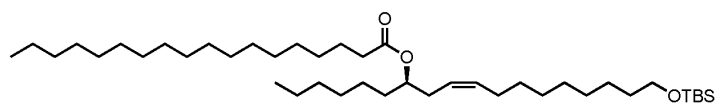


[0239] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl laurate (**4c**):

[0240] According to General Procedure A, silyl ether **3** (997 mg, 2.50 mmol), lauric acid (601 mg, 3.00 mmol), DCC (619 mg, 3.00 mmol) and DMAP (458 mg, 3.75 mmol) in CH₂Cl₂ (8 mL) provided ester **4c** (1.38 g, quantitative yield) as a clear, colourless oil.

*R*_f = 0.56 (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.56-5.41 (m, 1H), 5.41-5.26 (m, 1H), 4.90 (quint., *J* = 6.2 Hz, 1H), 3.61 (t, *J* = 6.6 Hz, 2H), 2.37-2.21 (m, 4H), 2.11-1.95 (m, 2H), 1.72-1.43 (m, 12H), 1.43-1.13 (m, 38H), 0.91 (br s, 15H), 0.07 (s, 6H).

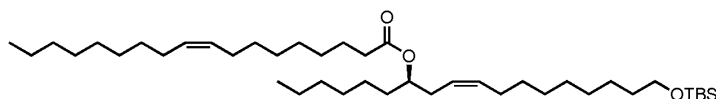


[0241] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl stearate (**4d**):

[0242] According to General Procedure A, silyl ether **3** (997 mg, 2.50 mmol), stearic acid (853 mg, 3.00 mmol), DCC (619 mg, 3.00 mmol) and DMAP (458 mg, 3.75 mmol) in 2:1 THF/CH₂Cl₂ (6 mL) provided ester **4d** (1.56 g, 94%) as a clear, colourless oil.

$R_f = 0.48$ (SiO₂, 90:10 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.57-5.41 (m, 1H), 5.41-5.25 (m, 1H), 4.90 (quint., $J = 6.3$ Hz, 1H), 3.61 (t, $J = 6.5$ Hz, 2H), 2.39-2.20 (m, 4H), 2.11-1.96 (m, 2H), 1.72-1.43 (m, 8H), 1.43-1.13 (m, 44H), 0.91 (br s, 15H), 0.07 (s, 6H).

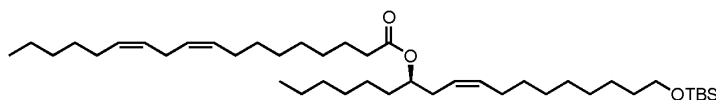


[0243] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl oleate (**4e**):

[0244] According to General Procedure A, silyl ether **3** (997 mg, 2.50 mmol), oleic acid (847 mg, 3.00 mmol), DCC (619 mg, 3.00 mmol) and DMAP (458 mg, 3.75 mmol) in CH₂Cl₂ (10 mL) provided ester **4e** (1.64 g, quantitative) as a clear, colourless oil.

$R_f = 0.41$ (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.56-5.25 (m, 4H), 4.90 (quint., $J = 6.2$ Hz, 1H), 3.61 (t, $J = 6.5$ Hz, 2H), 2.42-2.19 (m, 8H), 2.11-1.93 (m, 6H), 1.70-1.44 (m, 8H), 1.44-1.17 (m, 40H), 0.91 (br s, 15H), 0.06 (s, 6H).

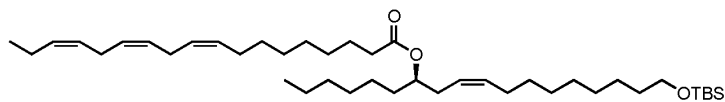


[0245] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl linoleate (**4f**):

[0246] According to General Procedure A, silyl ether **3** (847 mg, 2.12 mmol), linoleic acid (715 mg, 2.55 mmol), DCC (526 mg, 2.55 mmol) and DMAP (389 mg, 3.19 mmol) in CH₂Cl₂ (7 mL) provided ester **4f** (1.06 g, 76%) as a clear, colourless oil.

$R_f = 0.46$ (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.67-5.24 (m, 6H), 4.90 (quint., $J = 6.2$ Hz, 1H), 3.61 (t, $J = 6.6$ Hz, 2H), 2.79 (t, $J = 5.9$ Hz, 2H), 2.40-2.17 (m, 4H), 2.15-1.94 (m, 4H), 1.71-1.44 (m, 8H), 1.43-1.17 (m, 26H), 0.91 (br s, 15H), 0.07 (s, 6H).

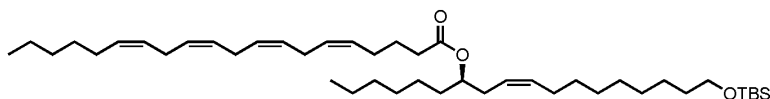


[0247] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl linolenate (**4g**):

[0248] According to General Procedure A, silyl ether **3** (997 mg, 2.50 mmol), linolenic acid (835 mg, 3.00 mmol), DCC (619 mg, 3.00 mmol) and DMAP (458 mg, 3.75 mmol) in CH₂Cl₂ (8 mL) provided ester **4g** (1.52 g, 92%) as a clear, colourless oil.

R_f = 0.34 (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.58-5.26 (m, 8H), 4.90 (quint., J = 6.2 Hz, 1H), 3.61 (t, J = 6.5 Hz, 2H), 2.83 (t, J = 5.8 Hz, 4H), 2.35-2.22 (m, 4H), 2.17-1.97 (m, 6H), 1.69-1.44 (m, 6H), 1.43-1.18 (m, 26H), 1.00 (t, J = 7.5 Hz, 3H), 0.91 (br s, 12H), 0.07 (s, 6H).

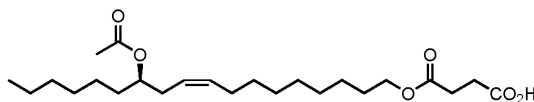


[0249] (*R,Z*)-18-((*tert*-Butyldimethylsilyl)oxy)octadec-9-en-7-yl arachidonate (**4h**):

[0250] According to General Procedure A, silyl ether **3** (797 mg, 2.00 mmol), arachidonic acid (670 mg, 2.20 mmol), DCC (227 mg, 2.20 mmol) and DMAP (366 mg, 3.00 mmol) in CH₂Cl₂ (7 mL) provided ester **4h** (730 mg, 53%) as a clear, colourless oil after flash column chromatography (99:1→95:5 hexanes/EtOAc).

R_f = 0.57 (SiO₂, 95:5 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.57-5.26 (m, 10H), 4.91 (quint., J = 6.3 Hz, 1H), 3.61 (t, J = 6.5 Hz, 2H), 2.94-2.84 (m, 6H), 2.38-2.22 (m, 4H), 2.20-1.96 (m, 6H), 1.71 (quint., J = 7.4 Hz, 2H), 1.63-1.46 (m, 4H), 1.45-1.16 (m, 26H), 0.91 (br s, 15H), 0.07 (s, 6H).



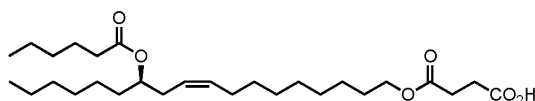
[0251] (*R,Z*)-4-((12-Acetoxyoctadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5a**):

[0252] According to General Procedure B, desilylation of silyl ether **4a** (1.79 g, 4.07 mmol) with HF•pyridine solution (1.52 mL, 12.2 mmol), pyridine (0.98 mL, 12.2 mmol) and THF (10 mL) gave the intermediate primary alcohol (1.34 g), which was subjected to acylation with succinic

anhydride (814 mg, 8.14 mmol), DMAP (1.24 g, 10.2 mmol) and CH₂Cl₂ (10 mL) to afford carboxylic acid **5a** (1.72 g, quantitative yield).

R_f = 0.23 (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.57-5.42 (m, 1H), 5.42-5.27 (m, 1H), 4.89 (quin., J = 6.2 Hz, 1H), 4.11 (t, J = 6.7 Hz, 2H), 2.76-2.57 (m, 4H), 2.11-1.97 (m, 2H), 2.05 (s, 3H), 1.72-1.46 (m, 4H), 1.46-1.16 (m, 18H), 0.90 (m, 3H).

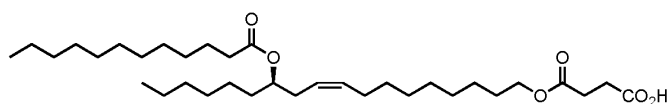


[0253] (*R,Z*)-4-((12-(Hexanoyloxy)octadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5b**):

[0254] According to General Procedure B, desilylation of silyl ether **4b** (2.35 g, 5.00 mmol) with HF•pyridine solution (1.86 mL, 15.0 mmol), pyridine (1.21 mL, 15.0 mmol) and THF (13 mL) gave the intermediate primary alcohol (2.01 g), which was subjected to acylation with succinic anhydride (1.00 g, 10.0 mmol), DMAP (1.53 g, 12.5 mmol) and CH₂Cl₂ (13 mL) to afford carboxylic acid **5b** (2.20 g, 92% yield).

R_f = 0.32 (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.56-5.42 (m, 1H), 5.41-5.27 (m, 1H), 4.90 (quint., J = 6.4 Hz, 1H), 4.11 (t, J = 6.5 Hz, 2H), 2.76-2.58 (m, 4H), 2.38-2.22 (m, 4H), 2.11-1.96 (m, 2H), 1.73-1.47 (m, 6H), 1.46-1.15 (m, 22H), 0.97-0.82 (m, 6H).

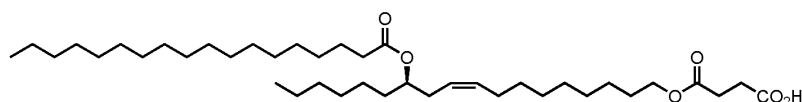


[0255] (*R,Z*)-4-((12-(Lauroyloxy)octadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5c**):

[0256] According to General Procedure B, desilylation of silyl ether **4c** (1.38 g, 2.50 mmol) with HF•pyridine solution (0.93 mL, 7.50 mmol), pyridine (0.60 mL, 7.50 mmol) and THF (8 mL) gave the intermediate primary alcohol (1.21 g), which was subjected to acylation with succinic anhydride (500 mg, 5.00 mmol), DMAP (764 mg, 6.25 mmol) and CH₂Cl₂ (8 mL) to afford carboxylic acid **5c** (1.33 g, 94%).

$R_f = 0.44$ (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.57-5.42 (m, 1H), 5.41-5.26 (m, 1H), 4.90 (quint., $J = 6.2$ Hz, 1H), 4.12 (t, $J = 6.6$ Hz, 2H), 2.78-2.59 (m, 4H), 2.37-2.22 (m, 4H), 2.11-1.96 (m, 2H), 1.73-1.45(m, 6H), 1.45-1.12 (m, 28H), 0.98-0.80 (m, 6H).

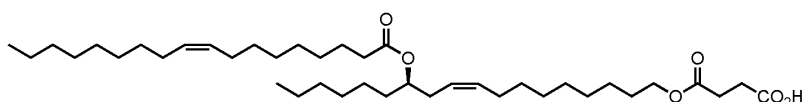


[0257] (*R,Z*)-4-oxo-4-((12-(Stearoyloxy)octadec-9-en-1-yl)oxy)butanoic acid (**5d**):

[0258] According to General Procedure B, desilylation of silyl ether **4d** (1.66 g, 2.50 mmol) with HF•pyridine solution (0.93 mL, 7.50 mmol), pyridine (0.60 mL, 7.50 mmol) and THF (8 mL) gave the intermediate primary alcohol (1.30 g), which was subjected to acylation with succinic anhydride (500 mg, 5.00 mmol), DMAP (764 mg, 6.25 mmol) and CH₂Cl₂ (8 mL) to afford carboxylic acid **5d** (1.29 g, 79% yield).

$R_f = 0.35$ (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.56-5.42 (m, 1H), 5.41-5.27 (m, 1H), 4.90 (quint., $J = 6.3$ Hz, 1H), 4.11 (t, $J = 6.5$ Hz, 2H), 2.77-2.58 (m, 4H), 2.39-2.19 (m, 4H), 2.12-1.95 (m, 2H), 1.73-1.45(m, 6H), 1.44-1.11 (m, 46H), 0.98-0.80 (m, 6H).

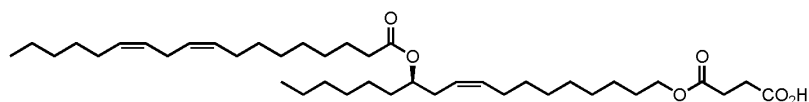


[0259] (*R,Z*)-4-oxo-4-((12-(Oleoyloxy)octadec-9-en-1-yl)oxy)butanoic acid (**5e**):

[0260] According to General Procedure B, desilylation of silyl ether **4e** (663 mg, 1.00 mmol) with HF•pyridine solution (0.37 mL, 3.00 mmol), pyridine (0.24 mL, 3.00 mmol) and THF (5 mL) gave the intermediate primary alcohol (546 mg), which was subjected to acylation with succinic anhydride (200 mg, 2.00 mmol), DMAP (305 mg, 2.50 mmol) and CH₂Cl₂ (5 mL) to afford carboxylic acid **5e** (630 mg, 97% yield).

$R_f = 0.42$ (SiO₂, 50:50 hexanes/EtOAc);

^1H NMR (300 MHz, CDCl_3): δ 5.57-5.25 (m, 4H), 4.90 (quint., $J = 6.2$ Hz, 1H), 4.11 (t, $J = 6.5$ Hz, 2H), 2.77-2.59 (m, 4H), 2.39-2.20 (m, 4H), 2.13-1.93 (m, 6H), 1.72-1.46 (m, 6H), 1.46-1.02 (m, 34H), 0.97-0.80 (m, 6H).

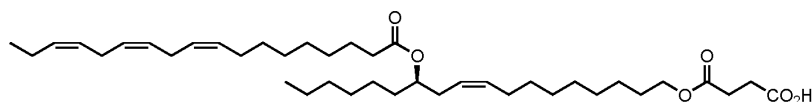


[0261] 4-(((*R,Z*)-12-(Linoleoyloxy)octadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5f**):

[0262] According to General Procedure B, desilylation of silyl ether **4f** (1.06 g, 1.60 mmol) with $\text{HF}\cdot\text{pyridine}$ solution (0.60 mL, 4.80 mmol), pyridine (0.39 mL, 4.80 mmol) and THF (8 mL) gave the intermediate primary alcohol (890 mg), which was subjected to acylation with succinic anhydride (320 mg, 3.20 mmol), DMAP (489 mg, 4.00 mmol) and CH_2Cl_2 (8 mL) to afford carboxylic acid **5f** (1.04 g, quantitative yield).

$R_f = 0.35$ (SiO_2 , 50:50 hexanes/ EtOAc);

^1H NMR (300 MHz, CDCl_3): δ 5.57-5.26 (m, 6H), 4.90 (quint., $J = 6.3$ Hz, 1H), 4.11 (t, $J = 6.7$ Hz, 2H), 2.79 (t, $J = 6.0$ Hz, 2H), 2.75-2.58 (m, 6H), 2.38-2.20 (m, 4H), 2.14-1.94 (m, 6H), 1.72-1.46 (m, 8H), 1.46-1.14 (m, 30H), 0.98-0.81 (m, 6H).

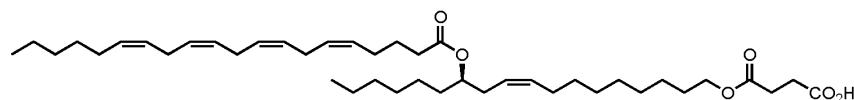


[0263] 4-(((*R,Z*)-12-(Linolenoyloxy)octadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5g**):

[0264] According to General Procedure B, desilylation of silyl ether **4g** (1.54 g, 2.34 mmol) with $\text{HF}\cdot\text{pyridine}$ solution (0.87 mL, 7.01 mmol), pyridine (0.57 mL, 7.01 mmol) and THF (6 mL) gave the intermediate primary alcohol (1.31 g), which was subjected to acylation with succinic anhydride (468 mg, 4.68 mmol), DMAP (714 mg, 5.84 mmol) and CH_2Cl_2 (6 mL) to afford carboxylic acid **5g** (1.47 g, quantitative yield).

$R_f = 0.35$ (SiO_2 , 50:50 hexanes/ EtOAc);

^1H NMR (300 MHz, CDCl_3): δ 5.56-5.25 (m, 8H), 4.90 (quint., $J = 6.2$ Hz, 1H), 4.11 (t, $J = 6.5$ Hz, 2H), 2.82 (t, $J = 5.7$ Hz, 4H), 2.37-2.22 (m, 4H), 2.16-1.95 (m, 6H), 1.74-1.46 (m, 6H), 1.46-1.15 (m, 30H), 0.99 (t, $J = 7.6$ Hz, 3H), 0.94-0.83 (m, 6H).

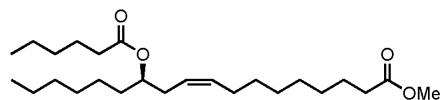


[0265] 4-(((*R,Z*)-12-(Arachidonoyloxy)octadec-9-en-1-yl)oxy)-4-oxobutanoic acid (**5h**):

[0266] According to General Procedure B, desilylation of silyl ether **4h** (711 mg, 1.04 mmol) with $\text{HF}\cdot\text{pyridine}$ solution (0.39 mL, 3.11 mmol), pyridine (0.25 mL, 3.11 mmol) and THF (5 mL) gave the intermediate primary alcohol (593 mg), which was subjected to acylation with succinic anhydride (201 mg, 2.01 mmol), DMAP (306 mg, 2.51 mmol) and CH_2Cl_2 (5 mL) to afford carboxylic acid **5h** (582 mg, 87% yield).

$R_f = 0.31$ (SiO_2 , 50:50 hexanes/EtOAc);

^1H NMR (300 MHz, CDCl_3): δ 5.58-5.24 (m, 10H), 4.90 (quint., $J = 6.2$ Hz, 1H), 4.11 (t, $J = 6.7$ Hz, 2H), 2.93-2.75 (m, 6H), 2.76-2.58 (m, 4H), 2.39-2.22 (m, 4H), 2.20-1.96 (m, 6H), 1.71 (quint., $J = 7.4$ Hz, 2H), 1.69-1.47 (m, 4H), 1.46-1.13 (m, 26H), 0.99-0.80 (m, 6H).

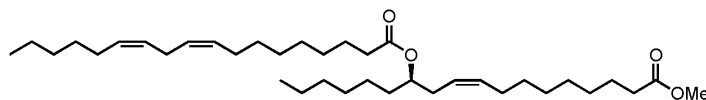


[0267] Methyl (12*R*)-hexanoyloxyoleate (**6a**):

[0268] According to General Procedure C, methyl ricinoleate (2.00 g, 6.40 mmol), hexanoic acid (898 mg, 7.68 mmol), DCC (1.58 g, 7.68 mmol) and DMAP (1.17 g, 9.60 mmol) in CH_2Cl_2 (10 mL) provided, after filtration through silica gel (95:5 hexanes/EtOAc), ricinoleate **6a** (2.52 g, 96% yield) as a clear, colourless oil.

$R_f = 0.62$ (SiO_2 , 70:30 hexanes:EtOAc);

^1H (300 MHz, CDCl_3): δ 5.54-5.42 (m, 1H), 5.40-5.28 (m, 1H), 4.90 (quint., $J = 6.2$ Hz, 1H), 3.69 (s, 3H), 2.37-2.23 (m, 6H), 2.11-1.97 (m, 2H), 1.72-1.48 (m, 6), 1.43-1.20 (m, 20), 0.96-0.84 (m, 6H).

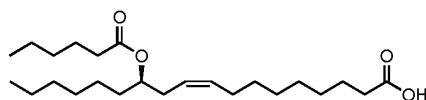


[0269] Methyl (12*R*)-linoleoyloxyoleate (**6b**):

[0270] According to General Procedure C, methyl ricinoleate (500 mg, 1.60 mmol), linoleic acid (538 mg, 1.92 mmol), DCC (396 mg, 1.92 mmol) and DMAP (293 mg, 2.40 mmol) in CH₂Cl₂ (5 mL) provided, after filtration through silica gel (95:5 hexanes/EtOAc), ricinoleate **6c** (875 g, 93% yield) as a light yellow oil.

R_f: 0.67 (SiO₂, 80:20 hexanes:EtOAc);

¹H (300 MHz, CDCl₃): δ 5.54-5.42 (m, 1H), 5.40-5.28 (m, 1H), 4.90 (quint., J = 6.2 Hz, 1H), 3.69 (s, 3H), 2.37-2.23 (m, 6H), 2.11-1.97 (m, 2H), 1.72-1.48 (m, 6), 1.43-1.20 (m, 20), 0.96-0.84 (m, 6H).

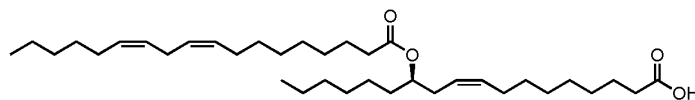


[0271] (12*R*)-Hexanoyloxyoleic acid (**7a**):

[0272] An argon-flushed round bottom flask was charged with methyl ester **6a** (1.97 g, 4.79 mmol, 1.00 equiv.) and *t*-BuOH (12 mL), then aqueous 2.0 M NaOH (1.80 mL, 3.60 mmol, 0.75 equiv.). After 17 h, the pH of the reaction solution was adjusted to 2 using aqueous 1 M HCl and extracted with Et₂O (3×30 mL). The combined organics were washed with water (1×30 mL), brine (1×30 mL), dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The residue was filtered through a plug of silica (98:2:0→50:45:5 hexanes:EtOAc:MeOH) to afford carboxylic acid **7a** (1.30 g, 92% yield) as a pale yellow oil.

R_f = 0.24 (SiO₂, 75:20:5 hexanes/EtOAc/MeOH);

¹H NMR (300 MHz, CDCl₃): δ 5.55-5.28 (m, 6H), 4.90 (quint., J = 6.2 Hz, 1H), 3.69 (s, 3H), 2.79 (t, J = 5.8 Hz, 2H), 2.40-2.21 (m, 6H), 2.16-1.93 (m, 6H), 1.72-1.46 (m, 8H), 1.46-1.18 (m, 32H), 1.00-0.80 (m, 6H).

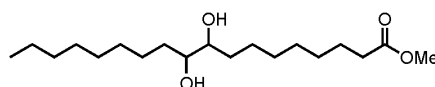


[0273] (12*R*)-Linoleoyloxyoleic acid (**7b**):

[0274] An argon-flushed round bottom flask was charged with methyl ester **6b** (5.97 g, 10.4 mmol, 1.00 equiv.) and *t*-BuOH (26 mL), then aqueous 2.0 M NaOH (4.70 mL, 9.30 mmol, 0.90 equiv.). After 17 h, the pH of the reaction solution was adjusted to 2 using aqueous 1 M HCl and extracted with Et₂O (3×30 mL). The combined organics were washed with water (1×30 mL), brine (1×30 mL), dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The residue was purified by flash column chromatography (SiO₂, 95:5:0→80:15:5 hexanes:EtOAc:MeOH) to afford carboxylic acid **7b** (4.48 g, 85% yield) as a pale yellow oil.

R_f: 0.35 (SiO₂, 75:20:5 hexanes/EtOAc/MeOH);

¹H (CDCl₃, 300 MHz): δ 5.55-5.28 (m, 6H), 4.90 (quint., J = 6.2 Hz, 1H), 2.79 (t, J = 6.0 Hz, 2H), 2.43-2.21 (m, 6H), 2.14-1.96 (m, 6H), 1.73-1.47 (m, 6H), 1.46-1.18 (m, 30H), 0.99-0.81 (m, 6H).



[0275] Methyl 9,10-dihydroxystearate (**8**):

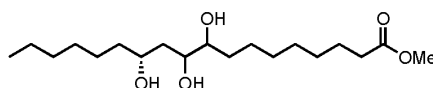
[0276] KOH (7.01 g, 125 mmol, 5.00 equiv.) was added to a rapidly stirred room temperature mixture of oleic acid (7.06 g, 25.0 mmol) and water (175 mL) in a 500 mL Erlenmeyer flask, then cooled to ~10 °C. A solution of KMnO₄ (7.11 g, 45.0 mmol, 1.80 equiv.) in water (75 mL) was added dropwise over 10 min. After stirring an additional 10-15 min, the reaction was quenched by addition of saturated aqueous NaHSO₃, then adjusted to pH ≤2 by addition of concentrated HCl with the aid of a cooling bath. The white, flocculent mixture was stirred for 1 h at room temperature, then the solids collected by suction filtration and dried in air overnight. The resulting white solids were hot gravity filtered and recrystallized from EtOH to afford the (±)-*syn*-9,10-dihydroxystearic acid as white crystals (5.86 g, 74% yield).

[0277] Concentrated H₂SO₄ (0.06 mL, 1.00 mmol, 0.05 equiv.) was added to a MeOH (50 mL) suspension of the above dihydroxy acid (6.33 g, 20.0 mmol) and the resulting mixture was heated

at reflux. After 14 h, the mixture was cooled to room temperature and concentrated on a rotary evaporator under reduced pressure and the resulting residue was partitioned between EtOAc and saturated aqueous NaHCO₃. The organic layer was washed with water (1×75 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure to afford methyl ester **8** (6.44 g, 97% yield) as a white solid.

R_f = 0.45 (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 3.68 (s, 3H), 3.61 (app br s, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.06-1.85 (app br s, 2H), 1.73-1.16 (m, 26H), 0.96-0.81 (m, 3H).



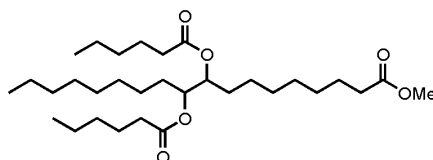
[0278] Methyl 9,10,12*R*-trihydroxystearate (**9**):

[0279] KOH (5.61 g, 100 mmol, 2.00 equiv.) was added to a rapidly stirred room temperature mixture of ricinoleic acid (14.9 g, 50.0 mmol) and water (500 mL) in a 1 L Erlenmeyer flask, then cooled to ~10 °C. A solution of KMnO₄ (13.4 g, 85.0 mmol, 1.70 equiv.) in water (250 mL) was added dropwise over 15 min. After stirring an additional 10-15 min, the reaction was quenched by addition of saturated aqueous Na₂SO₃, then adjusted to pH ≤2 by addition of concentrated HCl with the aid of a cooling bath. The white, flocculent mixture was stirred for 4 h at room temperature, then the solids collected by suction filtration and dried in air overnight. The resulting white solids were hot gravity filtered with EtOH to afford the crude 9,10,12-trihydroxystearic acid, which was used without further purification.

[0280] Concentrated H₂SO₄ (0.13 mL, 2.50 mmol, 0.05 equiv.) was added to a MeOH (120 mL) suspension of the above dihydroxy acid (6.33 g, 20.0 mmol) and the resulting mixture was heated at reflux. After 14 h, the mixture was cooled to room temperature and concentrated on a rotary evaporator under reduced pressure and the resulting residue was partitioned between warm EtOAc and saturated aqueous NaHCO₃. The organic layer was washed with water (1×75 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The resulting pale yellow solid was triturated four times with warm Et₂O to afford methyl ester **9** (9.52 g, 55% yield) as a white solid.

$R_f = 0.33$ (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 4.07-3.58 (m, 3H), 3.68 (s, 3H), 2.31 (t, $J = 7.5$ Hz, 2H), 1.86-1.14 (m, 24H), 0.90 (br t, 3H).

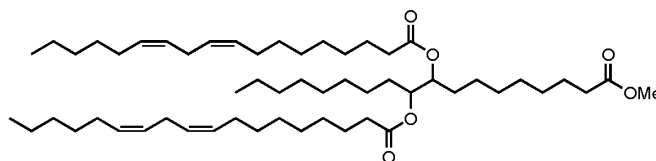


[0281] Methyl 9,10-dihexanoyloxystearate (**10a**):

[0282] DCC (2.27 g, 11.0 mmol, 2.20 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (13 mL) solution hexanoic acid (1.28 g, 11.0 mmol, 2.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath, diol **8** (1.65 g, 5.00 mmol) was added, followed by DMAP (1.53 g, 12.5 mmol, 2.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with Et₂O, stirred for 10 min, then filtered through Celite®. The filtrate was washed with aqueous 1 M HCl (2×30 mL), aqueous 1 M NaOH (2×30 mL), H₂O (1×30 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure to afford triester **10a** (2.61 g, quantitative yield) as a clear, colourless oil.

$R_f = 0.66$ (SiO₂, 70:30 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.08-4.92 (m, 2H), 3.68 (s, 3H), 2.40-2.20 (m, 6H), 1.74-1.44 (m, 12H), 1.44-1.13 (m, 28H), 1.01-0.80 (m, 9H).



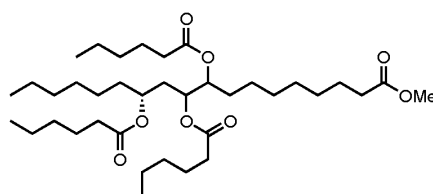
[0283] Methyl 9,10-dilinoleoyloxystearate (**10b**):

[0284] DCC (4.33 g, 21.0 mmol, 2.10 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (25 mL) solution linoleic acid (5.89 g, 21.0 mmol, 2.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled

again in an ice bath, diol **8** (3.30 g, 10.0 mmol) was added, followed by DMAP (3.05 g, 25.0 mmol, 2.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with hexanes, stirred for 10 min, then filtered through Celite®. The filtrate was concentrated on a rotary evaporator to yield the crude as a white semi-solid, which was purified by filtration through a plug of silica gel (95:5 hexanes/EtOAc) to afford the triester **10b** (7.24 g, 85% yield) as a clear colourless oil.

$R_f = 0.57$ (SiO₂, 70:30 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.49-5.27 (m, 8H), 5.05-4.94 (m, 2H), 3.68 (s, 3H), 2.79 (t, $J = 5.9$ Hz, 4H), 2.39-2.23 (m, 6H), 2.15-1.97 (m, 8H), 1.72-1.45 (m, 10H), 1.45-1.15 (m, 50H), 0.98-0.82 (m, 9H).

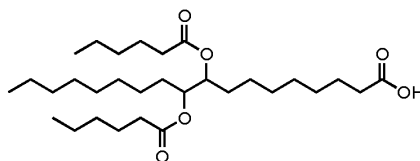


[0285] Methyl 9,10,12*R*-trihexanoyloxystearate (**11**):

[0286] DCC (2.64 g, 12.8 mmol, 3.20 equiv.) was added to a stirring, ice-cold CH₂Cl₂ (13 mL) solution hexanoic acid (1.49 g, 12.8 mmol, 3.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath, triol **9** (1.39 g, 4.00 mmol) was added, followed by DMAP (1.71 g, 14.0 mmol, 3.50 equiv.), and the reaction mixture was allowed to warm to room temperature over 14 h. The reaction mixture was diluted with hexanes, stirred for 10 min, then filtered through Celite®. The filtrate was washed with aqueous 1 M HCl (2×30 mL), aqueous 1 M NaOH (2×30 mL), H₂O (1×30 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure to afford triester **11** (1.99 g, 78% yield) as a clear, colourless oil.

$R_f = 0.77$ (SiO₂, 70:30 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 5.13-4.84 (m, 3H), 3.68 (s, 3H), 2.38-2.19 (m, 8H), 1.92-1.69 (m, 2H), 1.69-1.42 (m, 12H), 1.42-1.16 (m, 28H), 1.00-0.82 (m, 12H).

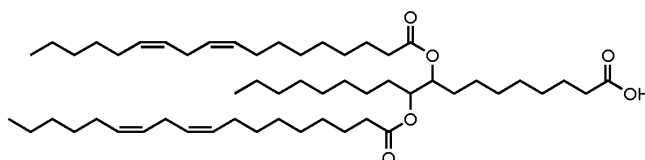


[0287] 9,10-Dihexanoyloxystearic acid (**12a**):

[0288] Aqueous 2.0 M KOH (0.91 mL, 1.82 mmol, 1.00 equiv.) was added to a room temperature *t*-BuOH (7 mL) solution of triester **10a** (1.05 g, 2.00 mmol, 1.10 equiv.) in a round bottom flask under argon. After stirring for 20 h, the reaction mixture was acidified to pH ≤ 2 by addition of aqueous 3 M HCl and extracted with Et₂O (3 $\times$ 20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The crude residue was purified by flash column chromatography (90:5:5 $\rightarrow$ 85:10:5 hexanes/EtOAc/MeOH) to afford carboxylic acid **12a** (802 mg, 86% yield) as a clear, colourless oil.

R_f = 0.22 (SiO₂, 85:10:5 hexanes/EtOAc/MeOH);

¹H NMR (300 MHz, CDCl₃): δ 5.08-4.93 (m, 2H), 2.36 (t, J = 7.8 Hz, 2H), 2.30 (t, J = 7.6 Hz, 4H), 1.72-1.44 (m, 10H), 1.44-1.16 (m, 30H), 0.97-0.83 (m, 9H).



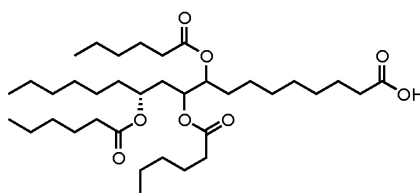
[0289] 9,10-Dilinoleoyloxystearic acid (**12b**):

[0290] Aqueous 2.0 M KOH (3.00 mL, 6.00 mmol, 1.00 equiv.) was added to a room temperature *t*-BuOH (7 mL) solution of triester **10b** (5.64 g, 6.60 mmol, 1.10 equiv.) in a round bottom flask under argon. After stirring for 20 h, the reaction mixture was acidified to pH ≤ 2 by addition of aqueous 3 M HCl and extracted with hexanes (3 $\times$ 75 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The crude residue was purified by flash column chromatography (90:10:0 $\rightarrow$ 85:10:5

hexanes/EtOAc/MeOH) to afford carboxylic acid **12b** (2.39 g, 68% yield) as a clear, colourless oil.

R_f = 0.33 (SiO₂, 85:10:5 hexanes/EtOAc/MeOH);

¹H NMR (300 MHz, CDCl₃): δ 5.49-5.25 (m, 8H), 5.07-4.93 (m, 2H), 2.79 (t, J = 5.9 Hz, 4H), 2.36 (t, J = 7.7 Hz, 2H), 2.30 (t, J = 7.5 Hz, 4H), 2.13-2.00 (m, 8H), 1.72-1.45 (m, 10H), 1.45-1.15 (m, 50H), 0.98-0.81 (m, 9H).



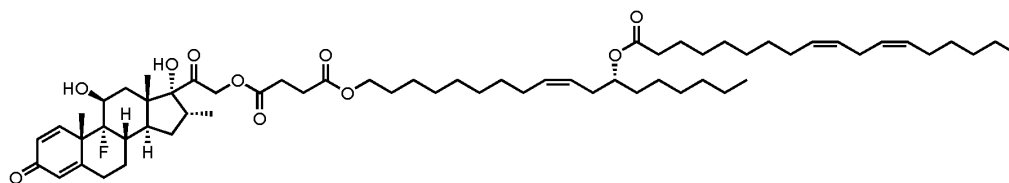
[0291] 9,10,12*R*-Trihexanoyloxystearic acid (**13**):

[0292] Aqueous 2.0 M KOH (1.47 mL, 2.94 mmol, 1.00 equiv.) was added to a room temperature *t*-BuOH (10 mL) solution of tetraester **11** (1.98 g, 3.10 mmol, 1.10 equiv.) in a round bottom flask under argon. After stirring for 20 h, the reaction mixture was acidified to pH $\leq$ 2 by addition of aqueous 3 M HCl and extracted with hexanes (3 $\times$ 30 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. The crude residue was purified by flash column chromatography (90:10:0 $\rightarrow$ 85:10:5 $\rightarrow$ 75:20:5 hexanes/EtOAc/MeOH) to afford carboxylic acid **13** (1.40 g, 78% yield) as a clear, colourless oil.

R_f = 0.32 (SiO₂, 80:15:5 hexanes/EtOAc/MeOH);

¹H NMR (300 MHz, CDCl₃): δ 5.13-4.82 (m, 3H), 2.42-2.18 (m, 8H), 1.92-1.69 (m, 2H), 1.69-1.43 (m, 12H), 1.43-1.14 (m, 28H), 0.99-0.81 (m, 12H).

[0293] Example E: Synthesis of INT-D045



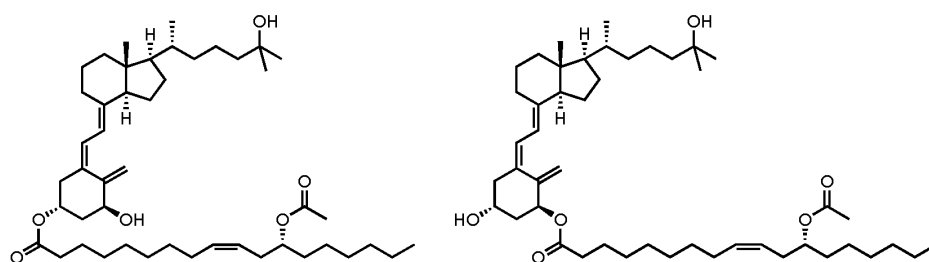
2-((8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-17-yl)-2-oxoethyl ((*R*,*Z*)-12-(linoleoyloxy)octadec-9-en-1-yl) succinate (**INT-D045**):

[0294] According to General Procedure D, dexamethasone (157 mg, 0.40 mmol), hemisuccinate **5f** (310 mg, 0.48 mmol), DCC (99 mg, 0.48 mmol), DMAP (73 mg, 0.60 mmol) and CH₂Cl₂ (2 mL) afforded, after flash column chromatography (SiO₂, 80:20→50:50 hexanes/EtOAc), **INT-D045** (278 mg, 68% yield) as a clear, colourless oil.

R_f = 0.50 (SiO₂, 50:50 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 7.22 (d, *J* = 10.1 Hz), 6.36 (dd, *J* = 10.2, 1.8 Hz), 6.13 (s, 1H), 5.56-5.25 (m, 6H), 4.93 (s, 2H), 4.89 (quint., *J* = 6.3 Hz), 4.46-4.31 (m, 1H), 4.10 (t, *J* = 6.8 Hz, 2H), 3.20-3.04 (m, 1H), 2.88-2.54 (m, 7H), 2.53-1.91 (m, 15H), 1.90-1.46 (m, 14H), 1.47-1.12 (m, 34H), 1.06 (s, 3H), 0.99-0.81 (m, 9H).

[0295] Example S: Synthesis of INT-D053



(1*R*,3*S*,*Z*)-3-hydroxy-5-(2-((1*R*,3*aS*,7*aR*,*E*)-1-((*R*)-6-hydroxy-6-methylheptan-2-yl)-7*a*-methyloctahydro-4*H*-inden-4-ylidene)ethylidene)-4-methylenecyclohexyl (*R*,*Z*)-12-acetoxyoctadec-9-enoate and (1*S*,5*R*,*Z*)-5-hydroxy-3-(2-((1*R*,3*aS*,7*aR*,*E*)-1-((*R*)-6-hydroxy-6-methylheptan-2-yl)-7*a*-methyloctahydro-4*H*-inden-4-ylidene)ethylidene)-2-methylenecyclohexyl (*R*,*Z*)-12-acetoxyoctadec-9-enoate (**INT-D053**): JZ-25-057, 029

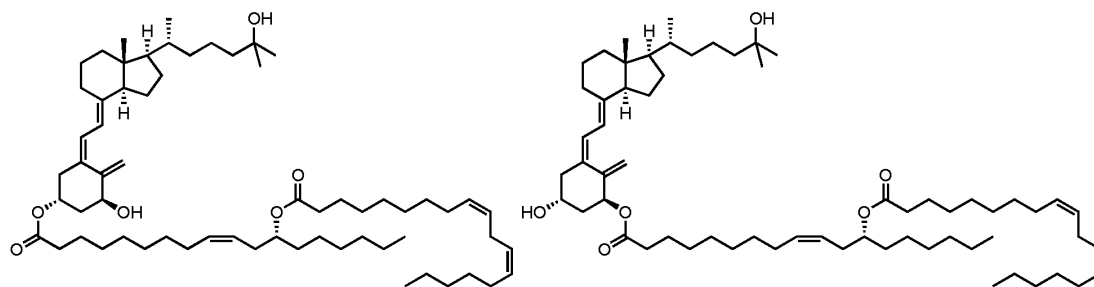
[0296] DCC (50 mg, 0.24 mmol, 1.20 equiv.) was added to a stirring, ice-cold 1:1 CH₂Cl₂/THF (4 mL) solution of (12*R*)-acetoxyoleic acid (82 mg, 0.24 mmol, 1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath and solid calcitriol (83 mg, 0.20 mmol) and DMAP (29 mg, 0.24 mmol, 1.20 equiv.) were added. The reaction mixture was allowed to warm up over 14

h, diluted with EtOAc, stirred for 10 min, then filtered through Celite®. The filtrate was concentrated to afford the crude as a pale yellow oil and subsequently purified by flash column chromatography (SiO₂, 80:20→65:35 hexanes/EtOAc) to afford an ~1:1 mixture of the 1- and 3-acylated conjugates (61 mg, 41% yield) as a clear, colourless oil.

R_f = 0.33 (SiO₂, 60:40 hexanes/EtOAc);

¹H NMR (300 MHz, CDCl₃): δ 6.44-6.25 (m, 2H), 6.02 (d, J = 11.2 Hz, 1H), 5.92 (d, J = 11.2 Hz, 1H), 5.56-5.40 (m, 3H), 5.40-5.27 (m, 4H), 5.26-5.16 (m, 1H), 5.07-4.97 (m, 2H), 4.87 (quint., J = 6.2 Hz, 2H), 4.45-4.34 (m, 1H), 4.23-4.10 (m, 1H), 2.89-2.74 (m, 2H), 2.68-2.51 (m, 2H), 2.48-2.18 (m, 11H), 2.17-1.77 (m, 25H), 1.76-1.13 (m, 90H), 1.12-0.99 (m, 2H), 0.99-0.80 (m, 13H), 0.55 (s, 3H), 0.52 (s, 3H).

[0297] Example T: Synthesis of INT-D068



(*R,Z*)-18-(((1*R*,3*S*,*Z*)-3-Hydroxy-5-(2-(((1*R*,3*aS*,7*aR*,*E*)-1-((*R*)-6-hydroxy-6-methylheptan-2-yl)-7*a*-methyloctahydro-4*H*-inden-4-ylidene)ethylidene)-4-methylenecyclohexyl)oxy)-18-oxooctadec-9-en-7-yl linoleate and (*R,Z*)-18-(((1*S*,5*R*,*Z*)-5-hydroxy-3-(2-(((1*R*,3*aS*,7*aR*,*E*)-1-((*R*)-6-hydroxy-6-methylheptan-2-yl)-7*a*-methyloctahydro-4*H*-inden-4-ylidene)ethylidene)-2-methylenecyclohexyl)oxy)-18-oxooctadec-9-en-7-yl linoleate (**INT-D068**):

[0298] DCC (50 mg, 0.24 mmol, 1.20 equiv.) was added to a stirring, ice-cold 1:1 CH₂Cl₂/THF (4 mL) solution of (12*R*)-linoleoyloxyoleic acid (135 mg, 0.24 mmol, 1.20 equiv.) in a round bottom flask under argon, then the ice bath was removed and the resultant stirred for 15 min. The reaction mixture was cooled again in an ice bath and solid calcitriol (83 mg, 0.20 mmol) and DMAP (29 mg, 0.24 mmol, 1.20 equiv.) were added. The reaction mixture was allowed to warm up over 14 h, diluted with EtOAc, stirred for 10 min, then filtered through Celite®. The filtrate was

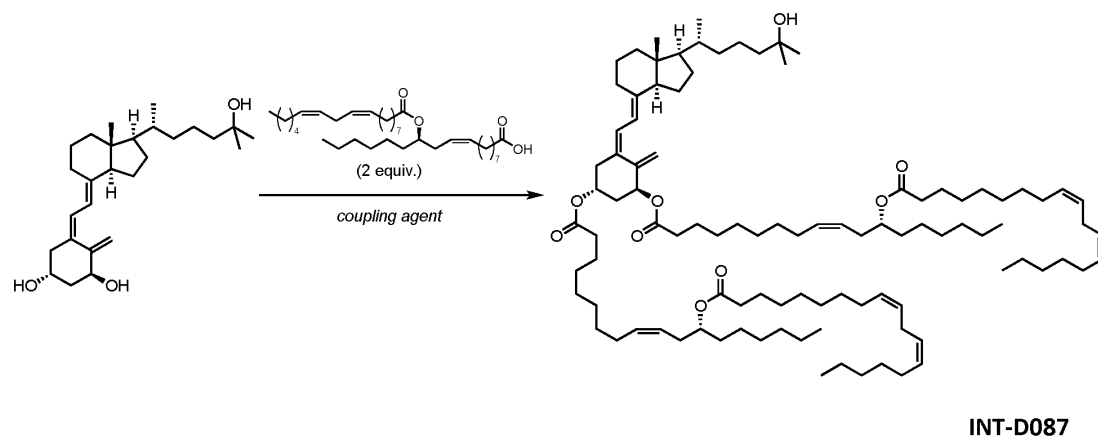
concentrated to afford the crude as a pale yellow oil and subsequently purified by flash column chromatography (SiO₂, 95:5→90:10→70:30 hexanes/EtOAc) to afford an ~1:1 mixture of the 1- and 3-acylated conjugates (75 mg, 39% yield) as a clear, colourless oil.

R_f = 0.26 (SiO₂, 70:30 hexanes/EtOAc);

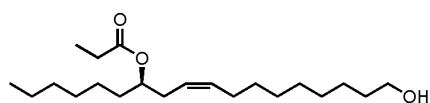
¹H NMR (300 MHz, CDCl₃): δ 6.43-6.26 (m, 2H), 6.02 (d, J = 11.2 Hz, 1H), 5.92 (d, J = 11.2 Hz, 1H), 5.57-5.26 (m, 15H), 5.26-5.16 (m, 1H), 5.07-4.97 (m, 2H), 4.88 (quint., J = 6.2 Hz, 2H), 4.47-4.34 (m, 1H), 4.23-4.10 (m, 1H), 2.89-2.70 (m, 6H), 2.68-2.52 (m, 2H), 2.47-2.20 (m, 15H), 2.16-1.77 (m, 25H), 1.77-1.12 (m, 118H), 1.12-1.01 (m, 2H), 1.00-0.79 (m, 19H), 0.55 (s, 3H), 0.52 (s, 3H).

[0299] Example W: Synthesis of Disubstituted Calcitriol, INT-D087

[0300] An example of a synthesis scheme for preparing a calcitriol lipid conjugate disubstituted with two lipid moieties is provided below:



[0301] Example X: Synthesis of Sirolimus prodrug (lipid conjugate), INT-D097



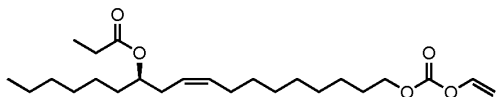
[0302] (12*R*)-(Propionyloxy)oleyl alcohol:

[0303] Pyridine (1.96 mL, 24.0 mmol, 1.30 equiv.) was added to an ice-cold PhH (47 mL) solution of ricinoleyl alcohol (2.65 g, 9.3 mmol, 1.00 equiv.) in a round bottom flask under argon, followed by dropwise addition of propionyl chloride (1.95 mL, 22.0 mmol, 1.20 equiv.). The reaction

mixture was stirred for 10 min, the ice bath removed, stirred for a further 45 min, then heated at 100 °C. After 4 h, the reaction was allowed to cool to room temperature, diluted with *t*-BuOMe, washed with aqueous 1 M HCl (1×35 mL), aqueous 1 M NaOH (1×25 mL), water (1×35 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator to afford 3.94 g of a cloudy oil as the crude diacylated intermediate.

[0304] The above material was dissolved in 5:1 EtOH-H₂O (6.6 mL) in a round bottom flask, KOH (517 mg, 9.20 mmol, 0.99 equiv.) was added and the resultant was allowed to stir at room temperature. After 18 h, the reaction mixture was diluted with water (30 mL), extracted with *t*-BuOMe (3×40 mL) and the combined organic extracts were washed with water (1×35 mL), brine (1×35 mL), then dried over Na₂SO₄ and concentrated on a rotary evaporator to afford a yellow oil as the crude. Purification was carried out by flash column chromatography (silica gel, 70:30 hexanes-EtOAc) to provide the title compound as a pale yellow oil (2.21 g, 71% yield over 2 steps).

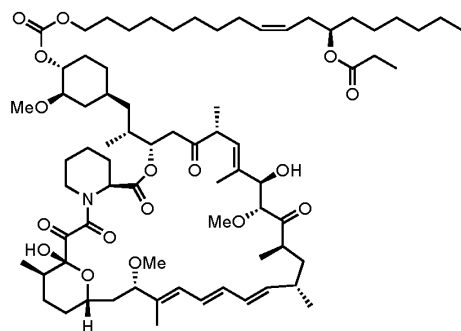
[0305] ¹H NMR (300 MHz, CDCl₃): δ 5.56-5.41 (m, 1H), 5.41-5.27 (m, 1H), 4.90 (quint., *J*=6.2 Hz, 1H), 3.66 (t, *J*=6.5 Hz, 2H), 2.40-2.22 (m, 4H), 2.12-1.96 (m, 2H), 1.65-1.22 (m, 23H), 1.15 (t, *J*=7.6 Hz, 3H), 0.89 (br t, *J*=6.6 Hz, 3H) ppm.



[0306] (12*R*)-(Propionyloxy)oleyl vinyl carbonate:

[0307] Vinyl chloroformate (0.46 mL, 5.00 mmol, 1.40 equiv.) was added dropwise to an ice-cold pyridine (12 mL) solution of (12*R*)-(propionyloxy)oleyl alcohol (1.23 g, 3.60 mmol, 1.00 equiv.) in a round bottom flask under argon. After 45 min, the ice bath was removed and the reaction mixture allowed to stir for a further 3 h. The reaction was acidified to pH≤2 by addition of aqueous 6 M HCl, extracted with CH₂Cl₂ (3×50 mL), then the combined organic extracts were washed with water (1×50 mL), brine, dried over Na₂SO₄ and concentrated on a rotary evaporator to afford a yellow oil as the crude. Purification was carried out by filtration through a plug of silica gel (70:30 hexanes-EtOAc) and the filtrate concentrated on a rotary evaporator to provide the title compound as slightly yellow oil. (1.34 g, 91% yield)

[0308] ^1H NMR (300 MHz, CDCl_3): δ 7.10 (dd, $J=13.9, 6.3$ Hz, 1H), 5.56-5.42 (m, 1H), 5.42-5.27 (m, 1H), 4.94 (dd, $J=13.8, 1.9$ Hz, 1H), 4.90 (quint., $J=6.1$ Hz, 1H), 4.60 (dd, $J=6.2, 1.9$ Hz, 1H), 4.21 (t, $J=6.6$ Hz, 2H), 2.39-2.23 (m, 4H), 2.11-1.96 (m, 2H), 1.71 (br quint., $J=6.7$ Hz, 2H), 1.63-1.48 (m, 2H), 1.46-1.21 (m, 18H), 1.15 (t, $J=7.6$ Hz, 3H), 0.89 (br t, $J=6.6$ Hz, 3H) ppm.



[0309] (12*R*)-(Propionyloxy)oleyl sirolimus 40-carbonate:

[0310] A dry thick-walled glass tube was charged with (12*R*)-(propionyloxy)oleyl vinyl carbonate (1.33 g, 3.24 mmol, 6.00 equiv.), sirolimus (494 mg, 0.54 mmol, 1.00 equiv.), Novozym 435 (494 mg, 100% w/w) and dry *t*-BuOMe (7.7 mL), then sealed with a TeflonTM screw cap and O-ring and heated at 60 °C in an oil bath. After 22 h, the reaction was removed from the heat, allowed to cool to room temperature, filtered through a pad of Celite[®], washing with *t*-BuOMe and concentrated on a rotary evaporator to provide the crude as a yellow oil. Purification was carried out by flash column chromatography (silica gel, 80:20→70:30→60:40 Me_2CO -pentane) to provide the title compound as a clear, colourless oil that solidified on standing (567 mg, 82% yield).

[0311] R_f : 0.53 (70:30 Me_2CO -pentane);

[0312] ^1H NMR (300 MHz, CDCl_3): δ 6.44-6.06 (m, 3H), 6.00-5.84 (m, 1H), 5.61-5.38 (m, 3H), 5.37-5.24 (m, 2H), 5.21-5.06 (m, 1H), 4.88 (quint., $J=6.2$ Hz, 1H), 4.81 (br s, 1H), 4.56 (m, 2H), 4.31-4.07 (m, 4H), 3.91-3.80 (m, 1H), 3.74-3.62 (m, 2H), 3.62-3.53 (m, 1H), 3.44-3.29 (m, 8H), 3.13 (s, 3H), 2.78-2.67 (m, 2H), 2.64-2.53 (m, 1H), 2.39-2.21 (m, 6H), 2.17 (s, 3H), 2.14-1.92 (m, 6H), 1.89-1.18 (m, 46H), 1.16-0.77 (m, 20H), 1.13 (t, $J=7.7$ Hz, 3H) ppm. Sirolimus is reported to have conformational isomers in CDCl_3 (Crull, et al. *J. Nat. Prod.* **2023**, 86, 1862).

[0313] Lipid nanoparticle (LNP) preparation

[0314] The lipids 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) or 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (PEG-DSPE) or 1,2-dimyristoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000 (PEG-DMG) were dissolved in ethanol. DSPC, DMPC, PEG-DSPE and PEG-DMG were purchased from Avanti Polar Lipids (Alabaster, AL), and cholesterol was obtained from Sigma (St Louis, MO).

[0315] Lipid conjugates containing immunomodulatory agents (referred to as “prodrugs” in the following examples) (see FIGs. 10A-10M) were synthesized as provided above and as previously described (e.g. see WO/2020/191477, which is incorporated by reference herein in its entirety).

[0316] Lipid conjugates (prodrugs) were dissolved in ethanol, isopropanol, DMSO or THF. LNP were prepared by rapidly mixing DSPC or DMPC, cholesterol, prodrugs, and PEG-DSPE (in a molar ratio of 49/40/10/1) with phosphate-buffered saline (PBS) using a cross-junction mixer. Formulations were dialyzed against PBS to remove residual ethanol. In cases where a peptide antigen, such as ovalbumin peptide 323-329 (OVA), was co-formulated within the LNP, the peptide is dissolved in PBS and rapidly mixed with the lipid phase. Dialysis or tangential flow filtration was used to remove all untrapped peptide.

[0317] To prepare LNPs that contain mRNA that codes for the antigen, ionizable lipid such as INT-A002 (see page 32 of co-owned WO 2021/026647; Application No. PCT/CA2020/051098, which is incorporated herein by reference), DSPC, cholesterol and PEG-DMG were dissolved in ethanol. Prodrugs were dissolved in ethanol, isopropanol, DMSO or THF. The mRNA was dissolved in 10 mM citrate or 25mM acetate buffer at pH 4.0. LNP were prepared by rapidly mixing the lipid components in ethanol (in molar ratio of 45/8.5/35/1.5/10 of INT-A002/DSPC/chol/PEG-DMG/prodrugs) with nucleic acids in aqueous buffer at a volumetric flow rate ratio of 1:3 (ethanol to aqueous, combined flow rate 28 ml/min) at room temperature. The product was then dialyzed against 1 X phosphate-buffered saline (PBS) at pH 7.4 for 24 hours to remove residual ethanol and to raise the pH.

[0318] The physiochemical properties of the LNPs prepared as described above were subsequently characterized. Particle size was determined by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern, UK) following buffer exchange into phosphate-buffered saline. Number-weighted size and distribution data was used. Lipid concentrations were determined by measuring

total cholesterol using the Cholesterol E enzymatic assay kit from Wako Chemicals USA (Richmond, VA). mRNA entrapment was determined using a modified Quanti-iT Ribogreen assay (ThermoFisher, Waltham, MA). LNP- mRNA systems were incubated in the presence or absence of 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO). Fluorescence intensities (Ex/Em: 480/520 nm) were compared to determine % mRNA entrapment.

[0319] Example 1: LNP efficiently accumulate in APCs in the pancreatic islets, pancreatic lymph nodes and spleen

[0320] This example demonstrates that LNPs provide a potential delivery platform to effectively deliver drugs and antigens to the APCs located in a subject. This may have the additional effect of limiting side-effects and reducing dose requirements by avoiding drug accumulation in other cell types and providing effective delivery to the APC populations.

[0321] In order to determine LNP accumulation in pancreatic APCs, mice received 2 injections, 24 hours apart, of LNP containing the fluorescent marker DiO injected at a dose of 600 mg/kg i.p. At 48 hours following the first injection, animals were euthanized and pancreatic islets and lymph nodes were harvested. Islets were hand-picked to 99% purity. Islet and lymph nodes were dispersed into single cell suspensions and stained for viability, CD45 (pan-immune cell marker) and CD11b and CD11c (APC markers), and the number of DiO positive cells was quantified by flow cytometry.

[0322] FIGs. 1A-D shows that through a simple injection, LNPs are taken up by APCs in both pancreatic islets and pancreatic lymph nodes. Furthermore, as shown in both the lymph node and islet, there is limited LNP uptake by other cell types, including endocrine cells in the islets, and non-APC immune cells (e.g., T cells) in the lymph node (Table 4).

[0323] Table 4. LNPs specifically accumulate in pancreatic APCs

Tissue	Cell type	DiO+ (% total population)
Pancreatic islets	Islet Macrophages	88 ± 3
	All other islet cells	0.84 ± 0.5
Pancreatic lymph nodes	Dendritic cells	74 ± 5
	Lymphocytes	9.7 ± 4

n=2-3 biological samples, 2-3 mice pooled per sample to obtain sufficient cell numbers

[0324] In order to compare the effect of LNP lipid composition on islet APC targeting, C57Bl/6J male mice were injected with 150 mg/kg DSPC/Chol or ionizable LNP with DiO, 24 hours prior to islet, pancreatic lymph node, and splenocyte isolation. Islets were hand-picked to 99% purity. Tissues were dispersed into single cell suspensions and stained for viability, CD45 (pan-immune cell marker) and CD11b and CD11c (APC markers), and the number of DiO positive cells was quantified by flow cytometry.

[0325] The pronounced accumulation in APCs is observed for both liposomes and LNP (DSPC/Chol or ionizable) in the islet, pancreatic lymph node and spleen (FIG. 2).

Example 2: LNP can efficiently co-encapsulate and stably retain lipid conjugates (prodrugs) of tolerizing agents

As shown in Example 1, both liposomes and LNP (DSPC/Chol and ionizable) can accumulate in APCs *in vivo*. Another non-limiting aspect of the disclosure provides LNPs with two or more different immunomodulatory agents co-formulated therein. The prodrugs are uniquely suited for co-formulation due to their lipophilic nature. The results not only show that more than one prodrug can be formulated in an LNP at high encapsulation efficiency, but also that the prodrugs can be stably retained within the LNPs. Moreover, the results herein show that the prodrug strategy is uniquely suited for the delivery of combination ratios of two or more immunomodulatory agents.

[0326] As shown in FIG. 3, both dexamethasone (D045) and calcitriol (D053, D068, D083) lipid prodrugs are stably retained within LNP after 2 hours of incubation in human plasma. While this example demonstrates the ability to prepare LNP containing up to 20 mol% of dexamethasone and calcitriol prodrugs, the LNPs of the disclosure have been shown to be capable of incorporating up to 99 mol% of the prodrugs. Therefore, using the prodrug strategy of the disclosure, co-formulation of additional immunomodulatory agents (e.g., such as but not limited to acetylsalicylic acid, mycophenolate, sirolimus and tacrolimus) can be achieved in the LNP formulations disclosed herein.

[0327] The results showing efficient entrapment of different lipid prodrugs in LNPs are set forth in Table 5. Tables 6, 7 and 8 below demonstrate that different ratios of these prodrugs can be achieved with no impact on entrapment efficiencies.

[0328] Table 5. Different lipid conjugates and combinations of lipid conjugates can be formulated into LNP

Dexamethasone Prodrug	Calcitriol Prodrug	Size	PdI	% Entrap (Dex/Calcitriol)
---	---	43	0.054	N/A
---	D053	55	0.070	-- / 98
---	D068	43	0.073	-- / 94
---	D083	51	0.066	-- / 97
---	D087	40	0.055	-- / 99
D034	---	50	0.043	97 / --
D034	D052	54	0.050	93 / 92
D034	D083	49	0.079	94 / 100
D045	---	47	0.051	96 / --
D045	D053	49	0.053	93 / 97
D045	D068	44	0.065	98 / 94
D045	D083	50	0.047	100 / 99
D045	D087	43	0.085	99 / 99

[0329] Table 6. D045 and D053 Combination LNP

D045:D053	Size	PdI	% Entrapment (Dex/Calcitriol)
10:10	51	0.051	100/100
3:10	53	0.058	100/97
1:10	51	0.065	93/97
10:3	49	0.049	100/98
10:1	48	0.037	99/94
3:3	52	0.069	94/88
1:1	56	0.047	100/97
Control (0:0)	44	0.037	N/A

[0330] Table 7. D045 and D068 Combination LNP

D045:D068	Size	PdI	% Entrapment (Dex/Calcitriol)
10:10	47	0.055	99/98
3:10	45	0.059	94/95
1:10	44	0.054	95/98
10:3	48	0.054	99/96
10:1	47	0.055	99/74
3:3	46	0.107	95/86
1:1	51	0.035	95/86

Control (0:0)	45	0.041	N/A
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[0331] Table 8. D045 and D083 Combination LNP

D045:D083	Size	PdI	Entrapment (Dex/Calcitriol)
10:10	53	0.034	98/98
3:10	55	0.046	85/97
1:10	55	0.050	100/98
10:3	51	0.044	97/91
10:1	48	0.055	96/97
3:3	54	0.049	97/83
1:1	59	0.033	100/76
Control (0:0)	45	0.053	N/A

[0332] Materials & Methods - Examples 3 to 7

[0333] Mice. NOD/ShiLtJ (001976) and C57BL/6J (000664) mice were purchased from Jackson Laboratories (Jackson Laboratories, Bar Harbor, ME) and housed under 12-h light 12-h dark conditions at room temperature, with ad libitum access to chow (Teklad #2918, Envigo) and water.

[0334] Cell Culture. BMDCs were generated as follows: bone marrow was harvested from the femurs and tibia of mice, red blood cells lysed with ammonium chloride solution (Stemcell Cat#07800) and 0.5 million cells seeded in 6 cm petri dishes at 0.125 million cells/mL in RPMI media supplemented with 10% FBS, 50 μ M β -mercaptoethanol (Cat#M3148-100ML), 2 mM L-Glutamine (Gibco Cat#20503-149), 1x NEAA (Gibco Cat#11140-050) and 1% pen/strep, 30 ng/mL GM-CSF (PeproTech Cat#315-03-100UG) and 30ng/mL IL-4 (PeproTech Cat#214-14-100UG) for 7 days (media replenished on days 3 and 5). On day 8, cells were harvested and 1 million cells/well seeded into 6 well plates at 1 million cells/mL. BMDCs were treated with LNPs (1 μ g/mL mRNA) for 48 h, in the presence or absence of 10 ng/mL LPS for the final 24 h. Subsequently, BMDCs were stained with CD80, CD86, MHC II, CD40, CD11c antibodies, along with fixable viability dye (FVD) and analyzed by flow cytometry. Cells were gated on FVD-CD11c⁺ and data are shown as histograms of mode-normalized cell counts vs fluorescence of indicated marker.

[0335] Human CD14⁺ monocytes were enriched from PBMC using EasySep™ huCD14 Positive Selection Cocktail II (Stemcell Cat#17858). CD14⁺ monocytes were seeded at 0.1 million cells/well into 96-well plates in X-Vivo w/o gentamicin and phenol red media (Lonza Cat#BEBP02-054Q) supplemented with 5% Human Serum (Wisent Bioproducts Cat#022-210), 1% pen/strep, 1% Glutamax (Cat#35050061), 1% sodium pyruvate (Gibco Cat#11360-070), 50 ng/mL Recombinant human GM-CSF (Stemcell Cat #78015.3) and 100 ng/mL Human Recombinant IL-4 (Stemcell Cat#78045) for 6 days. Cells were fed on day 3 and matured on day 6 with the addition of 50 ng/mL Recombinant human GM-CSF (Stemcell Cat#78015.3) and 100 ng/mL Human Recombinant IL-4 (Stemcell Cat#78045), 50 ng/mL Recombinant human tumour necrosis factor (TNF)- α (eBioscience Cat#BMS301), 1000 ng/mL Prostaglandin E2 (PGE2) (Tocris Cat#2296), 10 ng/mL Recombinant human IL-1 β (Stemcell Cat#78034.1), and 100 ng/mL Recombinant human IL-6 (Stemcell Cat#78148). On day 6, 50 ng/mL of Recombinant human interferon (IFN)- γ (Gibco Cat#PHC4033) was added to the culture for the final 24 h. DCs were treated with LNPs (3 μ g/mL mRNA) starting on day 3 for 4 d, in the presence or absence of maturation cytokines. Subsequently, DCs were stained with CD80, CD86, CD83, HLA-DR, CD14 and CD11c antibodies, along with fixable viability dye (FVD) and analyzed by flow cytometry. Cells were gated on FVD- CD14-CD11c⁺ and data are shown as histograms of mode-normalized cell counts vs fluorescence of indicated marker.

[0336] *mRNA*. 5'-methoxyuridine-modified mRNA (5moU) encoding full-length Ovalbumin (Ova) as a model/control antigen was purchased from Trilink (cat#L-7210). Sequences encoding either full-length diabetes relevant antigen (mouse preproinsulin 2; Ins2) or a multiple diabetes relevant antigen (multi-antigen) construct were codon optimized and synthesized by Trilink with their 5'UTR and 3'UTR and polyA tail and CleanCap Cap1 technology. The multi-antigen mRNA contains an open-reading frame encoding the Ins2 signal peptide followed by multiple MHC Class I and MHC Class II diabetes-relevant epitopes from prevalent autoantigens and hybrid insulin peptides in type 1 diabetes and NOD mice (Insulin, IA-2, IGRP, GAD65 and HIP2.5), each separated by short linker peptides, were codon optimized and synthesized as mRNA. The Multi epitope construct encoded a polypeptide of sequence SEQ ID NO: 90.

[0337] *LNP Formulation*. LNPs were formulated with INT's proprietary ionizable lipids and 5moU-modified mRNA using a rapid mixing process. Briefly, all lipid components were dissolved

in ethanol while the mRNA was dissolved in pH 4.0 citrate buffer. Tolerogenic LNP contain immunomodulatory prodrugs up to 10 mol% while control LNP do not. The ethanol and citrate solutions were injected into a T-junction mixture at a ratio of 1:3, respectively. The resultant LNP solutions were dialyzed overnight in pH 7.4 tris buffer to remove residual ethanol and to increase the pH to physiological levels. All LNP-mRNA were formulated at an amine-to-phosphate ratio (N/P) of 6. Lipid concentrations were determined by measuring cholesterol content (Cholesterol Total E Assay, Wako) and RNA entrapment and concentrations were determined using Ribogreen (Life Technologies) and/or measuring absorbance at 260nm. Prodrug entrapment was determined by analysis on the UPLC. Final LNP-mRNA were stored frozen at -70°C.

[0338] *LNP treatment.* Mice were administered LNP formulations at a dose of 5 or 10 µg mRNA/mouse via intraperitoneal injection unless otherwise indicated. Volume matched buffer solution was injected as vehicle control. For fast-refeed, mice were fasted overnight and subsequently refeed ad libitum for 45 minutes. Blood was collected from the saphenous vein prior to and after the refeed period.

[0339] *Flow cytometry.* Cells were prepared in single cell suspensions following standard protocols and stained for the markers indicated in each figure, following manufacturer instructions. Flow cytometry was conducted on BD LSRFortessa or Beckman Coulter Cytoflex and data analyzed with FlowJo software.

[0340] *Metabolic tests.* Blood glucose was measured via tail poke and measured with a OneTouch Ultra Glucometer (Life Scan Inc., Burnaby, Canada). For glucose tolerance tests, mice were fasted for 6 h and subsequently injected intraperitoneally with 2 g/kg body weight and measured at indicated time points.

[0341] **Example 3: Identification of lead tolerogenic small-molecule immunomodulator prodrugs (lipid conjugates) for inducing protection against autoimmune islet infiltration in the NOD mouse model of type 1 diabetes.**

[0342] The ability of antigen mRNA and immunomodulator to induce antigen specific immune tolerance was explored in the NOD mouse model of T1D. LNPs were formulated with an immunodominant diabetes-relevant antigen in NOD mice, Insulin 2 (Ins2), encoded by mRNA, along with varying compositions of immunomodulator prodrugs. LNPs carrying Ins2 mRNA only

were used as a control. These LNPs of varying immunomodulator compositions were tested in pre-diabetic NOD mice to determine which composition best prevented autoimmune infiltration of pancreatic islets (insulinitis). NOD/ShiLtJ female mice were injected intraperitoneally 2x, 16 days apart, starting at 4.3 weeks of age with 10 ug LNP carrying Insulin mRNA with/without immunomodulator prodrugs. Pancreases were collected at 11.9 weeks of age, fixed, paraffin embedded, sectioned and stained with H&E. Insulinitis was scored for all islets in 3 pancreas sections per mouse separated by 100 um, and averaged per section. n=5 mice per treatment group. As shown in Figure 4, two formulations were identified that significantly increased the number of uninfiltrated islets relative to vehicle-injected mice. These two lead formulations were comprised of Ins2 antigen mRNA with either D053 or a 1:1 combination of D034:D097 prodrugs. Importantly, control LNP (without any immunomodulators) did not improve the number of uninflamed islets compared to vehicle control.

[0343] Example 4: Tolerization of APCs *ex vivo* with LNPs co-delivering D034;D097 prodrugs with mRNA.

[0344] The ability of LNP-encapsulated prodrugs to tolerize APCs *ex vivo* was tested in this example. LNPs were formulated carrying 5moU modified mRNA with/without D034 and D097 prodrugs. In this system, the antigen-encoded by the mRNA is inconsequential as there is antigen-receptor immune component (T or B cells) in this assay. Nevertheless, mRNA is included to mimic the formulations that are used in systems where antigen is relevant and to control for the effect of mRNA-only containing LNPs on the immune response. NOD mouse BMDCs were treated with LNPs containing Ins2 mRNA with/without D034;D097 for 48 h and subsequently challenged with/without lipopolysaccharide (LPS) stimulation for 24 hours to determine whether prodrug formulations could prevent LPS-mediated activation (i.e. tolerize) BMDCs (Figure 5).

[0345] In more detail, BMDCs were generated from a NOD/ShiLtJ mouse according to standard protocols, and treated with LNPs (1 ug/mL mRNA) for 48 h, in the presence or absence of 10 ng/mL LPS for the final 24 h. Subsequently, BMDCs were stained with CD80, CD86, MHC II, CD40, CD11c antibodies, along with fixable viability dye (FVD) and analyzed by flow cytometry. Cells were gated on FVD- CD11c⁺. Data in Figure 5 are shown as histograms of mode-normalized cell counts vs fluorescence of indicated marker.

[0346] LNPs carrying both D034/D097 and mRNA attenuated the LPS-induced increase in cell surface activation markers including CD80, CD86, CD40 and MHC Class II in NOD BMDCs, relative to cells treated with matched mRNA-only LNPs (control LNP) or buffer only (vehicle). These data indicate that LNPs co-delivering D034/D097 and mRNA can maintain BMDCs in an immature/semi-mature/tolerogenic state *ex vivo*.

[0347] Similarly, LNPs co-delivering D034/D097 and mRNA to human monocyte-derived DCs *ex vivo* reduced cell surface expression of CD86, CD83 and HLA-DR in immature cells and reduced CD83 in cytokine-matured cells (Figure 6). In more detail, DCs were differentiated from CD14⁺ monocytes enriched from human PBMCs (PC23021) according to standard protocols, and treated with LNPs (3 ug/mL mRNA) for 4 d, in the presence or absence of maturation cytokines. Subsequently, DCs were stained with CD80, CD86, CD83, HLA-DR, CD14 and CD11c antibodies, along with fixable viability dye (FVD) and analyzed by flow cytometry. Cells were gated on FVD⁻ CD14⁺ CD11c⁺. Data in Figure 6 are shown as histograms of mode-normalized cell counts vs fluorescence of indicated marker. Data are representative of two independent experiments (PC23021 and PC23019) using different sources of human monocytes (PBMCs and commercially prepared frozen monocytes respectively) and different maturation cocktails.

[0348] Example 5: LNPs co-delivering D034/D097 and diabetes antigen mRNA increase tolerogenic T cell markers

[0349] To examine whether multi-cargo LNPs carrying D034/D097 and mRNA encoding diabetes-relevant antigen can induce antigen-specific tolerance *in vivo*, Treg frequency in NOD mice injected with LNPs was examined. Mice receiving a single injection of LNPs carrying D034/D097 and mRNA encoding diabetes-relevant antigen had a significant increase in splenic Foxp3⁺ and CTLA4⁺PD1⁺ CD4⁺ T cells 2 weeks post-injection (Figure 7) relative to vehicle injected NOD mice. In more detail, NOD/ShiLtJ female mice were injected intraperitoneally at 7.1 weeks of age with 5 ug LNP carrying Ins2 mRNA with/without immunomodulator prodrugs, Ova mRNA with/without immunomodulator prodrugs, or buffer (vehicle). Spleens were collected at 9.4 weeks of age for analysis of Foxp3⁺ CD4⁺ cells and markers of dysfunction (PD1, CTLA-4) by flow cytometry. Only a modest, non-significant increase was observed in mice injected with LNPs carrying Ova mRNA and D034/D097 or Ins2 mRNA only. These data indicate that while diabetes-relevant antigen and immunomodulator prodrugs may have slight tolerogenic effects, the

combination of the two components is synergistic and critical for the full effect and that the effect is antigen-dependent. Furthermore, no increase was observed in mice injected with LNPs carrying irrelevant antigen only (Ova mRNA). In addition, delivery of either Ova or Ins2 mRNA with a different immunomodulator composition (D053/D097) had minimal effect on T cell markers indicating that the choice of immunomodulator prodrugs is critical to inducing tolerance in this model of T1D.

[0350] Example 6: LNPs co-delivering immunomodulatory prodrugs and mRNA-encoded diabetes-relevant antigens limit the acute inflammatory response to LNP injections.

[0351] Part of what makes conventional mRNA-LNP systems effective vaccines is that they act as their own adjuvants by inducing acute proinflammatory immune responses that strengthen the subsequent development of an antigen-specific adaptive immune response. This acute inflammatory response is antigen-independent, and involves a non-specific, rapid response of immune cells to specific molecules in the LNP and the mRNA molecule itself (regardless of what the mRNA encodes). Notably, though base modifications to mRNA can greatly attenuate the acute immune response to the mRNA molecule, it is not completely diminished. The immune-stimulatory nature of conventional mRNA-LNP systems is undesirable for tolerance therapeutics as it could counteract any potential tolerogenic effect or even exacerbate an immune response to that antigen and exacerbate disease. Thus, this example examined whether co-loading of D034 and D097prodrugs (lipid conjugates) with mRNA can diminish their immune stimulatory nature and reduce the acute inflammatory response to LNP injection, by measuring serum cytokines at 0, 4 and 24 h following injection of LNPs formulated with Ova/multi-antigen mRNA and with/without immunomodulatory prodrugs D034 and D097 (Figure 8). In more detail, NOD/ShiLtJ female mice were injected intraperitoneally (5 µg/mouse) with LNP carrying 5moU modified Ova or multi-antigen mRNA with/without coloaded prodrugs D034/D097. Serum was collected at 0, 4 and 24 h post injection and cytokine/chemokine response measured via MesoScale Discovery multiplex assay. Data in Figure 8 represent mean + SD, n=3-5 per group. Mice injected with LNPs loaded with: multi-antigen mRNA + D034/D097, multi-antigen only, Ova mRNA+ D034/D097, Ova mRNA only or buffer only. Co-loading of immunomodulators attenuated the acute cytokine/chemokine response to Ova and multi antigen mRNA-LNPs in NOD mice, including MIP2, IL-6, MIP1α 4 hours post LNP injection.

[0352] Example 7: LNPs co-delivering D034/D097 and mRNA disease-relevant antigens induce antigen-specific protection in the NOD model of type 1 diabetes.

[0353] To determine whether LNPs carrying both D034/D097 and disease-relevant antigen encoded by mRNA could induce antigen-specific tolerance and protection from autoimmunity, NOD/ShiLtJ female mice were injected intraperitoneally from 5 to 15 weeks of age every 2 weeks with 5 ug of LNPs or buffer (vehicle) and monitored diabetes progression up to 30 weeks of age (Figure 9 and Table 9). Blood glucose was monitored 1-2x weekly for up to 30 weeks of age. Diabetes onset was defined as 2 consecutive blood glucose measures ≥ 20 mM. Mice were randomized to treatment groups, n=15/group. Mice injected with an LNP formulated with mRNA encoding multiple diabetes-relevant antigens (Multi) and D034/D097 were significantly protected from diabetes relative to vehicle-injected controls (P=0.002). By 30 weeks of age only 13.3% of mice treated with Multi+D034/D097 LNPs met the diabetes onset criteria, compared to 66.7% of vehicle-injected controls. Moreover, median diabetes onset in mice treated with Multi+D034/D097 LNPs could not be determined as only 2/15 of the mice turned diabetic by study endpoint, whereas median onset in vehicle-injected controls was 22.3 weeks of age.

[0354] Control mice treated with LNPs carrying Multi mRNA only were not protected from diabetes with a similar progression relative to vehicle-injected controls. Furthermore, Ova mRNA only and Ins2 mRNA only LNP treatment had a trend toward worsened diabetes progression relative to vehicle-injected controls. Thus, mRNA only LNPs were not protective and in some cases may accelerate disease onset, underlining the critical role of the immunomodulatory prodrug component (D034/D097) in mediating the protective effect of multi-cargo LNPs. In addition, Ova mRNA with D034/D097 prodrugs had only a slight trend toward protection relative to vehicle-injected control, indicating that prodrugs with irrelevant antigen are not sufficient for the full protective effect of our multi-cargo LNPs. This also highlights that the protection induced by Multi+D034/D097 LNPs is antigen specific. Thus, both antigen and immunomodulatory prodrugs are required for the tolerogenic effect of multi-cargo LNPs and these two components have a synergistic effect on delaying diabetes progression.

[0355] Table 9. Diabetes progression in NOD mice treated with multi-cargo LNPs

Group	# Mice Turned	% Diabetes (by 30 weeks)	Median Onset (age in weeks)	Onset Rate (%/week)*
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Vehicle	10	66.7	22.3	2.2
Multi+D034/D097	2	13.3	-	0.4
Multi	10	66.7	23.3	2.2
Ins2 + D034/D097	6	40.0	-	1.3
Ins2	14	93.3	21.3	3.1
Ova + D034/D097	8	53.3	29.0	1.8
Ova	11	73.3	21.3	2.4

[0356] Example 8:

[0357] To examine which routes of administration are efficacious for LNPs carrying D034/D097 and disease-relevant antigen encoded by mRNA, NOD/ShiLtJ female mice were injected with LNPs carrying both mRNA encoding multiple diabetes-relevant antigens (Multi, as described above) and 5 mol% each of D034/D097 (i.e. 5 mol% of D034 and 5 mol% of D097) via 5 different routes of administration: intravenous (i.v.), intraperitoneal (i.p.), subcutaneous (s.c.), intradermal (i.d.) and intramuscular (i.m.) (Figure 10) n=6-7/group. All routes of administration were compared to untreated NOD/ShiLtJ controls. Compared to Example 7, which used 6 injections, only 3 injections were used in this study, and the treatment started at a more advanced stage of autoimmune disease (injections at 9, 11 and 13 weeks of age). Blood glucose was monitored weekly and diabetes defined as 2 consecutive blood glucose measures > 20 mM and mice were monitored up to 30 weeks of age. As shown in Figure 10, all routes of administration were efficacious in treating diabetes, even though treatment was initiated in advanced stages of autoimmunity.

[0358] Example 9:

[0359] To test whether LNPs carrying D034/D097 and disease-relevant antigen encoded by mRNA could be used to treat established autoimmune disease in addition to prophylactic use, diabetic NOD/ShiLtJ female mice were treated with LNPs beginning at diagnosis of diabetes (2 consecutive blood glucose > 13 mM). NOD female mice were aged and allowed to spontaneously progress to diabetes onset at which point injections of LNP or buffer control was initiated. On the day of diagnosis (day 0), mice received subcutaneous insulin pellets which slowly release exogenous insulin for a duration of 14 days to temporarily normalize blood glucose levels. The

insulin therapy serves two functions: 1) to rest beta cells such that residual beta cells at diagnosis can be salvaged, and 2) to mimic the clinical new-onset type 1 diabetes patient population more closely, where patients are treated with exogenous insulin from diagnosis. The insulin pellets deplete by approximately 14 days and control of blood glucose levels from that point is attributed to endogenous beta cells; this time frame is validated by the return to hyperglycemia in controls. In addition to insulin pellets, on the day of diagnosis (day 0), mice received intradermal injections of either 5 µg mRNA in LNP carrying Multi mRNA and 1/9 mol% of D034/D097 respectively, or buffer (n=4/group) as follows: 3 injections within 10 days of diagnosis including day 0, and 5 injections occurring once weekly thereafter for a total of 8 injections. Blood glucose was tracked for 10 weeks post diagnosis and progression to overt diabetes defined as 2 consecutive blood glucose measures > 15 mM. The results are shown in Figure 11. All buffer-injected mice progressed to overt diabetes, most of them progressing rapidly after insulin pellet cessation. In contrast, 100% of LNP-treated mice remained normoglycemic for the duration of the study. Thus, in addition to prophylactic use, these data show that LNPs carrying relevant mRNA-encoded antigen and both D034/D097 can be used to treat established diabetes. These data also demonstrate that the intradermal route of administration is effective in this model.

[0360] Example 10:

[0361] Type 1 diabetes is a complex autoimmune disease including both CD8 and CD4 T cells. Therefore, to examine whether mRNA encoding both MHC Class I and Class II epitopes was necessary for efficacy of Multi+D034/D097 LNPs in the NOD mouse model of type 1 diabetes, several mRNA constructs were compared for prevention of diabetes in NOD mice following injection of LNPs carrying mRNA and 5 mol% each of D034/D097. NOD/ShiLtJ female mice were injected intramuscularly with 5 µg mRNA in LNP also carrying D034/D097 at 11.5 weeks of age, followed by a second intramuscular injection of the same formulation at 2.5 µg mRNA at 14.5 weeks of age. This represents treatment initiation at very advanced autoimmune stage but prior to diagnosis of disease. Control mice received buffer injections only. Blood glucose was monitored weekly for up to 38 weeks of age and diabetes defined as 2 consecutive blood glucose measures > 20 mM, n=6-8/group. This study was carried out longer than the typical 30 week endpoint to allow for assessment of the durability of tolerance following this low-dose, 2 injection treatment regimen. The results are shown in Figure 12. Mice receiving LNPs carrying D034/D097

and Multi mRNA—which carries both Class I and Class II epitopes (see design A-B-C-L1-D-E-F in Table 10, below)—had reduced diabetes incidence relative to buffer controls. mRNA encoding only Class I (design A-B-C-E-F in Table 10) or only Class II (design A-B-D-E-F in Table 10) epitopes were not effective. The order of Class I and Class II epitopes in the mRNA construct did not robustly alter efficacy (compare design A-B-C-L1-D-E-F versus A-B-D-L1-C-E-F). Collectively these data show that treatment of autoimmune disease involving both CD8 and CD4 components, such as diabetes, benefits from inclusion of both Class I and Class II epitopes in the mRNA. In addition, these data demonstrate that LNPs carrying D034/D097 and relevant antigen mRNA are effective at preventing and/or delaying diabetes with intramuscular injections initiated at a late stage of autoimmunity (11.5 weeks) and with only 2 total injections given 4 weeks apart and with a lower second dose.

[0362] Table 10: Table of mRNA constructs used in Examples 10-14

Disease model	Design	Open reading frame sequence	Protein sequence
Experimental autoimmune encephalitis (EAE) = Multiple sclerosis	A-B-D-E-F	ATGGCTTGTCTCTGGAGCTTTAGTTGGCCAAG CTGTTTTTGTCCCTGCTTCTCCTTCTCTTGC AACTGTCTTGTTCCTACGCACCTGGAAGAAC GCTACTGGCATGGAGGTTGGCTGGTATAGATC ACCTTTCAGTAGGGTCGTACACCTCTACCGGA ACGGCAAAGACCAGGACGCAGAGCAAGCTATT GTTGGCATCGTAGCTGGGCTTGCCGTGTTGGC TGTCGTGGTGATTGGCGCAGTTGTGGCAACCG TAATGTGTCGCCGGAAGAGCAGCGGCGGCAAG GGGGATCTTATTCTCAAGCAGCCTCCTCTGA CAGTGCCCAAGGGAGCGACGTTAGCCTCACAG CTTAA (SEQ ID NO: 153)	MACLWSFSWPSCFLS LLLLLLQLSCSYAPG KNATGMEVGWYRSPF SRVHLYRNGKDQDA EQAIVGIVAGLAVLA VVVIGAVVATVMCRR KSSGGKGSYSQAAS SDSAQGSVDVSLTA (SEQ ID NO: 154)
Experimental autoimmune thyroiditis (EAT) = Hashimoto's thyroiditis	A-B-D-E-F	ATGACAGCACTCGTGCTGTGGGTTAGTACCCT TCTCTCCTCTGTCTGTCTGTAGCAGCAGTGG ACCTCCTCATTTGGGGTTCTCAGGACGACGGG CTGATAAACCGAGCCAAGGCTGTCAAACAGTT TGAAGAAAGCCAGGGCCCTGGACCTGGCTCCG TGCCCTCAGCCGATAGCCCTCTCGTGCACTGC CTCACTGACTGCGCTAACGACGAAGCTTGTAG CTTCCTTACTGTTGGACCTGGACCAGGCGCTG CCACATCCAACCTTTTCTATGGCCCAGGACTTC TGCTTGCAACAGTGTCCAGACACCAGGATTG CTTGGTCACAACAGGCCAGGACCAGGCTTCA TGTATCACGTTCCCGAAAGTTACGGCCACGGA TCTCTGGAATTGCTCGCCGACGTGCAGTACGC TTTCGGAAGTGGTGGACCCGACCTGGGTGCC	MTALVLWVSTLLSSV CLVAAVDLLIGGSQD DGLINRAKAVKQFEE SQGPGPSVPSADSP LVQCLTDCANDEACS FLTVPGPGAATSNF SMAQDFCLQQCSRHQ DCLVTTGPGPGFMYH VPESYGHGSLELLAD VQYAFGLPGPGPGCH RDGHYQTVQCQTEGM CWCVDAQGREPGPG FATPWPDPFIPGAGGE SYKELSAQLPNRQGL

		ATCGCGACGGACATTACCAGACTGTACAGTGT CAGACCGAAGGCATGTGCTGGTGCCTAGACGC TCAGGGAAGAGAAGGACCAGGGCCTGGGTTTG CTACACCTTGGCCTGATTTTATACCAGGTGCC GGAGGCGAGTCTTACAAAGAATTGTCTGCTCA ACTCCCCAATCGCCAAGGTCTGAAGGGCCCTG GACCAGGGGATCTTGAGGTTGGCCCCGGCCTT GAGGAAGACCTCTCCGGGAGTCTTGAGCCCGT GCCTAAATCATACTCTAAAGGACCTGGACCCG GCTTCGAGCCAACCGGCTTTCAGAACGTCCTC AGTGGTTTGTACTCTCCAGTTGTTTTAGTGC AAGCGGCGCAAATTTGACAGACACACATACAT ATTGTCTTCTGGCCTGTGACAACGACGGACCT GGACCAGGCGGCATGGTTCCTAGGTCAGACTT TCCAGGGGATATGGCAACTGAATTGTTCTCCC CAGTCGATATAACCCAAGTGATAGTCGGCCCA GGACCAGGCTGTGAGACAGTGGGAATTACGC ACCCGTGCAGTGCAGCTGCAACGGGTCCAGT GCTGGTGCCTGGATACCGAGGGGATGGGACCC GGACCTGGGTGTGCCGACAGCCAAGGGAGGGA GCTCGCTGAAACCGGACTGGAGCTGCTGCTGG ACGAAATTTACGACACTATCTTTGCTGGACCT GGACCAGGCACTTGGCGGATACTCGATTGCGG CAGTGAAGATACAGAGGTTACACCTATCCCT TCGGCTGGTATCAGAAACCCGCTGTGGGACCC GGACCCGGACTGGCTGCTGCTGTCTGGTACTA CAGTTTGGAGCACTCTACTGACGACTACGCCA GTTTCAGCCGCGCCCTGGAAAACGCCGGCCCT GGACCTGGCGCTCAGTATCCAAGCCTCCAAGA CGTGCCCCAAGTTGTTTTGGAAGGGGCAACAA CCCCACCAGGAGAAAACATCTTCCTCATTGTA GGTATAGTAGCAGGTTTGGCTGTCTTGCCGT CGTTGTTATCGGGGCCGTAGTTGCAACCGTTA TGTGCAGGAGGAAATCTAGTGGGGGCAAAGGG GGCAGTTACTCCCAAGCAGCCTCTTCTGATTC CGCACAAGGGAGTGACGTAAGTCTCACAGCTT AA (SEQ ID NO: 155)	KGPGPGDLEVGPGLE EDLSGSLEPVPKSYS KGPGPGFEPTGFGQNV LSGLYSPVVSASGA NLTDHTYCLLACDN DGP GPGMVPRSDFP GMATELFSVDITQ VIVGPGPGCQDSGNY APVQCDLQVRQCWCV DEGMGPGPGCADSQ GRELAETGLELLLDE IYDTIFAGPGPTWR ILDGSEDTEVHTYP FGWYQKPAVGPGL AAAVWYYSLEHSTDD YASFSRALENAGPGP GAQYPSLQDVPQVVL EGATTPGENIFLIV GIVAGLAVLAVVIG AVVATVMCRRKSSGG KGGSYSQAASSDSAQ GSDVSLTA (SEQ ID NO: 156)
Delayed-type hypersensitivity (DTH) and gluten-induced enteropathy = Celiac disease	A-B-G-F	ATGAAGACATTTCTTATTCTCGTCTTGCTGGC CATAGTAGCAACAACCGCCACTACTGCTGTAA GGTTCCCCGTCCCTCAACTCCAGCCCCAAAAC CCCTCTCAGCAACAACCTCAAGAGCAAGTCCC CCTCGTCCAGCAGCAGCAATTCTCGGTCAAC AACAGCCATTTCTCTCCTCAACAACCATATCCT CAGCCACAGCCCTTCCCATCCAGCTTCCCTA TTTGCAACTTCAGCCTTTTCCCCAACCTCAAC TTCCCTACAGCCAGCCACAGCCATTCCGCCCA CAACAGCCTTACCCACAACCTCAACCACAGTA CTCCAGCCACAGCAGCCTATCTCACAGCAGC	MKTFLILVLLAIVAT TATTAVRFPVQLQP QNPSQQQPQEQVPLV QQQQLGQQQFPFPQ QPYQPQFPFSPQLPY LQLQFPFPQLPYSQ PQFPRPQPYQPQFP QYSQPQQPISQQQQQ QQQQQQQQQQQQIL QQILQQQLIPCMDVV LQQHNIAGRSQVLQ

		AGCAGCAGCAGCAACAGCAGCAGCAACAGCAA CAACAGCAACAACAAATACTCCAACAGATCTT GCAACAACAGCTCATCCCTTGTATGGACGTCG TGTTGCAGCAACATAATATAGCTCACGGCCGA AGCCAAGTCCTTCAACAGTCCACATATCAGCT GCTGCAAGAACTCTGCTGTCAGCATCTCTGGC AAATCCCCGAGCAAAGCCAGTGTCAGGCCATT CACAACGTGGTCCACGCTATCATCCTCCATCA ACAGCAGAAACAGCAGCAGCAACCCTCCAGCC AGGTCTCTTTCCAACAGCCTCTTCAGCAATAC CCATTGGGGCAGGGCAGCTTCAGACCTTCTCA GCAAAATCCACAGGCACAAGGCTCAGTGCAAC CACAGCAGCTGCCACAGTTTGAAGAAATCCGG AACCTCGCTTTGCAGACTCTTCTGCCATGTG CAACGTGTATATACCCCCTATTGTACTATCG CCCCCTTCGGGATTTTCGGAACCAACTAA (SEQ ID NO: 157)	QSTYQLLQELCCQHL WQIPEQSQCQAIHNV VHAIILHQQKQQQQ PSSQVSFQQPLQQYP LGQGSFRPSQQNPQA QGSVQPQQLPQFEEI RNLALQTLPAACNVY IPPYCTIAPFGIFGT N (SEQ ID NO: 158)
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[0363] Example 11:

[0364] To confirm that LNPs carrying D034/D097 and relevant antigen encoded by mRNA is also effective in other autoimmune diseases, the well-established MOG35-55 induced mouse model of experimental autoimmune encephalitis (EAE) was used, which is a model for multiple sclerosis. EAE was induced in C57BL/6 female mice on day 0 with an emulsion containing 200 ug MOG35-55 peptide in Complete Freund's Adjuvant and booster injections with 200 ng pertussis in PBS given on days 0 and 2. Following disease induction on day 0, mice were treated with 2 intramuscular injections delivering 5 µg myelin oligodendrocyte glycoprotein (MOG) mRNA (see design A-B-D-E-F in Table 10) in LNPs carrying 5 mol% each of D034/D097, or control LNPs or buffer at days 1 and 5 post disease induction and monitored for clinical signs of EAE. EAE signs were scored following well established methodology for this model for 19 days. Results for this example are shown in Figure 13. Data are mean +/- SEM, n=10/group. As shown in Figure 13, MOG mRNA combined with D034/D097 in LNP delayed and attenuated clinical signs of EAE in mice compared to vehicle (buffer) injected controls. Moreover, this effect was far more pronounced compared to treatment with LNPs carrying irrelevant Ova mRNA with D034/D097, and compared to MOG mRNA only in LNP, demonstrating that both MOG mRNA and immunomodulatory lipid conjugate components synergize to drive protection. Thus, as observed in the type 1 diabetes model, the combination of both relevant antigen mRNA and D034/D097 is important for eliciting strong immune tolerance in autoimmunity.

[0365] Example 12:

[0366] To further confirm that LNPs carrying D034/D097 and relevant antigen encoded by mRNA is effective in other autoimmune diseases, a mouse model of experimental autoimmune thyroiditis to model Hashimoto's thyroiditis was used. Experimental autoimmune thyroiditis was induced in SJL/J female mice by intravenous injections of 40 µg recombinant thyroglobulin (Tg) protein with LPS as adjuvant on days 0 and 7. Mice were treated with two intramuscular injections of either buffer control or 5 µg of a multi-epitope Tg mRNA construct (see design A-B-D-E-F in Table 10) in LNPs carrying 1 mol% D034 and 9 mol% D097 at days -5 and 10 post disease induction. Naive mice were not induced with Tg protein and did not receive LNP treatment. Thyroids and spleens were collected 28 days post disease induction. Immune infiltration of thyroid was quantified and scored by H&E staining of multiple non-serial thyroid sections per mouse and splenic Tregs (CD4+CD25+Foxp3+) were analyzed by flow cytometry as a percent of CD4+ T cells. For Figure 14 panel A, non-diseased mice did not receive Tg induction or buffer/LNP injections and were used as a baseline control. All other groups were induced with Tg. Statistical analyses performed by one way ANOVA, n=7-9 per group. Mice receiving LNPs carrying thyroglobulin (Tg) mRNA and D034/D097 had significantly reduced immune thyroid infiltration compared to buffer-treated controls and compared to mice treated with either Tg mRNA only LNPs or irrelevant antigen (Ova) +D034/D097 LNP (Figure 14 panel A). For Figures 14 panel B, statistical analyses were done by one-way ANOVA with Dunnet test, n=6/group. The frequency of splenic Tregs was increased by treatment of Tg mRNA combined with D034/D097 in LNP compared to untreated controls (Figure 14 panel B). Treatment with Tg mRNA only LNP or irrelevant Ova mRNA with D034/D097 LNP did not increase Treg frequency (Figure 14 panel B), indicating this to be an antigen-dependent effect requiring both antigen and immunomodulatory lipid components, similar to the results obtained in EAE and type 1 diabetes models.

[0367] Example 13:

[0368] To further confirm LNPs carrying D034/D097 and relevant antigen encoded by mRNA for other autoimmune diseases, a mouse model of delayed hypersensitivity reaction to the antigen gliadin is used to model celiac disease in humans. C57BL/6J female mice, fed a gluten-free diet, are sensitized by subcutaneous injection of 100 µg gliadin emulsified in Complete Freund's adjuvant on day 0. Mice are treated with 3 intramuscular injections of either buffer, or 5 µg of a

full-length alpha gliadin mRNA construct (see design A-B-G-F in Table 10) with D034/D097 in LNP, or control LNP at days -1, 5 and 8 post gliadin-sensitization. Control LNPs include LNP containing gliadin mRNA without D034/D097, and/or LNP containing irrelevant antigen with and without D034/D097. Mice are challenged with a 10 µg intradermal gliadin or irrelevant antigen ovalbumin in the pinna on day 14 and pinna thickness before and 24 h post challenge are measured to determine ear swelling. Mice treated with LNP carrying gliadin mRNA and D034/D097 will have reduced ear swelling and increased splenic Treg frequencies compared to mice treated with control LNP.

[0369] Example 14:

[0370] A mouse model of gluten-induced enteropathy is used to model celiac disease in humans. C57BL/6J female mice fed a gluten-free diet are sensitized by injection of 100 µg gliadin emulsified in Complete Freund's adjuvant at the base of the tail followed by a booster injection of 50 µg gliadin emulsified in Incomplete Freund's adjuvant 15 days apart. Memory CD4⁺ T cells are isolated from the spleens of these mice 1 month after sensitization and adoptively transferred to immunocompromised Rag1 knockout female mice (also fed a gluten-free diet) by intraperitoneal injection of 3×10^5 memory T cells. Gluten-induced enteropathy is induced in adoptive transfer recipient mice by placing them on gluten-containing diet on day 0 (day of adoptive transfer). As described, exposure to gluten-containing diet in this model elicits an effector response in the adoptively transferred, gliadin-memory T cells, causing enteropathy and weight loss. Mice are subjected to 3 total intramuscular injections of either buffer, or 5 µg of a full-length alpha gliadin mRNA construct (see design A-B-G-F in Table 10) with D034/D097 in LNP, or control LNP on week 1, 2 and 3 post adoptive transfer. Naïve controls remain on gluten-free diet throughout the study. Body weight is monitored for 8 weeks post diet, at which point spleens are collected for flow cytometry analysis of Tregs and intestines collected and assessed for pathology by histological examination. Gluten-fed mice treated with LNPs carrying gliadin mRNA and D034/D097 will have attenuated disease signs (attenuated weight loss and enteropathy) and increased splenic Treg frequencies. This effect will not be phenocopied in gluten-fed mice receiving either irrelevant antigen mRNA and D034/D97 containing LNPs or gliadin mRNA only LNPs, which will not significantly attenuate disease or increase Tregs relative to buffer-injected gluten-fed control mice.

[0371] Although the invention has been described and illustrated with reference to the foregoing examples, it will be apparent that a variety of modifications and changes may be made without departing from the invention.

CLAIMS

1. An immunomodulatory combination comprising:

a first lipid conjugate comprising a first immunomodulatory agent covalently linked to a first lipophilic moiety by a first cleavable linkage or through a first cleavable linker, wherein the first immunomodulatory agent is a corticosteroid;

a second lipid conjugate comprising a second immunomodulatory agent covalently linked to a second lipophilic moiety by a second cleavable linkage or through a second cleavable linker, wherein the second immunomodulatory agent is an mTOR/mTORC inhibitor; and

one or more nucleic acids encoding one or more epitopes targeted by autoreactive T cells, wherein the one or more epitopes comprise one or more class II epitopes, optionally wherein the one or more epitopes are from a plurality of autoantigens and/or antigens that trigger recognition of autoantigens by autoreactive T cells in an autoimmune disease or condition;

wherein the first lipid conjugate, the second lipid conjugate, and the one or more nucleic acids are co-formulated together in a delivery vehicle, or are formulated in two or three separate delivery vehicles.

2. The immunomodulatory combination of claim 1, wherein:

the mTOR/mTORC inhibitor is sirolimus, everolimus, temsirolimus, zotarolimus, ridaforolimus, deforolimus, umirolimus, or DL001, 3HOI-BA-01, 4EGI-1, ABTL-0812, Apitolisib (GDC-0980), Astragaloside IV, AZD8055, BGT226 (NVP-BGT226) maleate, Bimiralisib (PQR309), CC-115, Chrysophanic Acid, Compound 401, CZ415, Dactolisib (BEZ235), DHM25, ETP-46464, GDC-0349, Gedatolisib (PKI-587), GNE-477, GNE-493, GSK1059615, JR-AB2-011, KU-0063794, Lanatoside C, MHY-1685, MTI-31, mTOR inhibitor-1, Nitazoxanide, NU7441 (KU-57788), Omipalisib (GSK2126458), Onatasertib (CC 223), OSI-027, Palomid 529 (P529), Paxalisib (GDC-0084), PF-04691502, PI-103, PP30, PP121, PQR620, Samotolisib (LY3023414), Sapanisertib (MLN0128), SF2523, Tacrolimus (FK506), Torin 1, Torin 2, Torkinib (PP242), Vistusertib (AZD2014), Voxelisib (XL765), VS-5585 (SB2343),

W922, WAY-600, WYE-125132 (WYE-132), WYE-354, or WYE-687, optionally sirolimus; and/or

the corticosteroid is alclometasone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, fluclorolone, fludrocortisone, flugestone, flumetasone, flunisolide, fluocinolone, fluocinonide, fluocortin, fluorometholone, fluperolone, fluprednisolone, flurandrenolide, fluticasone, formocortal, halcinonide, halometasone, hydrocortisone, loteprednol, medrysone, meprednisone, methylprednisolone, mometasone, paramethasone, prebediolone acetate, prednicarbate, prednisolone, prednisone, prednylidene, rimexolone, tixocortol, triamcinolone, or ulobetasol, optionally dexamethasone.

3. The immunomodulatory combination of claim 1 or 2, wherein the lipid conjugate has a cLogP value of 5-20, 6-18, 9-18, or 9-17.

4. The immunomodulatory combination of any one of claims 1 to 3, wherein the delivery vehicles are lipid nanoparticles and/or liposomes, optionally wherein the delivery vehicles are lipid nanoparticles, wherein the one or more nucleic acids are entrapped within a lipid nanoparticle or liposome that comprises a lipid that has or is ionizable to produce a positive net charge.

5. The immunomodulatory combination of claim 4, wherein the lipid conjugates in the lipid nanoparticles are 5-20%, 7-15%, 9-12%, or about 10% of the total lipids, optionally wherein the ratio of the first lipid conjugate to the second lipid conjugate is 1:9, 9:1, or 5:5.

6. The immunomodulatory combination of any one of claims 1 to 5, wherein the one or more epitopes further comprises one or more class I epitopes, optionally wherein the one or more class I epitopes comprise a plurality of class I epitopes, each class I epitope separated by a class I linker peptide, optionally wherein the class II epitopes are separated from the class I epitopes by a class II linker.

7. The immunomodulatory combination of any one of claims 1 to 6, wherein the one or more class II epitopes comprise a plurality of class II epitopes, each class II epitope separated by a class II linker peptide.

8. The immunomodulatory combination of any one of claims 1 to 7, wherein the plurality of epitopes comprises a full length antigen.

9. The immunomodulatory combination of any one of claims 1 to 8, wherein the one or more nucleic acids comprises a first nucleic acid encoding one or more class I epitopes, optionally wherein the class I epitopes are separated by class I linker peptides, and a second nucleic acid encoding one or more class II epitopes, optionally wherein the class II epitopes are separated by class II linker peptides.

10. The immunomodulatory combination of any one of claims 1 to 9, wherein each of the one or more nucleic acids is an mRNA independently configured according to one of the following formulas:

A-B-C-L¹-D-L²-E-F (Formula IIa)

A-B-D-L¹-C-L²-E-F (Formula IIb)

A-B-C-L²-E-F (Formula IIc)

A-B-D-L²-E-F (Formula IId)

A-B-C-L¹-D-F (Formula IIe)

A-B-D-L¹-C-F (Formula II f)

A-G-F (Formula IIg)

A-B-C-F (Formula IIh)

A-G-L²-E-F (Formula Iii)

A-B-D-F (Formula IIj)

A-B-G-F (Formula IIk)

A-B-G-L²-E-F (Formula III)

wherein:

A is a 5' untranslated region (5'-UTR);

B is a signal peptide, optionally a signal peptide from the same antigen/autoantigen as one of the one or more epitopes;

L¹ is a cleavable or non-cleavable linker, optionally a class II linker, optionally GPGPG (SEQ ID NO: 26);

C encodes one or more class I epitopes, each class I epitope separated by a class I linker peptide and optionally flanked on both sides by 0-25 flanking amino acids;

L² is an optional linker;

D encodes one or more class II epitopes, each class II epitope separated by a class II linker peptide and optionally flanked on both sides by 0-25 flanking amino acids;

E encodes a polypeptide comprising an endolysosomal trafficking sequence motif, optionally an MHC-I trafficking domain (MITD), invariant chain (CD74), LAMP1, LAMP2, or DC-LAMP;

F is a 3' untranslated region (3'UTR) further comprising a polyadenine tail; and

G is a full length antigen/autoantigen.

optionally wherein C comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class I epitopes, and D comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 class II epitopes.

11. The immunomodulatory combination of any one of claims 1 to 10, wherein the one or more epitopes is a plurality of epitopes, and wherein each epitope in the plurality of epitopes is an epitope of an autoantigen targeted by autoreactive T cells in type 1 diabetes (T1D),

optionally wherein the plurality of epitopes comprises full-length and/or epitopic fragments from one or more, optionally two or more, of the following: preproinsulin, mature insulin components (A chain, B chain, and/or C-peptide), glutamic acid decarboxylase 65 (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2β), Zinc transporter 8 (ZnT8), heat shock protein 60 (Hsp60), and hybrid insulin peptides (HIPs),

optionally wherein the epitopes are epitopic fragments from autoantigens selected from the group consisting of: preproinsulin, glutamic acid decarboxylase 65 (GAD65), islet-specific

glucose-6-phosphatase catalytic subunit-related protein (IGRP), islet antigen 2 (IA-2), islet antigen 2 beta (IA-2 β), zinc transporter 8 (ZnT8), and hybrid insulin peptides (HIPs).

12. The immunomodulatory combination of any one of claims 1 to 9, wherein each of the one or more epitopes comprises an epitope of one or more antigens/autoantigens targeted by autoreactive T cells in one of the following:

Multiple Sclerosis (MS), optionally wherein the one or more antigens/autoantigens comprises one or more of myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin basic protein (MBP), and/or α B-crystallin;

celiac disease (CD), optionally wherein the one or more antigens/autoantigens comprises gliadin;

autoimmune thyroiditis, optionally Hashimoto's thyroiditis, optionally wherein the one or more antigens/autoantigens comprises one or both of thyroid peroxidase and/or thyroglobulin;

vitiligo, optionally wherein the one or more antigens/autoantigens comprises one or more of Pmel17, Melanin-Concentrating Hormone Receptor 1, and/or Tyrosinase; and

inflammatory bowel disease (IBD), optionally wherein the one or more antigens/autoantigens comprises flagellin.

13. A method for treating a subject having an autoimmune disease involving autoreactive T cells, the autoreactive T cells comprising both CD4⁺ T cells and CD8⁺ T cells, the method comprising administering the immunomodulatory combination as defined in any one of claims 1 to 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially.

14. A method for treating a subject having type 1 diabetes comprising administering the immunomodulatory combination as defined in any one of claims 1 to 11 to the subject, wherein the one or more nucleic acids encoding the plurality of epitopes, and the lipid conjugate(s) are administered together or sequentially.

15. A method for treating Multiple Sclerosis (MS) in a subject, comprising administering the immunomodulatory combination as defined in any one of claims 1 to 10 and 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the

lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in MS, optionally wherein the one or more autoantigens comprise one or more of myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin basic protein (MBP), and/or α B-crystallin.

16. A method for treating celiac disease in a subject, comprising administering the immunomodulatory combination as defined in any one of claims 1 to 10 and 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in celiac disease and/or antigens that trigger recognition of autoantigens by autoreactive T cells in celiac disease, optionally wherein the one or more autoantigens comprise gliadin.

17. A method for treating thyroiditis in a subject, comprising administering the immunomodulatory combination as defined in any one of claims 1 to 10 and 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in thyroiditis, optionally wherein the one or more autoantigens comprise one or both of thyroid peroxidase and/or thyroglobulin.

18. A method for treating vitiligo in a subject, comprising administering the immunomodulatory combination as defined in any one of claims 1 to 10 and 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in vitiligo, optionally wherein the one or more autoantigens comprise one or more of Pmel17, Melanin-Concentrating Hormone Receptor 1, and/or Tyrosinase.

19. A method for treating inflammatory bowel disease (IBD) in a subject, comprising administering the immunomodulatory combination as defined in any one of claims 1 to 10 and 12 to the subject, wherein the one or more nucleic acids encoding the one or more epitopes, and the lipid conjugate(s) are administered together or sequentially, wherein each of the one or more epitopes comprises an epitope of one or more autoantigens targeted by autoreactive T cells in IBD

and/or antigens that trigger recognition of autoantigens by autoreactive T cells in IBD, optionally wherein the one or more autoantigens comprise flagellin.

20. An mRNA construct comprising:

5'UTR sequence;

a sequence encoding a signal peptide;

a multi-epitope coding sequence encoding a multi-epitope polypeptide, the multi-epitope polypeptide comprising three or more epitopes independently selected from the group consisting of: Class I epitope of Insulin B chain, Class I epitope of GAD65, Class I epitope of IGRP, Class I epitope of ZnT8, Class II epitope of Insulin B chain, Class II epitope of GAD65, Class II epitope of HSP60, Class II epitope of IA-2, Class II epitope of IA-2beta, and Class II epitope of ZnT8, wherein epitopes in the multi-epitope polypeptide are separated from each other by cleavable or noncleavable linkers, optionally wherein class I epitopes are separated by class I linker peptides and class II epitopes are separated by class II linker peptides, optionally wherein class I and class II linkers are separated by a class II linker, optionally wherein each epitope is flanked on both sides by 0-25 flanking amino acids, optionally wherein the multi-epitope comprises a signal peptide, and wherein the epitopes comprise epitopes from a plurality of antigens/autoantigens;

3'UTR sequence;

polyadenine tail; and

optionally, a sequence encoding a polypeptide comprising an endolysosomal trafficking sequence motif positioned 5' to the 3'UTR sequence.