(51) **International Patent Classification:**
A61K 39/00 (2006.01) *A61K 31/593* (2006.01)
A61P 3/10 (2006.01) *A61K 38/28* (2006.01)
A61K 31/592 (2006.01)

(21) **International Application Number:**
PCT/SE2015/050651

(22) **International Filing Date:**
4 June 2015 (04.06.2015)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
1450678-6 4 June 2014 (04.06.2014) SE
1451315-4 4 November 2014 (04.11.2014) SE

(71) **Applicant: DIAMYD MEDICAL AB** [SE/SE]; Kungs-gatan 29, S-111 56 Stockholm (SE).

(72) **Inventors: ESSEN-MÖLLER, Anders**; C/o Diamyd Medical AB, Kungsgatan 29, S-111 56 Stockholm (SE). **LUDVIGSSON, Johnny**; C/o Linköpings Universitet, S-581 83 Linköping (SE).

(74) **Agents: ZACCO SWEDEN AB** et al.; P.O. Box 5581, Valhallavägen 117N, S-114 85 Stockholm (SE).

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

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report:**
28 January 2016

---

(54) **Title:** NOVEL COMBINATIONS FOR ANTIGEN BASED THERAPY

(57) **Abstract:** The present invention relates to a method for prevention and/or treatment of an autoimmune disease, comprising ad-ministering a composition, said composition comprising at least one beta cell autoantigen, to a subject The subject may have a serum vitamin-D level above 50 nanomole/liter or the composition may be administered by intralymphatic injection or injection directly into a lymph node, or over a period of weeks, months, or years. The invention also relates to a composition comprising a plurality of particles, each having immobilised on its surface at least one first and at least one second antigen, wherein the first antigen is a beta cell autoantigen, and the second antigen is either a tolerogen or a beta cell autoantigen, andto composition comprising i) at least one beta cell autoantigen, and at least one of iia) an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and iib) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor. The invention also relates to pharmaceutical kits and to medical use of beta cell autoantigens.

Novel Combinations for Antigen Based Therapy

<u>Cross-reference to related applications</u>

This international application claims priority from Swedish patent application 1450678-6, filed on 4 June 2014, and Swedish patent application 1451315-4, filed on 4 November 2014, which are hereby expressly incorporated by reference in their entirety.

<u>Field of the invention</u>

The present invention generally relates to the field of immunology and in particular immunotherapy. More particularly, the present invention pertains to the prevention and/or treatment of autoimmune diseases such as type 1 diabetes or autoimmune diabetes. The present invention provides compositions and combinations which are particularly useful in the prevention and/or treatment of such disease. Also provided are methods for combination therapies for treatment and/or prevention of autoimmune disease.

<u>Background of the invention</u>

The immune defence system, like many defence systems consists of numerous and mostly interactive parts. There is the innate system, that basically is thought to react via pattern recognition receptors, such as Toll-like receptors, to rather well-known and common microbial antigens (ag); and there is the acquired system which can adapt to respond to a large variety of foreign invaders and moreover create and maintain memory cells that quickly react in the event new encounters occur with the same ag.

In autoimmune disease, the adaptive immune system commonly reacts to at least one self-antigen, (auto-antigen, aag). Although not fully understood, autoimmunity may be triggered in several ways. Examples of discussed possible triggers include but are not limited to innate responses to viruses, diets, vitamins, stress, microbiomes, vaccines, antibiotics and other drugs. Genetic disposition is an additional important factor.

In the case of endogenously produced autoantigens, these are processed inside a cell and peptides thereof are transported to the cell surface in association with MHC-I molecules and presented to various cells including naïve CD8+ Tcells; already mature cytotoxic CD8+ Tcells; and/ or CD8+ memory effector Tcells. To activate a naïve CD8+ Tcell to mature into a cytotoxic Tcell, a secondary co-stimulatory signal associated with CD28 is needed.

An autoantigen that is not inside a cell is normally taken up by an Antigen Presenting Cell (APC) such as a Dendritic Cell (DC) or macrophage, and processed peptides will be transported in association with MHC-II molecules and presented to naïve, mature or memory CD4+ Tcells. Also here is a second co-stimulation signal involving CD28 needed to activate the naïve CD4+ Tcell into a form of Thelper (Th) cell. Usually two forms of Th cells are discussed: Th1; Th2.

What type of Th cell that is generated upon the encounter between the APC-MHCII-aag complex and the CD4+ Tcell receptor (TCR) depends on several factors including the maturity of the DC and the

surrounding milieu, including but not limited to availability of IL-10, at the time of presentation. Whereas a Th1 cell secrets inflammatory cytokines including IFNg, and stimulates an inflammatory cell mediated response, Th2 cells typically secret IL4 and stimulate a humoral response where BCs produce non-cytolytic and neutralizing antibodies. Antibodies for "coating" and complement fixing (antibody dependent cellular toxicity) are usually stimulated by the Innate system.

Each B-lymphocyte (BC) expresses its unique and specific B cell receptor (BCR) in the form of an immobilized antibody molecule. Whereas TCs recognize cognate ag – as a processed peptide in the context of an MHC molecule, B cells recognize antigens in their native form (such unprocessed antigens do not normally interact with T cell activation), and with help from mostly Th2 signalling they differentiate into short lived antibody producing plasma cells, 10% of which are thought to become long-lived antigen-specific memory BCs.

It is commonly thought in the field, that insulin and insulin associated molecules, are early molecules to be involved in the process that leads to autoimmune diabetes including type 1-diabetes and latent autoimmune diabetes in the adult. Large clinical trials have been performed and are currently being performed using different insulin derived formulations in order to induce tolerance and stop or prevent the disease progression. Many other beta cell antigen molecules and peptides thereof including but not limited to GAD65 have been tried for the same purpose, and others, not limited to insulin B-chain and pro-insulin and variations thereof, are being prepared for clinical development.

During development of the immune system in man, self-antigen reactive lymphocytes are thought to be clonally deleted to discriminate self from non-self. Non-self reactive T lymphocytes are saved and go on to differentiate into naïve CD4+ or CD8+ Tcells, that can be activated upon encounter with their specific antigen. Some self-reactive lymphocytes do however escape the deletion process. More so if the self-antigen is sequestered like for example inside a cell. In the case of insulin however, as an example of an abundant molecule, relatively few insulin-reactive lymphocytes do escape the deletion process and as a consequence relatively few naïve insulin reactive CD4+ or CD8+ Tcells exist.

Naturally occurring abundant molecules, such as for example insulin, are not usually adsorbed and presented by DCs unless this process is initiated by activated CD8+ T cells and/or TLR-signalling. Therefore, and what may not have been noted in the field, the autoimmune process leading to diabetes may in many cases be initiated by escaped CD8+ naïve insulin reactive lymphocytes that recognize aag directly associated with MHC class-1 on cells of the target organs. In the case of the genetically predisposed, an insulin reactive "escaped" naïve CD8+ thymocyte can accordingly trigger a an inflammatory process including generation of CD8+ cytotoxic cells which in turn may induce cytokines that alert the innate system including raising causing BCs to produce insulin antibodies. Some beta cells will succumber and release sequestered antigens, such as for example GAD65, for which an abundant amount of naïve CD4+ and CD8+ TCs exist. GAD65 may in addition have commonalities with certain viral structures that further stimulates TLR-like signalling.

While the autoimmune reaction processes, counter inflammatory mechanisms develop, such as for example expression of CTLA4 molecules and IL-10 secretion. Since in the case of insulin, the ADC-MHCII contribution to the emerging autoimmune disease is weak, such compensatory mechanisms induce tolerance to insulin in an autoantigen-specific manner. GAD molecules however is taken up, processed DCs and GAD peptides are presented to naïve CD4+TCs, activating them into Thelper

cells. Thus a transcient immunity reaction to insulin is replaced by a more robust reaction towards GAD. This explains why in many genetically predisposed small children the first autoantibody often is to insulin, and why such antibodies often disappear as disease progresses.

Mocellin, Cytokine Growth Factor Rev. 2004, The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle, describes how Interleukin-10 (IL-10) is a pleiotropic cytokine that modulates the function of several adaptive immunity-related cells. Although generally considered an immunosuppressive molecule, IL-10 possesses immunostimulatory properties in vivo models. As an example locally produced IL-10 by pancreatic islet cells stimulate progression of autoimmunity in the NOD mouse, whereas systemic administration may have a beta cell preserving and anti-inflammatory effect. A summary on the relationship between IL-10 and infectious diseases, autoimmunity, allergy, cancer and transplantation involving adaptive immunity is provided.

Russel et al, Islets. 2014, The impact of anti-inflammatory cytokines on the pancreatic β-cell, more recently presented evidence that anti-inflammatory molecules such as interleukin (IL)-4, IL-10 and IL-13 can exert a direct influence of β-cell function and viability and that the circulating levels of these cytokines may be reduced in type 1 diabetes and proposed that targeting of anti-inflammatory pathways might offer therapeutic potential in type 1-diabetes.

Calcinaro et al, Diabetologia. 2005, Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse, concluded thatearly oral administration of probiotic bacteria prevented diabetes development in NOD mice and was associated with increased IL-10 expression in the pancreas, where IL-10-positive islet-infiltrating mononuclear cells were detected.

Van Dongen et al, Int J Cancer. 2010 Aug 15;127(4):899-909. doi: 10.1002/ijc.25113. Anti-inflammatory M2 type macrophages characterize metastasized and tyrosine kinase inhibitor-treated gastrointestinal stromal tumors (GIST). reports that tumors treated with the tyrosine kinase inhibitors imatinib and sunitinib,induced secretion of anti-inflammatory IL-10 in macrophage cultures, indicating that treatment with these inhibitors might contribute to an immune suppressive microenvironment in GIST. Overall, data revealed GIST as an active site of tumor-immune interaction in which suppressive mechanisms overrule potential antitumor responses. Tyrosine kinase inhibitors might promote this negative balance.

Louvet et al, Proc Natl Acad Sci U S A. 2008, Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice,reports how tyrosine kinase (TK) inhibitors offer opportunities for the treatment of autoimmune diseases. Treatment with imatinib (Gleevec) prevented and reversed T1D in NOD mice. Similar results were observed with sunitinib (Sutent). Another TK inhibitor, PLX647 showed only marginal efficacy whereas a soluble form of platelet-derived growth factor receptor (PDGFR), PDGFRbetaIg, rapidly reversed diabetes. Imatinib treatment led to durable remission and long-term efficacy and tolerance is likely to depend on inhibiting a combination of tyrosine kinases supporting the use of selective kinase inhibitors for the treatment of T1D.

Agostinoet al, J Oncol Pharm Pract. Epub 2010, Effect of the tyrosine kinase inhibitors (sunitinib, sorafenib, dasatinib, and imatinib) on blood glucose levels in diabetic and nondiabetic patients in general clinical practice, describes how tyrosine kinase inhibitors (TKIs) influenced the blood

glucose (BG) concentrations in patients treated with dasatinib, imatinib, sorafenib, and sunitinib. All showed significant declines in BG. Although the mechanism for the hypoglycemic effect of these drugs is unclear, c-kit and PDGFRβ were common target kinases.

Adorini, Ann N Y Acad Sci. 2003, Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes, reports that 1,25-Dihydroxyvitamin D(3) induces DCs with a tolerogenic phenotype, characterized by decreased expression of CD40, CD80, and CD86 co-stimulatory molecules, low IL-12, and enhanced IL-10 secretion. Treatment with 1,25-(OH)(2)D(3) induced tolerance to mouse islet allografts associated with impaired development of type 1 CD4(+) and CD8(+) cells and an increased percentage of CD4(+)CD25(+) regulatory cells and also inhibited diabetes development at non-hypercalcemic doses, suggesting Vitamin D as an approach for treatment of type 1-diabetes.

Heine et al, Eur J Immunol. 2008, 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells, reports that 1,25-dihydroxyvitamin D(3) (calcitriol) inhibits expression of IgE by B cells and enhances expression of IL-10 by dendritic cells and T cells. The molecular link in activated B cells between vitamin D signaling, expression of IL-10, and their ability to produce calcitriol from its precursor, suggest that 25-hydroxyvitamin D(3)) can be used as a modulator of immune responses.

Niiro et al, Biochem Biophys Res Commun. 1998, MAP kinase pathways as a route for regulatory mechanisms of IL-10 and IL-4 which inhibit COX-2 expression in human monocytes, describes how mitogen-activated protein kinases (MAPKs) are activated and play an important role in regulating the expression of pro-inflammatory molecules in monocytes/macrophages. Lipopolysaccharide (LPS)-stimulated human monocytes induce COX-2 protein and COX-2 mRNA expression as well as the signal-regulated protein kinase (ERK)2 and p38 MAPK in monocytes. The induction of COX-2 mRNA, COX-2 protein, and prostaglandin (PG)E2 by LPS was inhibited by the specific inhibitors of ERK and p38 MAPK. Interleukin (IL)-10 similarly inhibited COX-2 expression. LPS-induced phosphorylation and activation of ERK2 and p38 MAPK were significantly inhibited by IL-10, suggesting that the inhibition by IL-10 of the LPS-induced expression of pro-inflammatory molecules could be ascribed to the regulatory effects of both cytokines on MAPK activation.
Obermajer et al, Blood. 2011, Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells, describes how dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs) show opposing roles in the immune system.

Cyclooxygenase 2 (COX2) is the key regulator of PGE(2) synthesis and a determining factor in redirecting the development of CD1a(+) DCs to CD14(+)CD33(+)CD34(+) monocytic MDSCs. Exogenous PGE(2) and such diverse COX2 activators as lipopolysaccharide, IL-1β, and IFNγ all induce monocyte expression of COX2, blocking their differentiation into CD1a(+) DCs and inducing endogenous PGE(2), IDO1, IL-4Rα, NOS2, and IL-10, typical MDSC-associated suppressive factors. The disruption of COX2-PGE(2) feedback using COX2 inhibitors or EP2 and EP4 antagonists suppresses the production of MDSC-associated suppressive factors and the CTL-inhibitory function of fully developed MDSCs from cancer patients. The central role of COX2-PGE(2) feedback in the induction and persistence of MDSCs highlights the potential for its manipulation to enhance or suppress immune responses in cancer, autoimmunity, or transplantation.

Lieb, Med Hypotheses. 2007, Antidepressants, prostaglandins and the prevention and treatment of cancer, reports that a putative mechanism of carcinogenesis are up-regulation of cyclooxygenase, the synthesis and expression of oncogenes, viral activation, signal disruption, failed apoptosis, tumor initiation and promotion, angiogenesis, metastasis, immunosuppression, telomerase activity

5   andautoimmunity. All are regulated by prostaglandins. Observable and radiographic regression of cancer has been documented in patients taking non-steroidal, anti-prostaglandin drugs such as indomethacin and ibuprofen.

Kim, et al, Immune Netw. 2010, Cyclooxygenase Inhibitors, Aspirin and Ibuprofen, Inhibit MHC-restricted Antigen Presentation in Dendritic Cells, reports that NSAIDs have immunomodulatory

10   effects on T and B cells and that ibuprofen inhibit MHC class I and class II-restricted presentation of antigen in dendritic cells (DCs). Ibuprofen did not inhibit the phagocytic activity of DCs, the expression level of total MHC molecules and co-stimulatory molecules on DCs. Ibuprofen rather increased the expression level of total MHC molecules and co-stimulatory molecules on DCs. The results demonstrate that ibuprofen inhibits the intracellular processing of the phagocytosed antigen,

15   and suggest that prolonged administration of NSAIDs in high doses may impair the capability of DCs to present antigens in association with MHC molecules.

Gribben et al, Immunology 1994, CTLA4 mediates antigen-specific apoptosis of human T cells, describes how the CTLA4 molecule is a Tcell restricted molecule induced by TCR or CD28 activation. During an ongoing immune response there is a balance between proinflammatory and non-

20   proinflammatory signals. Cross-linking of CD28 or the common binding region of CD28/CTLA4 by mabs or the natural ligands B7-1 and 2 provides a positive costimulatory signal resulting in proinflammatory IL-2 upregulation. In fact crosslinking of CTLA4 can provide a weak costimulatory signal to CD28. After Tcell activation CD28 expression is down-regulated and CTLA4 up-regulated. At such times crosslinking of CTLA4 in the absence of CD28 costimulation may induce deletion of

25   previously activated Tcells, which indicates that CTLA4 can both costimulate and induce deletion depending on the activation state of the Tcell.

Posadas et al, Clinical Immunology 2009, Abatacept in the treatment of rheumatoid arthritis, describes that in rheumatoid arthritis (RA), Tcells and several other cells including dendritic cells DCs, macrophages and fibroblasts express markers of activation such as CD28 and cytotoxic T

30   lymphocyte antigen 4 (CTLA4). In the peripheral blood naïve Tcells need two signals to become activated to their full functional potential: i) an antigen in the context of an MHC molecule on the antigen presenting cell (APC) presented to the corresponding antigen specific Tcell receptor (TCR) on the Tcell; and ii) ligation of CD28 on the Tcell with CD80/86 on APCs. Stimulation of naïve Tcells by cognate antigen without costimulation results in Tcell anergy, whereas ligation of costimulatory

35   molecules on the Tcells in the absence of cognate antigen has no effect on the Tcell.

One of the surface molecules that are upregulated on the activated Tcell after successful stimulation with the two signals is CTLA4. It binds to CD80/80 with higher avidity than CD28. This not only blocks CD28 from binding but CTLA4 also induces inhibitory signals into the newly activated Tcell.

Abatacept (an Fc modified CTLA4 immunoglobulin) is a Tcell depleting, immunomodulating, fusion

40   protein consisting of the extracellular portion of human CTLA4 and the heavy chain of human IgG1. It blocks the costimulatory signal involved in activation of naïve Tcells. Its ligation with CD80/86 on APCs may also interfere with and reduce CD80/86 induced IL-6, which may downregulate

inflammatory cytokines such as IL-1beta, IFNgamma, and IL-17. Further Abatacept ligation with CD80/86 may induce indoleamine dioxygenase (IDO) in APCs, which may in turn may induce anergy in Tcells, as well as downregulate paracrine activation of naïve Tcells by activated Tcells.

Contradictory opinions have been presented regarding Abatacepts ability to influence the recall, restimulation, of memory CD4+ Tcells. CD80/86 expressed on B cells may yet be a further path where abatacept may carry out an immunomodulating function.

Patakas et al, abstract #723 ACR/ARHP, 2013, Abatacept is Highly Effective At Inhibiting T cell Priming and Induces a Unique Transcriptional Profile In CD4+ T Cells, showed how sc priming with ovalbumin in the presence of abatacept produced large amounts of IL-2, thereby resembling naïve T cells exposed to antigen for the first time. These Tcells cells included less Tregs and CD4+ Tcells than other (not primed or naïve) T cell populations, and their state were accompanied by an inhibition of the activation of dendritic cells at the transcriptional level. Patakas concluded that while abatacept significantly modulates the T-DC communication resulting in defective cell priming this is distinct from anergic tolerance.

Orban et al, Lancet 2011, Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial, , reports that abatacept significantly preserves endogenous insulin secretion as measured by stimulated C-peptide, over the initial 6 months as compared with placebo, then declines in parallel with the placebo group. The early beneficial effect is thought to be due to that abatacepts blocks the costimulatory B80/86-CD28 signal preventing activation of naïve Tcells. The after 6 months successive decline in beta cell function was speculated to be due to that continuous T cell activation diminished as the disease progress.

Although Orban mentions that activation of naïve T cells need two signals, whereas one is comprised by an antigen presented in the context of an MHC molecule on an APC; and the other is a costimulatory signal involving the CD28 molecule on the Tcell, no specific antigen was used in the trial. This may be ground for speculation that while abatacept initially and successfully prevents activation of naïve Tcells into cytotoxic Tcells , this may eventually be counteracted and/or compensated for by other parts of the immune system, such as for example increased secretion of IL-2, and when this has occurred, the window for inducing tolerance to a specific antigen is missed.

Orban et al, Diabetes Care 2013, Costimulation Modulation With Abatacept in Patients With Recent-Onset Type 1 Diabetes: Follow-up 1 Year After Cessation of Treatment, subsequently reported continued beneficial effect one year after cessation of drug administration although differences between the active arm and placebo diminished. Thus did 35% of patients in the active arm have a peak stimulated C-peptide >0.2 nmol/l compared to 30% for placebo subjects at 36 months after commencement of treatment. It was pointed out that there was a lack of effect with abatacept treatment in DR3-negative patients.

The two Orban studies mentioned above indicate that the immune regulation of abatacept as a stand alone therapy in recent onset type 1 diabetes patients is not sufficient to have a lasting beta cell function preservation effect.

Verhagen et al, PLOS 2014, CTLA-4 Modulates the Differentiation of Inducible Foxp3+ Treg Cells but IL-10 Mediates Their Function in Experimental Autoimmune Encephalomyelitis, concludes that presence of IL-10 is an important factor for enhancing the suppressive effect of Tregs.

GAD65 (the 65 kd isoform of Glutamic Acid Decarboxylase), is a major beta cell auto-antigen, as it is produced in the islets with increased release as response to beta cell stimulation. This protein has been shown to deeply influence the autoimmune immune process. Numerous studies have shown that GAD can prevent diabetes in experimental animals. The similarity of GAD with viral proteins may be important for the therapeutic action. The observed effect, even after the start of the immune process, suggests that it might be possible to expect the same effect in humans. Recombinant GAD was formulated in aluminiumhydroxide (alum) and in a phase II study in LADA patients, the administration of one low dose, Diamyd 20 µg, led to improved beta cell function for up to 2 years compared to the placebo treated group, with no side effects. Also other doses were tried: 4 µg showed no effect, 100 µg showed a similar effect as 20 µg, while 500 µg showed no effect. Association with change in the ratio of CD4+CD25+/ CD4- CD25- cells was found, indicating a mechanism for the effect. With this background a Phase II study in recent onset Type 1 diabetic patients 10-18 years was performed. Patients were randomized to either 20 µg GAD-alum (Diamyd) sc at Day 1 and 30, or placebo. The effect still after 30 months was remarkable, and clearly both statistically and clinically significant, with about half of the C-peptide decline in the GAD treated group compared with the placebo group. Patients with a diabetes duration < 3 months had a remarkably good effect with no or minimal decline of beta cell function during the follow-up of the first 15 months. Still after 48 months patients treated with < 6 months duration had significantly preserved C-peptide and no adverse events.

Subsequently Phase III trials were initiated in European in the US. In the European trial 334 patients were recruited into three arms, one arm with GAD-alum (Diamyd) 20 µg at Day 1,30, 90 and 270, another arm with GAD-alum 20 µg at Day 1 and 30, and placebo at Day 90 and 270 and a third arm with Placebo at Day 1,30,90 and 270. Although a positive trens was seen (16% efficacy, p=0.1) the primary endpoint, serum C-peptide AUC after a Mixed Meal Tolerance Test (MMTT) at 15 months was not met. This prompted early closure of the phase III trials. However, the European Phase III trial did show statistically significant efficacy in some pre-specified subgroups. Furthermore, 45 Swedish patients had passed the 30 month's visit when the study was stopped, and those 15 patients who had received two doses of GAD-alum (Diamyd) 20 µg showed a significant preservation of C-peptide after 30 months compared with placebo. It was noted that these Swedish patients were the ones without efficacy at 15 months, while efficacy was found at 15 months in the non-Nordic patients. It was concluded that while partly effective, GAD-alum may need to be combined with other compounds in order to become part of a clinically effective treatment regimen for type 1 diabetes.

Denes et al, Diabetes Technol Ther. 2010, Autoantigens plus interleukin-10 suppress diabetes autoimmunity, reported that whereas recombinant vaccinia virus (rVV) strains expressing the immunomodulatory cholera toxin B subunit (CTB) fused to a fragment of the autoantigen glutamic acid decarboxylase (GAD65) or the immunosuppressive cytokine interleukin-10 (IL-10) were independently able to generate only low levels of immune suppression of type 1 diabetes mellitus (T1DM) in the NOD mouse, a vaccinia virus (VV)-mediated combination of CTB::GAD fusion and IL-10 proteins showed to be a more effective and durable immunotherapeutic strategy for T1DM.

Robert et al, Diabetes. 2014, Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by Lactococcus lactis reverses diabetes in recent-onset NOD mice, more recently showed that oral delivery of live Lactococcus lactis (LL) bacteria for controlled secretion of the T1D autoantigen GAD65370-575 peptide and the anti-inflammatory cytokine interleukin-10, in combination with

5   short-course low-dose anti-CD3,preserved functional β-cell mass in recent-onset NOD mice.

Skyler et al, Diabetes 2011, Stopping Type 1 Diabetes, Attempts to Prevent or Cure Type 1 Diabetes in Man (which including references in entirety is incorporated herein), a combination regimen involving an immune modulator and an antigen-specific therapy provides a rational for a longer lasting treatment of T1D.

10   Staeva et al, DIABETES, VOL. 62, JANUARY 2013, Recent Lessons Learned from Prevention and Recent-Onset Type 1 Diabetes Immunotherapy Trials (which including references in entirety is incorporated herein), report the state of the art of current thinking and recommends evaluating combination therapies including self-antigens and anti-inflammatory compounds.

Skyler, DIABETES TECHNOLOGY & THERAPEUTICS, Volume 16, Supplement 1, 2014, Immune

15   Intervention for Type 1 Diabetes, 2012–2013, (which in entirety is incorporated herein), reviews recent trials in the field.

Rigby et al, Current Opinion Endocrinol Diabetes Obes 2014, 21:271–278 , Targeted immune interventions for type 1 diabetes: not as easy as it looks! (which including references in entirety is incorporated herein) describes many of the efforts to combat autoimmune diabetes and

20   concludes that despite nearly a dozen trials with many hundreds of participants, no monotherapy has been found and that: a) different subpopulations of patients with T1D seem to respond differently to immune interventions, suggesting significant heterogeneity; b) an effective therapy must combine inhibition of Teff cells (by depletion, enhanced suppressibility, or both), with stimulation of Tregs (by increased frequency or function, including ablation of the

25   proinflammatory milieu); and this may require combinations comprising a Teff-depleting agent, a Treg-boosting agent, and an antigen.

As referenced above several monotherapies and combination regimens including a variety of compounds have shown promising effects in prevention and treatment of autoimmune diabetes

30   in the mouse model. However, transfer of such regimens to man have all failed to show a clinically meaningful effect. Thus there remains a formidable need to relieve societies and patients from the devastating disease autoimmune diabetes and its long term complications. It is a subject of the present invention to disclose methods and compositions for prevention and treatment for the autoimmune component of T1D and LADA, paving the way for endogenous, or

35   by other methods accomplished, increase of functional beta cell mass.

03 Mar 2020

2015268960

Summary of the invention

In one aspect, the present invention provides a method for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent autoimmune diabetes in a subject, comprising administering to the subject a composition
5   comprising glutamic acid decarboxylase (GAD), by injection directly into a lymph node.

In another aspect, the present invention provides use of a composition comprising glutamic acid decarboxylase (GAD) in preparation of a medicament for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent
10   autoimmune diabetes in a subject, wherein said composition is administered to the subject by injection directly into a lymph node.

In one aspect, the present invention relates to a method for prevention and/or treatment of an autoimmune disease, comprising administering a composition, said composition comprising at
15   least one beta cell autoantigen, to a subject having a serum vitamin-D level above 50 nanomole/liter.

8A

The present invention discloses the method of using anti-inflammatory compounds and autoantigens in combination with methods to render dendritic antigen presenting cells (APCs) more "tolerizing" when presenting said autoantigens to the immune system.

The present invention discloses methods where combinatorial regimens including anti-inflammatory compounds, autoantigens and vitamin D can be effectively used as prevention and treatment methods for T1D and other autoimmune diseases.

In a further aspect, the present invention relates to a method for prevention and/or treatment of an autoimmune disease, comprising administering to a subject a composition, said composition comprising at least one beta cell autoantigen, by intralymphatic injection or injection directly into a lymph node.

In a further aspect, the present invention relates to a method for prevention and/or treatment of an autoimmune disease, comprising administering to a subject at least one beta cell autoantigen, in increasing doses over a period of weeks, months, or years.

In a further aspect, the present invention relates to a composition comprising a plurality of particles, each having immobilised on its surface at least one first and at least one second antigen, wherein the first antigen is a beta cell autoantigen, and the second antigen is either a tolerogen or a beta cell autoantigen, the composition further optionally comprising pharmaceutically acceptable adjuvants, excipients, solvents, and/or buffers.

In a further aspect, the present invention relates to a composition comprising

       i) at least one beta cell autoantigen, and at least one of

       iia) an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and

       iib) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor;

    and optionally pharmaceutically acceptable adjuvants, excipients, solvents, and/or buffers.

In a further aspect, the present invention relates to a pharmaceutical kit comprising

       i) a composition comprising a beta cell autoantigen, and at least one of

       iia) a composition comprising an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and

       iib) a composition comprising a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

In a further aspect, the present invention relates to a beta cell autoantigen for use in a method according to the invention.

In a further aspect, the present invention relates to a method for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent autoimmune diabetes in a subject, comprising administering to the subject a composition comprising glutamic acid decarboxylase (GAD), by injection directly into a lymph node.

5   In a further aspect, the present invention relates to a use of a composition comprising glutamic acid decarboxylase (GAD) in preparation of a medicament for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent autoimmune diabetes in a subject, wherein said composition is administered to the subject by injection directly into a lymph node.

10   Preferred embodiments of the invention are set out in the dependent claims.

Definitions

All terms and expressions as used herein are intended to have the meaning given to them by the person skilled in the art at the filing date of the present application, unless any other expression is evident from the context of this disclosure. However, for the sake of clarity, some terms and
15   expressions are explicitly defined below.

An "autoantigen" or "self-antigen" is an endogenous tissue constituent that has the ability to interact with autoantibodies and cause an immune response. A "beta cell autoantigen" is an autoantigen originating from pancreatic beta cells.

An "autoantibody" is an antibody that reacts with autoantigens of the organism that produced
20   them.

The term "Vitamin D" includes vitamin D_2 and vitamin D_3. "Vitamin D analogs" include without prejudice Ergocalciferol, Dihydrotachysterol, Alfacalcidol, Calcitriol, Colecalciferol, and Calcifediol, and combinations thereof, as well as any other vitamin D analog classified in group A11CC of the Anatomical Therapeutic Chemical Classification System.

25   The term "cyclooxygenase inhibitors", or "cox inhibitor", relates to compounds that combine with cyclooxygenase and thereby prevent its substrate-enzyme combination with arachidonic acid and the formation of eicosanoids, prostaglandins, and thromboxanes. A subgroup of the cyclooxygenase inhibitors is the cyclooxygenase-2 inhibitors, which have specificity for cyclooxygenase-2.

30   The term "TNF alpha inhibitor" relates to compounds that inhibits the action of Tumour Necrosis Factor alpha (TNF alpha), and includes adalimumab, certolizumab, etanercept, golimumab, infliximab, as well as any other compound classified in group L04AB of the Anatomical Therapeutic Chemical Classification System.

An "epitope" is the surface portion of an antigen capable of eliciting an immune response and of
35   combining with the antibody produced to counter that response, or a Tcell receptor.

The term "gamma-amino butyric acid analogs" includes vigabatrin and baclofen.

10

Expressions using the singular "a", "an" and the like shall be construed as including the plural.

As used herein, "coadministration" refers to administering the compounds of the regimen of the present invention so that their dosing regimens overlap. They do not need to be administered at the same time.

5   The abbreviation "T1D" stands for "Type 1 Diabetes".

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification

10   relates.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

15

Detailed description of the invention

At the time when a first autoantibody to insulin appears in a genetically predisposed individual, such as for example but not limited to a DR3-haplotype individual and no other autoantibodies are detected, this may mean that GAD-reactive CD8+ and CD4+ TCs have not yet been activated,

20   which

offers a window of opportunity to completely stop the disease with anti-inflammatory and/or lymphocyte differentiation inhibitors alone without the use of a beta cell antigen, as the autoimmunity to insulin is likely to be self-eliminated by counteracting mechanisms. In this situation with few if any GAD-reactive activated CD4+ Thelper cells yet produced, it is important to direct

5   activation away from the pro-inflammatory Th1 type. In GAD antibody positive individuals and/or diabetes patients, GAD and other beta cell antigens formulated in alum, usually considered as a Th2 driving adjuvant, may be added for antigen specific tolerization purposes. While this may produce more Th2-cells and Tregs than pro-inflammatory cells, several factors including internal environmental factors may nullify tolerizing effects. Therefore it may not be sufficient to use Th2

10   driving formulations of autoantigens as monotherapies and the present invention discloses compositions and combination regimens to enhance beta cell tolerization protocols.

Previous attempts to induce immune regulation in autoimmune diseases by administering monotherapies such as GAD and insulin antigens; anti-inflammatory compounds such as antibodies to CD3, CD20; Thymoglobulin, abatacept, alefacept or TNFalpha inhibitors such as etanercept have

15   shown some efficacy, but the duration of these effects or their efficacy after disease presentation are limitations for bringing these approaches into the clinic. The present invention discloses a novel strategy for treatment and prevention of autoimmune diseases in Vit D enforced DCs act synergistically with autoantigen and anti-inflammatories in order to provide various degrees of effect.

20   The present invention thus provides means (e.g., methods, compositions and combinations) for the prevention and/or treatment of autoimmune diseases, such as autoimmune diabetes.

The present invention utilizes the knowledge that Vitamin D (Vit D) enforces immature dendritic cells (DCs) to be more tolerogenic, which so far has not proved to be sufficient to alter the course of any autoimmune disease, and combines administration of Vit D with administration of a self-antigen,

25   associated with said autoimmune disease, in a timely fashion, thus enforcing the tolerogenic immunomodulation of the administered self-antigen.

To further enhance the tolerogenic milieu at which DCs present self-antigens to the immune system in a subject with adequate Vit D serum concentration, the present invention discloses the finding that a combination regimen using a common anti-inflammatory compound together with a

30   autoantigen is suitable for treatment and prevention of T1D.

Beyond its potential synergistic effects, another advantage of co-administration of the compounds of the present invention relates to the problem that autoimmune disorders often include autoimmune responses against multiple self-antigens. It may be difficult to attempt to anergize or delete all of the autoaggressive lymphocytes using direct antigen-specific tolerization with all of their

35   cognate antigens, because all of the cognate antigens may not be known. For example, Type 1 diabetes (T1D) is thought to be caused by autoaggressive lymphocytes that enter the islets of Langerhans, where they destroy .beta.-cells. Activation of such cells is probably multi-factorial involving a genetic predisposition, environmental triggers such as viruses and maybe damage to the pancreas (islets cells), for example caused by a local pro-inflammatory reaction.

40   Since the autoaggressive process is usually fairly advanced when pre-diabetic human individuals are identified by screening for islet-cell antibodies, one can assume that aggressive responses to more

than one islet-antigen will be ongoing during this stage of the disease. Furthermore, chronic non-specific systemic immune suppression is not considered an option, since diabetes frequently affects young individuals and lifelong immune suppression is associated with side effects that are unacceptable compared to even insulin therapy alone.

5   Therefore, a curative immune-based intervention with specificity and low systemic side effects is very desirable. The present methods circumvent the problem of multiple self-antigenic targets, because the coadministration of the compounds of the present invention may be sufficient to re-establish tolerance to multiple self-antigens that are targets in an autoimmune disorder.

The present methods also circumvent the problems of chronic non-specific systemic immune
10   suppression, because the coadministration of the compounds of the present invention can reestablish long-term tolerance without the need for continuous life-long dosing.

Without being bound by theory, the invention provides that the coadministration of the compounds of the invention has the potential to synergistically establish long-term tolerance in part by inducing
15   the activation/expansion of regulatory T-cells (and also regulatory antigen presenting cells (APCs)).

A number of different phenotypes of regulatory T cells have been described. They can arise after thymectomy and can be induced after systemic immune modulation. Their effector functions are not fully known. They appear to be part of the immune system's intrinsic balance and their loss results in severe immune dysregulation and autoimmunity. Th2-like regulators with defined antigen specificity
20   have been described. They are thought to act as bystander suppressors and arise after antigen-specific immunization. Homann et al., J. Immunol. (1999) 163:1833-8. Depending on their effector function they have been termed Th3 (TGF-.beta. producers). These cells are antigen specific lymphocytes with specialized effector functions and do not behave like Th2 cells. Applying the so-called Th1/Th2 paradigm to these cells can therefore be misleading.
25
Bystander suppression relates to the phenomenon of antigenic spreading. Antigenic spreading is thought to be an essential component during the progression of local autoimmune processes. One can therefore assume that when patients have several autoantibodies, the autoaggressive response may involve many self-antigens (or "autoantigens"). Since a majority of the autoantigens might not
30   be identified for a particular autoimmune disorder, it is not possible to tolerize each autoaggressive specificity with a therapeutic regimen that involves knowledge of the respective MHC restriction element and peptide. The induction of regulatory cells by the present methods has several advantages in this situation. It is known for example, that regulatory T cells in T1D can act locally in the lymph nodes and islets as bystander suppressors, which means that they can suppress
35   aggressive lymphocytes with other auto-antigenic specificities. This can occur by modulating antigen presenting cells (APCs), for example, by secretion of cytokines with immune modulatory function. Thus, such bystander suppressor T regulatory cells can dampen autoaggression to several other autoantigens without knowing their precise specificity.

Presence of a first autoantibody to an abundant 1st antigen or self-antigen such as for example
40   insulin in a genetically predisposed individual, while no autoantibodies to at least one more sequestered (less abundant) 2nd self-antigen(s) are detected, may offer a window of opportunity to stop a potential emerging disease process in its tracks using anti-inflammatory means and/or

lymphocyte differentiation inhibitors alone. In fact as the inflammatory response to such weak 1st self-antigen may be fuelled by relatively few, from the clonal deletion process in thymus escaped insulin specific auto reactive CD8+ and CD4+ Tcells (TCs), inflammation may fade and said 1st antigen may in some embodiments be used as a tolerogen inducing tolerance to other, perhaps more
5   sequestered or uncommon autoantigens, for which more escaped naïve auto reactive CD8+ and CD4+ Tcells (TCs) are available for activation.

In situations where autoimmunity has been triggered to more potent autoantigens such as for example GAD65 in the case of Autoimmune Diabetes, and which is able to drive the disease process it may not be sufficient to use anti-inflammatory means and/or lymphocyte differentiation inhibitors
10   alone, without the use of said potent autoantigens for induction of active tolerization. On the other hand, use of one potent autoantigens only, for example GAD65 formulated in an adjuvant such as aluminiumhydroxide (here "alum"), usually considered a Th2 driving adjuvant, may result in activation of not only regulatory components, but also of proinflammatory and/or cytotoxic molecules and cells, not limited to molecules belonging to the innate immune system, Macrophages,
15   Dendritic Cells, B-cells (BCs), T-cells (TCs) or other factors including environmental factors that may contribute to counteracting tolerization. Moreover, other less Th2 driving adjuvant formulations may be used, such as saline or human serum albumin. Therefore it may not be sufficient to use formulations of single autoantigens as monotherapies and the Present Invention discloses compositions and methods using at least two autoantigens to enhance the specific tolerization effect
20   of administered autoantigens formulated in alum, saline or human serum albumin.

The Present Invention provides in one aspect at least one pharmaceutical Composition comprising at least one antigen. The at least one pharmaceutical Composition according to the invention may therefore in some embodiments comprise at least two antigens. According to certain other embodiments, the at least one Composition comprises at least three autoantigens. According to
25   certain other embodiments, the at least one Composition comprises at least four autoantigens. According to certain other embodiments, the antigens may be formulated in separate Compositions. Most preferred is that all antigens according to the invention are formulated in the same Composition. In some embodiments all antigens are autoantigens, in some other embodiments some or all antigens are antigens (tolerogens) to which the immune system has developed a
30   regulatory response and thereby able to influence the reaction to other autoantigens in a tolerant way.

It is a subject of the present invention to disclose Compositions, regimens and methods enabling induction of tolerance to self structures being attacked in autoimmune disease. In one specific embodiment at least two antigens are associated with one carrier particle, thus exposing one
35   immune cell from the adaptive immune system to at least two antigens with various influence on said immune cell resulting in a modified response to the self structure subject to the autoimmune attack.

By adsorbing, binding or incasing a protein or peptide antigen to a carrier particle to which the immune system reacts, and inserting the particles into human tissue by e.g. subcutaneous injection,
40   the protein or peptide can be targeted for ingestion by the immune system. Likely this ingestion is performed by Dendritic Cells or Macrophages (Antigen Presenting Cells, APCs) . Some of the proteins or peptides ingested in this process will be presented to the adaptive immune system by

HLA (MHC) proteins and an adaptive immune reaction will follow. The adaptive response will be influenced by the activity levels of the innate immune system, and by immune responses that exist towards any antigens that are presented by the HLA proteins in the process. These influencing proteins can be the particle bound peptides or proteins, or native proteins, autoantigens or

5   tolerogens (antigens to which the immune system reacts in a tolerogenic way) that are ingested and presented in the same process. If these proteins are ingested by the same immune cell they will be presented together by the HLA proteins on the cell surface.

The presentation of non-native peptides to the immune system may by influence from signals from the innate immune system in response to tissue disruption, be ofTh2 character. Endogenous

10   antigens including autoantibodies and RNA-nucleotides etc may do the same. A native protein, or a protein to which a response already exists, will set off signaling by the adaptive immune cells, which will influence the total immune response, and might activate tolerogenic signals or a preexisting Th1 reaction. If the signaling induced by an injection procedure and by the interaction of naïve immune cells and antigen presenting immune cells, is stronger than the pre-existing response, a new immune

15   response including memory will form.

If a combination of proteins resting on the same particle carriers are injected and ingested and presented by the same APC, the effects will be additive by expanding the number of possible adaptive immune cells to interact with, leading to an increased chance of forming a novel immune reaction in spite of a possible pre-existing reaction to one of the proteins.

20   If a protein autoantigen is injected together with another protein (native or foreign) on the same particle carrier to which an immune response already exists, the immune response to the antigen will be largely influenced by the response that the immune system already has established to the accompanying antigens. If the accompanying antigens are native proteins to which central regulatory T-cells exist (such as for example IL10, insulin, Humans Serum Albumin, Hemoglobin) the

25   response will be driven towards tolerance. If these proteins are antigens that the person has already been immunized with, such as is often the case with Diptheria or Tetanus toxoid, the immune response will be influenced by these existing reactions (which mostly are inflammatory reactions).

The present invention includes methods for treating autoimmunity and/or establishing or inducing tolerance by the coadministration of the compounds of the invention. Besides autoimmune

30   diseases, present methods may also be used to establish tolerance to allergens, where allergenic peptides or proteins are coadministered with compounds of the invention where antigens instead of being self antigens are allergens (antigens) specific to the allergic disease.

It is understood that the details given herein with respect to one aspect, in particular detailsabout the autoantigens, Influencing antigens, the IL-10 inducing compound, and compound that reduces

35   the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes, the timing and mode of administration, apply mutatis mutandis to all other aspects of the invention.

Methods

In one aspect, the present invention relates to a method for prevention and/or treatment of an

40   autoimmune disease, comprising administering a composition, said composition comprising at least

one beta cell autoantigen, to a subject having a serum vitamin-D level above 50 nanomole/liter. Each of the at least two molecules may thus influence the reaction of the adaptive immune cell to which the antigens are presented.

The subject may have a serum D-vitamin level between 50-150 nanomole/liter, such as 60-100 nanomole/liter, 75-100 nanomole/liter or 100-150 nanomole/liter.

The method may comprise a pretreatment of the subject to adjust the serum vitamin-D level, and such pretreatment may comprise administration of vitamin-D and/or vitamin-D analogs, and/or exposure to UVB-radiation, preferably for between 7 to 90 days before administration of the composition comprising at least one beta cell autoantigen to said subject.

The method may further comprise administration of vitamin-D and/or vitamin-D analogs in an amount of 7000-70000 IU/week for 3-48 months.

The beta cell autoantigen may be a beta cell autoantigen as discussed below under the heading "Autoantigens".

The method may further comprise administration of a cyclooxygenase inhibitor, as discussed below under the heading "Cyclooxygenase inhibitors".

The method may further comprise administration of a CTLA4 compound, as discussed below under the heading "CTLA4 compounds".

The method may further comprise administration of a TNFalpha inhibitor, as discussed below under the heading "TNFalpha inhibitors".

The present invention provides a method for the prevention and/or treatment of an autoimmune disease in an individual in need thereof, the method comprising administering to said individual:

a)   for specific antigen tolerization purposes, at least one autoantigen or fragments thereof; or nucleic acids, plasmids or vectors coding for such molecules related to at least one of the autoimmune and inflammatory diseases as listed above. In one embodiment, autoantigen is administered when serum vitamin D levels are between 50 and 150nM/l, more preferably between 75 and 100 nM/l and most preferably between 100 -150nM/l; and

b)   for interfering with APCs ability to mature, administering to said individual at least one IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, gamma-amino butyric acid, gamma-amino butyric acid analogs and tyrosine kinase inhibitors; as listed above. In one embodiment the IL-10 induction is enhanced or accomplished by use of UVB-light exposure; and

c)   for interfering with the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes, administering a compound such as a NSAID compound; a CTLA-4 compound; or a TNFalpha inhibitor; as listed above.

The present invention relates in some aspects to methods for prevention and/or treatment of autoimmune disease, such as Type 1 diabetes (T1D) and autoimmune diabetes.

The invention discloses a method for treatment of autoimmune disease, such as T1D and autoimmune diabetes, the method comprising administering to a subject with said disease:

(a) a course of Vit D for enforcing the ability of antigen presenting dendritric cells to present antigen peptides to the immune system in a tolerizing manner;

5   b) an autoantigen, such as GAD65, formulated in a pharmaceutical carrier administered in an amount sufficient to restore or induce tolerance to the autoantigen; and optionally

c) a therapeutic dose of an anti-inflammatory compound, for example a cyclooxygenase inhibitor such as Ibuprofen, or a more pronounced cox-2 or cox-1 inhibitor.

The course of Vit D preferably starts 15 to 90 days prior to administration of autoantigen, or 7 to 90

10   days prior to administration of autoantigen, and is given in liquid or tablet form in doses corresponding to 7000 to 70000 iu per week for a period of 3 to 48 months.

The pretreatment with vitamin D aims to elevate the treated subject's serum levels of vitamin D to above about 50 nanomole/liter, or above 60, 75, or 100 nanomole/liter. The pretreatment may be dispensed with if the subject already has serum levels of vitamin D at these levels.

15   In another embodiment of the invention the serum concentration of Vit D can be enhanced by means of phototherapy. In this case subjects will be exposed to ultraviolet B radiation preferably between 10-120 minutes daily for 15 to 90 days prior to administration of autoantigen. The phototherapy should continue for a period of 3 to 48 months.

Preferred doses of the autoantigen is between two and four administrations, at least two weeks

20   apart, more preferably one month apart, of each between 10 and 200 µg antigen if given by injection. If administered orally the preferred doses are between 500mg and 5g daily for a period of between three months and 48 months. Ibuprofen is preferably administered in daily doses of 100 to 800mg for a period of 60 to 150 days during which period administration of autoantigen takes place.

The autoantigen can be administered by intralymphatic injection, injection directly into a lymph

25   node, subcutaneous injection, intramuscular injection, intraperitoneal injection, intravenous injection, subcutaneous injection, intranasal, transmucosal or sublingual application; or orally, including administration as tablets, pellets, granules, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions, sprays or as reconstituted dry powdered form with a liquid medium. The anti-inflammatory compound and the autoantigen can be administered in/with a pharmaceutically

30   acceptable carrier, excipient or diluent.

In one embodiment of the invention and in the case of treatment or prevention of T1D, injection of autoantigens are made subcutaneous in unilateral or bilateral areas of the stomach to enable an increased traffic of APC cells presenting autoantigen peptides to pancreatic lymph nodes.

According to certain other embodiments, the at least one autoantigen is administered is

35   subcutaneous, such as in the stomach close to the pancreatic draining lymph nodes. In some embodiments, the volume for subcutaneous injection of the antigens is between 0.2 and 2 ml, more preferred between 0.4 and 0.6ml.

According to certain other embodiments, the at least one autoantigen is administered via intradermal injection.

According to certain embodiments, where the at least one antigen is administered via subcutaneous injection in a "vaccine-like" fashion, at least 4 - 250ug, more preferably 10-100ug most preferably 10-50ug doses of each of the at least one antigen, is administered per treatment occasion.

According to certain embodiments, where the at least one antigen is administered via subcutaneous injection in an "allergy-like" fashion where various increasing dosage shedules of each used autoantigen may be used. Such a method may comprise administering the beta cell autoantigen in increased doses over a period of weeks, months, or years.

In one embodiment of the composition containing the beta cell autoantigen is administered 1-4 weeks apart, such as 2-4 weeks apart or 2 weeks apart, in an initial treatment period of 3 to 4 months, and optionally 2-3 months apart in a continued treatment period of 6-9 months.

In one embodiment, the amount of beta cell autoantigen is increased from 1-5 µg per administration at the beginning of the treatment period to about 40-100 µg per administration in the final administrations.

In one embodiment, the method comprises subcutaneous injections of beta cell autoantigen (e.g. GAD65) of increasing doses, starting at Baseline (Month 1) as follows: 0.4; 0.8; 2; 3.2; 4; 6.4; 8; 12; 16; 20; 24, 32, 40 µg, autoantigen weekly, where after 40 µg autoantigen will be administered from week 15 through 27 week at 2, 4 and 8 weeks interval. 40 µg autoantigen will thereafter be administered every 8-12 weeks for 1 year. Vitamin D may be administered as described above (from Day 1)

A preferred dosing regimen includes increasing autoantigen doses of 1 ug, 5 ug, 20 ug, 50 ug, two injections of each dose 4 weeks apart which would be 28 weeks treatment GAD-alum. An alternatively preferred dosing schedule includes increasing doses such as 4ug, 8ug, 16ug, 20ug, 40ug on days 0, 15, 30, 45, 60 and subsequently 40 ug on days 120, 180, 270.

In one embodiment of this invention administration of autoantigen is made directly into the lymph nodes or into the lymphatic system to allow resident APCs to present antigen peptides to the immune system. If administration of autoantigens is made directly into a lymph node or into the lymphatic system, the dose will preferably be between 1 and 15 µg per autoantigen, more preferred between 2 and 10 µg per autoantigen or 2 to 5 µg per autoantigen. Formulation in alum is preferred.

According to certain embodiments, the at least one autoantigen is administered intrainguinal, intra-lymph node or intralymphatic. In some embodiments, the volume for intra-inguinal injection of the antigens is between 0.05 and 0.2 ml, more preferred between 0.05 and 0.15 ml.

According to certain embodiments, where the at least one antigen is administered by intralymph-node or intralymphatic injection a preferred dosage is between 1-15ug, more preferred between 2-10, and most preferred between 2-5ug per injection and autoantigen used, such administrations taking place at least 2 times, more preferred at least 3 times and most preferred at least 4 times, at least 14 days apart, more preferably at least 30 days apart.

According to certain embodiments, where the at least one antigen is administered intravenously, at least 100-10000 ug of each antigen is administered per treatment occasion at least twice, at least one week apart..

According to certain embodiments, where the at least one antigen is administered orally, at least 0.5-5g of each antigen is administered per treatment occasion at least once every week.

According to certain embodiments, the at least one antigen is formulated separately or together with the other antigens as the case may be, in alum, saline or human serum albumin.

According to certain embodiments, the at least one pharmaceutical composition comprises at least two antigens on the same carrier particle. According to certain other embodiments, the at least one pharmaceutical Composition comprises at least two antigens on different carrier particles. According to certain embodiments, the at least two antigens are administered separately and at different times and frequencies, regimens and formulations that are suitable for the specific antigens. More preferably the at least two antigens are formulated in the same pharmaceutical Composition and therefore administered simultaneously.

In some embodiments the at least one antigen is formulated in an adjuvant such as alum. In more particular embodiments the at least one antigen is formulated in saline or human serum albumin.

According to certain embodiments, the at least one antigen and the at least one IL-10 inducing compound are administered simultaneously. According to certain embodiments, the at least one antigen and the at least one IL-10 inducing compound are administered separately.

According to certain embodiments, the at least one IL-10 inducing compound is administered simultaneously with the at least one antigen. According to certain embodiments, the administration of the at least one IL-10 inducing compound commences between 1-14 days prior to the first administration of the at least one antigen. According to certain embodiments, the administration of the at least one IL-10 inducing compound commences at least 2 preferably at least 10 weeks, prior to the first administration of the at least one autoantigen.

According to certain embodiments that include treatment periods with Vitamin D, between 500 and 10000 IU, more preferably between 1000 and 3000 IU of vitamin D, such as Vitamin D3, are administered per day.

According to certain embodiments that include pretreatment periods with Vitamin D, between 7,000 - 100,000 IU of Vitamin D, such as Vitamin D3 are administered per week, prior to first administration of the at least one antigen, and as a maintenance dose of 500-2000 IU per day thereafter.

In some embodiments the treatment period of Vitamin D is between 60 and 420 days after 1st administration of antigen.

The compound that reduces the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes, such as a NSAID compound; a CTLA-4 compound; or a TNFα inhibitor may be administered simultaneously or separately with the at least one antigen and/or the at least one IL-10 inducing compound.

In some embodiments, the compound that reduces the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes is a NSAID compound, such as a COX-inhibitor.

According to certain embodiments, the treatment period with NSAIDs starts at least 2 weeks prior to first administration of the at least one antigen.

According to certain embodiments, when the COX inhibitor is Ibuprofen, at least one 400 to 1000 mg doses are administered per day during the NSAID treatment period.

The NSAID treatment period is at least between 4 and 14 weeks, more preferred between 4 and 8 weeks

In the present methods, the anti-inflammatory compound should be administered orally or by injection.

In some embodiments, the compound that reduces the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes is a TNFα inhibitor.

Blocking TNFalpha reduces the activation state of the immune response and decreases the activity of DCs and other immunocytes. Further, because TNFalpha disrupts FSC and GC architecture in lymphoid tissue and impairs B cell functions, the activation of antigen-specific effector T cells and autoantibody production is reduced. Recent diabetes preclinical data show that beta cell antigen delivered by quiescent DCs can induce peripheral T cell unresponsiveness, down-modulate ongoing beta cell destruction and arrest beta cell destruction. Therefore, under a combined autoantigen - TNFalpha inhibitor therapy autoantibody levels and effector T cell activity and numbers will be relatively reduced while the number of autoantigen-specific Tregs number and function will be at least maintained maintained. It takes months to modify these immunologic processes and document these effects, but during this time, due to its acute beta-cell-protective and metabolic effects, TNFalpha inhibition will also preserve beta cells directly. Because GAD65 is known to be one of the primary autoantigens in T1DM, this approach produces a deviation of the diabetes autoimmune response towards a regulatory phenotype of sufficient critical mass to lead to a significant and prolonged effect on beta cell preservation.

Thus, in one aspect of the present invention, a TNF-alpha inhibitor not limited to for example infliximab, adalimumab, golimumab, and etanercept, is used as an anti-inflammatory compound. A preferred dose when etanercept is used is between 0.2 and 1mg/kg SQ, once or twice per week for a period of between 2 to 9 months.

According to certain embodiments, when the TNFα inhibitor is Etanercept, the FDA-approved dosage of 5 mg/kg at Weeks 0, 2, and 6 is preferred. In another embodiment the dose is the same as used in the Phase I TNFα inhibitor monotherapy trial (0.4 mg/kg (max 25 mg) SQ twice weekly × 26 weeks). In most preferred another embodiment only two doses are used, max 25mg/dose in combination treatment regimen including vitamin D and autoantigens.

According to certain embodiments, the TNFα inhibitor is administered prior to the first administration of the at least one antigen.

In some embodiments, the compound that reduces the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes is a CTLA-4 compound, such as abatacept. According to particular embodiments, when the compound is abatacept doses of at least 2-20mg/kg abatacept, is administered per treatment occasion, starting within +/- 7 days around the time of first administration of the at least one antigen.

According to certain embodiments, the CTLA-4 compound is administered simultaneously with the first administration of the at least one antigen.

According to certain embodiments, administration of the at least one antigen and the compound that reduces the immune system's ability to activate naïve TCs and BCs and recall responses from activated and memory lymphocytes, is repeated on day 14, 28 and 45 +/- one week as the case may be to ensure blockage of CD28 on TCs, and where day one marks the first administration of the at least one IL-10 inducing compound.

In one aspect, the invention provides a method to treat one or more symptoms associated with T1D. Symptoms associated with T1D include, but are not limited to, reduced insulin production, reduced insulin sensitivity, high blood glucose levels, destruction of insulin producing cells, and abnormal C peptide levels.

The methods of the invention may be directed towards the treatment and prevention of not only T1D but generally for autoimmune diseases and disorders. For example, subjects suffering from Grave's disease, Hashimoto's thyroiditis, hypoglyceimia, multiple sclerosis, mixed essential cryoglobulinemia, systemic lupus erthematosus, Rheumatoid Arthritis (RA), Coeliac disease, T1D, or any combination thereof. In these cases, for the disease relevant autoantigens are to be included as autoantigens in the treatment methods. In one aspect of the invention, the subjects suffer from autoimmune responses that involve T-cells or B-cells that have an antigenic specificity, or T-cell receptor (TCR) and/or B-cells that have T-cell receptor (TCR) or B-cell receptor (BCR) antigen specificity for an autoantigen.

According to certain embodiments, the individual to be treated according to the presentinvention is a mammal. According to particular embodiments, the individual to be treated according to the present invention is a human. According to certain embodiments, the individual to be treated according to the present invention is an infant. According to particular embodiments, the individual to be treated according to the present invention is an adolescent. human. According to particular embodiments, the individual to be treated according to the present invention is an adult human.

In some embodiments the human treated subject is above 4 years of age

In other embodiments the human treated subject is 8 years or above.

In other embodiments the human treated subject is 10 years or above.

In some embodiments the human treated subject is 18 years or below.

In some embodiments the human treated subject is 4-10, or 4-18, or 8-18, or 10-18 years of age.

In other embodiments the human treated subject is 18 years or above.

In some embodiments the human treated subject is 18-30 years of age.

Compositions

The present invention also discloses a composition comprising a plurality of particles, each having
5   immobilised on its surface at least one first and at least one second antigen, wherein the first antigen is a beta cell autoantigen, and the second antigen is either a tolerogen or a beta cell autoantigen, the composition further optionally comprising pharmaceutically acceptable adjuvants, excipients, solvents, and/or buffers.

According to certain embodiments, the at least one autoantigen is formulated in an adjuvant.
10   According to particular embodiments, the adjuvant is alum. In other particular embodiments the at least one antigen is formulated in saline or human serum albumin. In more particular embodiments the autoantigen may be administered as plasmids or encoded by a viral vector such as adeno-associated virus vectors or herpes simplex virus vectors.

According to certain embodiments, the at least one autoantigen is as discussed below.

15   The compositions according to the invention may comprise more than one autoantigen. Thus, according to certain embodiments, the composition comprises at least two autoantigens. According to certain other embodiments, the composition comprises at least three autoantigens. According to certain other embodiments, the composition comprises at least four autoantigens.

Therefore, according to particular embodiments, the composition comprises at least GAD, such as
20   GAD-65, and insulin. According to other particular embodiments, the composition comprises at least insulinoma antigen-2 and insulin. According to other particular embodiments, the composition comprises at least ZnT8 and insulin. According to other particular embodiments, the composition comprises at least IGRP and insulin. According to other particular embodiments, the composition comprises at least chromogranin A and insulin.

25   According to other particular embodiments, the composition comprises at least GAD, such as GAD-65, and B chain insulin. According to other particular embodiments, the composition comprises at least insulinoma antigen-2 and B chain insulin. According to other particular embodiments, the composition comprises at least ZnT8 and B chain insulin. According to other particular embodiments, the composition comprises at least IGRP and B chain insulin. According to other particular
30   embodiments, the composition comprises at least chromogranin A and B chain insulin.

According to other particular embodiments, the composition comprises at least GAD, such as GAD-65, and proinsulin. According to other particular embodiments, the composition comprises at least insulinoma antigen-2 and proinsulin. According to other particular embodiments, the composition comprises at least ZnT8 and proinsulin. According to other particular embodiments, the composition
35   comprises at least IGRP and proinsulin. According to other particular embodiments, the composition comprises at least chromogranin A and proinsulin.

According to other particular embodiments, the composition comprises at least insulin and B chain insulin. According to other particular embodiments, the composition comprises at least insulin and

proinsulin. According to other particular embodiments, the composition comprises at least B chain insulin and proinsulin.

According to particular embodiments, the composition comprises at least GAD, such as GAD-65, insulin and B chain insulin. According to other particular embodiment, the composition comprises at least GAD, such as GAD-65, insulin and proinsulin. According to yet other particular embodiments, the composition comprises at least GAD, such as GAD-65, B chain insulin and proinsulin. According to yet other particular embodiments, the composition comprises at least insulin, B chain insulin and proinsulin.

According to other particular embodiments, the composition comprises GAD, such as GAD-65, insulin, B chain insulin and B chain insulin.

According to certain embodiments, the at least one autoantigen, such as the at least two autoantigens or at least three autoantigens, is formulated in an adjuvant.

The autoantigens may be formulated in adjuvants such as aluminiumhydroxid, MAS-1, Human Serum Albumin, Lipid-emulsions.

According to particular embodiments, the adjuvant is alum.

In one embodiment, the invention relates to a composition comprising

i) at least one beta cell autoantigen, and at least one of

iia) an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and

iib) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor;

and optionally pharmaceutically acceptable adjuvants, excipients, solvents, and/or buffers.

The present invention also provides a composition (e.g. a pharmaceutical composition) comprising i) at least one autoantigen and ii) at least one IL-10 inducing compound.

More particularly, the present invention provides a composition (e.g. a pharmaceutical composition) comprising i) at least one autoantigen selected from the group consisting of insulin, B chain insulin, proinsulin, and beta cell autoantigens, and ii) at least one IL-10 inducing compound selecting from the group consisting of vitamin-D, vitamin-D analogs and tyrosine kinase inhibitors.

The composition according to the invention may comprise more than one IL-10 inducing compound. Thus, according to certain embodiments, the composition comprises at least two IL-10 inducing compounds. According to certain other embodiments, the composition comprises at least three IL-10 inducing compounds. According to certain other embodiments, the composition comprises at least four IL-10 inducing compounds.

Therefore, according to particular embodiments, the composition comprises at least Vitamin-D and a tyrosine kinase inhibitor. According to other particular embodiments, the composition comprises at least a Vitamin-D analog and a tyrosine kinase inhibitor.

According to more particular embodiments, the composition comprises at least Vitamin-D and dasatinib. According to other more particular embodiments, the composition comprises at least Vitamin-D and bosutinib. According to other more particular embodiments, the composition comprises at least Vitamin-D and saracatinib. According to other more particular embodiments, the composition comprises at least Vitamin-D and imatinib. According to other more particular embodiments, the composition comprises at least Vitamin-D and sunitinib.

According to more particular embodiments, the composition comprises at least a Vitamin-D analog and dasatinib. According to other more particular embodiments, the composition comprises at least a Vitamin-D analog and bosutinib. According to other more particular embodiments, the composition comprises at least a Vitamin-D analog and saracatinib. According to other more particular embodiments, the composition comprises at least a Vitamin-D analog and imatinib. According to other more particular embodiments, the composition comprises at least a Vitamin-D analog and sunitinib.

According to certain embodiments, the composition further comprises iii) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

According to particular embodiments, such compound is a cyclooxygenase inhibitor, such as a non-steroidal anti-inflammatory drug (NSAID). According to more particular embodiments, the NSAID is selected from the group consisting of Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac, Nabumetone, Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid, Salsalate (Disalcid), Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, and Nimesulide.

In one embodiment of the Present Invention, the at least one Composition including the protein/peptide antigen, or combination of protein/peptide antigens, will be formulated by mixing the individual GMP produced protein/peptide solutions together in a Formulation Buffer. The combined protein solution will then be sterile filtered into a closed formulation vessel with constant mixing, after which a sterile solution of adjuvant will be added to the formulation vessel. The protein containing particles will then be aseptically added to sterile and depyrogenated glass injection vials or prefilled syringes and sealed. Protein adsorbed articles can be drawn aseptically from each vial for injection into patient or subject tissue.

The proportions of combined proteins in the formulation can vary although most preferred is equal proportions by gram weight. If used, the proportion of Influencing Antigen should be at least equal to the combined mass of the other antigen proteins or peptides. The proportion of Influencing antigen can be greater than the combined mass of the other antigens. The Formulation Buffer can be an Isotonic Phosphate Buffered Mannitol buffer. The adjuvant can be Aluminum hydroxide (alum), a liposome, poly(lactide-co-glycolide) microparticles, or saline.

The medicaments, pharmaceutical Compositions or therapeutic combinations according to the present invention may be in any form suitable for the application to humans and/or animals, preferably humans including infants, children and adults and can be produced by standard

procedures known to those skilled in the art. The medicament, (pharmaceutical) Composition or therapeutic combination can be produced by standard procedures known to those skilled in the art, e.g. from the table of contents of "Pharmaceutics: The Science of Dosage Forms", Second Edition, Aulton, M.E. (ED. Churchill Livingstone, Edinburgh (2002); "Encyclopedia of Pharmaceutical

5   Technology", Second Edition, Swarbrick, J. and Boylan 15 J.C. (Eds.), Marcel Dekker, Inc. New York (2002); "Modern Pharmaceutics", Fourth Edition, Banker G.S. and Rhodes C.T. (Eds.) Marcel Dekker, Inc. New York 2002 y "The Theory and Practice of Industrial Pharmacy", Lachman L., Lieberman H. And Kanig J. (Eds.), Lea & Febiger, Philadelphia (1986). The respective descriptions are hereby incorporated by reference and form part of the disclosure. The terms "medicament",

10   "pharmaceutical Composition" and "pharmaceutical formulations" may be used interchangeably.

The medicament, pharmaceutical Composition or therapeutic combination according to the present invention may further comprise one or more pharmaceutically acceptable excipients. Carriers, diluents and excipients which are suitable for the preparation of a medicament, pharmaceutical Composition or therapeutic combination according to the present invention are well known to those

15   skilled in the art, e.g. from the "Handbook of Pharmaceutical Excipients" Sixth Edition, Raymond C. Rowe, Paul J. Sheskey and Marian E Quinn (Eds.),American Pharmaceutical Association (July 2009), which is hereby incorporated by reference and forms part of the disclosure.

Autoantigens

20   Autoantigens suitable for use in the methods, compositions, and kits according to the present invention are beta cell autoantigens.

These include: glutamic acid decarboxylase (GAD65 or GAD 67 or GAD32) (Baekkeskov et al., Nature (1990) 347: 151)); Insulin (Palmer et al., Science (1983) 222: 1337; including the B9-23 peptide comprising amino acids 9-23 of the insulin B chain (Daniel et al., Proc. Natl. Acad. Sci. USA (1995) 93:

25   956-960; Wong et al., Nat. Med. (1999) 5: 1026-1031) Proinsulin; including the B24-C36 peptide comprising amino acids 24-36 spanning the proinsulin B-chain C-peptide junction (Chen et al., J. Immunol. (2001) 167: 4926-4935; Rudy et al., Mol. Med., (1995) 1: 625-633); HSP60 (heat shock protein 60, Raz et al., Lancet (2001), 358:1749-53); ICA512/IA-2 (islet cell antigen 512; Rabin et al., J. Immunol. (1994) 152: 3183), insulinoma antigen-2, ZnT8, islet-specific glucose-6-phosphate catalytic

30   subunit-related protein (IGRP), chromogranin A, B chain insulin, preproinsulin, proinsulin II, a proinsulin peptide without a cytotoxic T-lymphocyte epitope, insulin C13-A5 peptide, islet cell antigen p69, or any peptide, derivative including citrullinated forms iDS, and corresponding nucleotides of the above.

For an alignment of insulin sequences between species, see Table I in Homann et al., J. Immunol.

35   (1999) 63: 1833-1838.

According to particular embodiments, the beta cell autoantigen is GAD, such as GAD-65 or GAD-67, including fragments thereof, derivatives thereof, or a nucleic acid coding therefor.

According to more particular embodiments, the beta cell autoantigen is GAD-65, a fragment thereof, derivative thereof, or a nucleic acidcoding therefor. According to more particular embodiments, the

beta cell autoantigen is GAD-65. According to other particular embodiments, the beta cell autoantigen is a fragment derived from GAD-65 (i.e. a GAD65 fragment.

According to other particular embodiments, the beta cell autoantigen is insulinoma antigen-2.

According to other particular embodiments, the beta cell autoantigen is ZnT8.

5   According to other particular embodiments, the beta cell autoantigen is islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP).

According to other particular embodiments, the beta cell autoantigen is chromogranin A.

According to certain other embodiments, the beta cell autoantigen is insulin.

According to certain other embodiments, the beta cell autoantigen is B chain insulin.

10   According to certain other embodiments, the beta cell autoantigen is proinsulin.

According to certain other embodiments, the beta cell autoantigen is preproinsulin.

In some aspects of the present invention, a tolerogen is administered to the treated subject. A tolerogen is an antigen that induces a state of specific immunological unresponsiveness to
15   subsequent challenging doses of the antigen. Suitable tolerogens for use in the present invention are native endogenous human proteins and other molecules that are abundantly available and exposed to the immune system and generally recognized as self. Examples of tolerogens include IL-10, Human Serum Albumin or hemoglobin, or gamma-amino butyric acid.

The autoantigens or tolerogens for use in the invention may be administered as full-length proteins,
20   or they may alternatively be administered as fragments or variants of such full-length proteins with the proviso that the fragments or variants of autoantigens have at least one epitope conserved relative to the original autoantigen and are effective in the methods according to the present invention.

A fragment of a protein autoantigen or tolerogen has the same amino acid sequence as the original
25   autoantigen or tolerogen, but lacks at least one N-terminal or C-terminal amino acid residue. A fragment of an autoantigen should comprise at least one relevant epitope of the original autoantigen. Autoantigen and tolerogen fragments preferably have a length of at least 8 amino acids, such as at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids.

A variant of a protein autoantigen or tolerogen may have an amino acid sequence that is less than
30   100% identical to the original autoantigen or tolerogen, such as 99%, 95%, 90%, 85%, 80%, 70%, 60% or 50% identical (as compared by a protein sequence alignment tool, e.g. Clustal Omega available from the European Bioinformatics Institute, Hinxton, GB), while, for autoantigens, at the same time having at least one relevant epitope conserved relative to the original autoantigen. Variants may also have shorter (i.e. be a fragment) or longer amino acid sequences as compared to the original
35   antigen or tolerogen.

In the invention, the administration of an autoantigen or tolerogen can involve an autoantigen or tolerogen that comprises a protein or a peptide fragment of the original protein. It may also involve the administration of a variant of the autoantigen or tolerogen.

Further, the protein or peptide can be introduced into a subject as a protein or peptide in a pharmaceutically acceptable carrier or the protein or peptide can be encoded by an expression vector, where the expression vector is introduced (for example, see the Examples where the self-antigen is expressed in a subject by a pCMV-expression vector). Such an expression vector may be a nucleic acid, such as DNA or RNA, and may be delivered by needle injection, gene guns, jet injection or with the aid of cytofectin as known in the art. The nucleic acid may be formulated in saline, on gold beads, in liposomes or in lipid formulations. For further description regarding the administration of self-antigens via their expression vectors, (and for specific antigens that can be administered) see U.S. Patent Publication US 2002/0107210, which is hereby incorporated by reference.

IL-10 inducing compounds

In some aspects, the methods, compositions and kits of the present invention use IL-10 inducing compounds.

According to certain embodiments, the at least one IL-10 inducing compound is vitamin-D, such as 1,25-Dihydroxyvitamin D.

According to certain other embodiments, the at least one IL-10 inducing compound is a vitamin-D analog, such as TX527.

According to certain other embodiments, the at least one IL-10 inducing compound include enhancement of serum vitamin D by means of UVB radiation.

According to certain other embodiments, the at least one IL-10 inducing compound is a tyrosine kinase inhibitor, such as dasatinib, bosutinib, saracatinib, imatinib, sunitinib, or combinations thereof.

According to particular embodiments, the tyrosine kinase inhibitor is dasatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is bosutinib.

According to other particular embodiments, the tyrosine kinase inhibitor is saracatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is imatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is sunitinib.

According to other particular embodiments, the tyrosine kinase inhibitor is a combination of at least two of dasatinib, bosutinib, saracatinib, imatinib and sunitinib. For example, the tyrosine kinase inhibitor may be a combination of dasatinib and bosutinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and saracatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and imatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a

combination of dasatinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and saracatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and imatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of

5   bosutinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of imatinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib, bosutinib and saracatinib.

The Composition according to the invention may comprise more than one IL-10 inducing compound. Thus, according to certain embodiments, the Composition comprises at least two IL-10 inducing

10   compounds. According to certain other embodiments, the Composition comprises at least three IL-10 inducing compounds. According to certain other embodiments, the Composition comprises at least four IL-10 inducing compounds.

Cyclooxygenase inhibitors

In some aspects, the methods, compositions and kits of the present invention use one or more

15   cyclooxygenase inhibitors.

These cyclooxygenase inhibitors may be non-steroidal anti-inflammatory drugs (NSAID). According to more particular embodiments, the NSAID is selected from the group consisting of Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac,

20   Nabumetone, Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid, Salsalate (Disalcid), Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, and Nimesulide.

According to certain embodiments, the cyclooxygenase inhibitor is a propionic acid derivative, such

25   as Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin or Loxoprofen.

According to certain embodiments, the cyclooxygenase inhibitor is a Acetic acid derivative, such as Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac or Nabumetone.

According to certain embodiments, the cyclooxygenase inhibitor is a Salicylate, such as Aspirin

30   (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid or Salsalate.

According to certain embodiments, the cyclooxygenase inhibitor is an enolic acid (Oxicam) derivative, such as Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam or Isoxicam.

According to certain embodiments, the cyclooxygenase inhibitor is an anthranilic acid derivative, such as Mefenamic acid, Meclofenamic acid, Flufenamic acid or Tolfenamic acid.

35   According to certain embodiments, the cyclooxygenase inhibitor is selective COX-2 inhibitor, such as Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib or Etoricoxib.

According to more particular embodiments, the cyclooxygenase inhibitor is Ibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Dexibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Naproxen.

According to more particular embodiments, the cyclooxygenase inhibitor is Fenoprofen

According to more particular embodiments, the cyclooxygenase inhibitor is Ketoprofen.

5   According to more particular embodiments, the cyclooxygenase inhibitor is Dexketoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Flurbiprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Oxaprozin.

According to more particular embodiments, the cyclooxygenase inhibitor is Loxoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Indomethacin.

10   Ibuprofen mainly blocks cox-2 but to some extent also cox-1. Ibuprofen has a somewhat broader
         effect on the immune system than a narrow IL-1 blocker and a quite pronounced anti-inflammatory
         effect without serious risks. Use of Ibuprofen dampens beta cell inflammation and enables the Vit D
         enforced DCs to induce tolerance to peptides from autoantigens presented to T-cells, thereby
         protecting the beta cells in the subject.

15

CTLA4 compounds

In some aspects, the methods, compositions and kits of the present invention use a CTLA-4
compound, such as a cytotoxic T-lymphocyte–associated antigen 4 immunoglobulin.

According to more particular embodiments, the CTLA-4 compound is abatacept.

20   Abatacept (an Fc modified CTLA4 immunoglobulin) is a Tcell depleting, immunomodulating, fusion
         protein consisting of the extracellular portion of human CTLA4 and the heavy chain of human IgG1. It
         blocks the costimulatory signal involved in activation of naïve Tcells. Its ligation with CD80/86 on
         APCs may also interfere with and reduce CD80/86 induced IL-6, which may downregulate
         inflammatory cytokines such as IL-1beta, IFNgamma, and IL-17. Further Abatacept ligation with
25   CD80/86 may induce indoleamine dioxygenase (IDO) in APCs, which may in turn may induce anergy
         in Tcells, as well as downregulate paracrine activation of naïve Tcells by activated Tcells. CD80/86
         expressed on B cells may yet be a further path where abatacept may carry out an
         immunomodulating function.

30   _TNFalpha inhibitors_

In some aspects, the methods, compositions and kits of the present invention use a TNF alpha
inhibitor, such as Adalimumab, Certolizumab, Etanercept, Golimumab or Infliximab. According to
more particular embodiments, the TNF alpha inhibitor is Adalimumab. According to other more
particular embodiments, the TNF alpha inhibitor is Certolizumab. According to other more particular

embodiments, the TNF alpha inhibitor is Etanercept. According to other more particular embodiments, the TNF alpha inhibitor is Golimumab. According to other more particular embodiments, the TNF alpha inhibitor is Infliximab.

Kits

5   The invention also provides kits relating to the methods of the invention. For example, in one aspect, a kit can comprise: (a) an anti-inflammatory compound; (b) an autoantigen; and (c) vitamin D and optionally d) instructions for the regimen of the invention.

The present invention also discloses a pharmaceutical kit comprising

i) a composition comprising a beta cell autoantigen, and at least one of

10   iia) a composition comprising an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and

iib) a composition comprising a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

15   The present invention also discloses a pharmaceutical kit comprising

i) a composition comprising a beta cell autoantigen, and at least one of

iia) a composition comprising an IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid, and gamma-amino butyric acid analogs; and

20   iib) a composition comprising a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

The present invention also discloses the use of Compositions comprising at least two of:

25   i)   at least one antigen or fragments thereof; or nucleic acids coding for such molecules related to at least one of the autoimmune and inflammatory diseases in the group as listed herein; and

ii)   at least one IL-10 inducing compound selected from the group: vitamin-D such as 1,25-Dihydroxyvitamin D. According to certain other embodiments, the at least one IL-10 inducing compound is a vitamin-D analog, such as TX527. According to certain other embodiments, the at least one IL-10 inducing compound is a tyrosine kinase inhibitor, such as dasatinib, bosutinib, saracatinib, imatinib, sunitinib, or combinations thereof; and

iii)   at least one compound that reduces the immune system's ability to activate naïve TCs and BCs and to recall responses from activated and memory lymphocytes, such as a COX-inhibitor; a CTLA-4 compound; or a TNF alpha inhibitor; as listed herein);

in the manufacture of a Kit including at least two pharmaceutical Compositions (i.e. medicaments).

One of the at least two pharmaceutical Compositions can comprise the at least one antigen formulated in alum, saline or human serum albumin as an injectable in a prefilled vial or syringe, and another of the at least two pharmaceutical Compositions may comprise the IL-10 inducing compound in the form of tablets for oral administration.

Some embodiments of the Present Invention provide a Kit including at least two pharmaceutical Compositions, comprising i) at least one antigen selected from the group listed herein and ii) at least one compound that reduces the dendritic cells' ability to activate naïve lymphocytes or recall activated or memory lymphocytes selected from the group: COX-inhibitors; CTLA-4 compounds; and TNF alpha inhibitors. As an example, one of the at least two pharmaceutical Compositions can comprise the at least one antigen formulated in alum, saline or human serum albumin as injectable in a prefilled vial or syringe, and another of the at least two pharmaceutical Compositions may comprise one compound selected from the group COX-inhibitors; a CTLA-4 compound; and TNFα inhibitors.

Some embodiments of the Present Invention provide a Kit including at least three pharmaceutical Compositions, comprising i) at least one antigen selected from the group listed herein; ii)at least one IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs and tyrosine kinase inhibitors. and iii) at least one compound that reduces the dendritic cells' ability to activate naïve lymphocytes or recall activated or memory lymphocytes selected from the group: COX-inhibitors; CTLA-4 compounds; and TNF alpha inhibitors. As an example, one of the at least three pharmaceutical Compositions can comprise the at least one antigen formulated in alum, saline or human serum albumin as an injectable in a prefilled vial or syringe, and another of the at least three pharmaceutical Compositions may comprise at least one IL-10 inducing compound selected from the group consisting of vitamin-D, vitamin-D analogs, gamma-amino butyric acid, gamma-amino butyric acid analogs, and tyrosine kinase inhibitors, and a third of the at least three pharmaceutical Compositions may comprise one compound selected from the group COX-inhibitors; a CTLA-4 compound; and TNFα inhibitors.

Medical use

The composition of the present invention may be used in therapy, in particular in the prevention and/or treatment of an autoimmune disease. Accordingly, the composition of the present invention may be a pharmaceutical composition.

According to certain embodiments, the composition is for use as medicament, such as for use in the prevention and/or treatment of an autoimmune disease.

According to particular embodiments, the composition is for use in the prevention and/or treatment of type 1 diabetes, such as type 1.

According to other particular embodiments, the composition is for use in the prevention and/or treatment of autoimmune diabetes.

According to other particular embodiments, the composition is for use in the prevention and/or treatment of latent autoimmune diabetes.

5   According to yet other particular embodiments, the composition is for use in the prevention and/or treatment of recurrence of autoimmune diabetes, such as recurrence of autoimmune diabetes in an individual (e.g. a human) with autoimmune diabetes that has been subject to islet cell transplantation or other cell therapies including stem cell therapy.

The present invention provides in a further aspect an autoantigen in combination with at least one
10   IL-10 inducing compound for use in the prevention and/or treatment of an autoimmune disease.

More particularly, the present invention provides an autoantigen selected from the group consisting of insulin, B chain insulin, proinsulin, and beta cell autoantigens, in combination with at least one IL-10 inducing compound, such as at least one IL-10 inducing compound selecting from the group consisting of vitamin-D, vitamin-D analogs and tyrosine kinase inhibitors, for use in the prevention
15   and/or treatment of an autoimmune disease.

According to certain embodiments, the autoantigen is a beta cell autoantigen, such as, glutamic acid decarboxylase (GAD), insulinoma antigen-2, ZnT8, islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP) or chromogranin A.

According to particular embodiments, the beta cell autoantigen is GAD, such as GAD-65 including
20   fragments thereof, derivatives thereof, or a nucleotide coding therefor.

According to more particular embodiments, the beta cell autoantigen is GAD-65, a fragment thereof, derivative thereof, or a nucleotide coding therefor. According to more particular embodiments, the beta cell autoantigen is GAD-65. According to other particular embodiments, the beta cell autoantigen is a fragment derived from GAD-65 (i.e. a GAD65 fragment.

25   According to other particular embodiments, the beta cell autoantigen is insulinoma antigen-2.

According to other particular embodiments, the beta cell autoantigen is ZnT8.

According to other particular embodiments, the beta cell autoantigen is islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP).

According to other particular embodiments, the beta cell autoantigen is chromogranin A.

30   According to certain embodiments, the autoantigen is insulin.

According to certain other embodiments, the autoantigen is B chain insulin..

According to certain other embodiments, the autoantigen is proinsulin.

According to certain embodiments, the autoantigen is formulated in an adjuvant.

According to particular embodiments, the adjuvant is alum.

According to certain embodiments, the at least one IL-10 inducing compound is vitamin-D, such as 1,25-Dihydroxyvitamin D.

According to certain other embodiments, the at least one IL-10 inducing compound is a vitamin-D analog, such as TX527.

According to certain other embodiments, the at least one IL-10 inducing compound is a tyrosine kinase inhibitor, such as dasatinib, bosutinib, saracatinib, imatinib, sunitinib, or combinations thereof.

According to particular embodiments, the tyrosine kinase inhibitor is dasatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is bosutinib.

According to other particular embodiments, the tyrosine kinase inhibitor is saracatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is imatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is sunitinib.

According to other particular embodiments, the tyrosine kinase inhibitor is a combination of at least two of dasatinib, bosutinib, saracatinib, imatinib and sunitinib. For example, the tyrosine kinase inhibitor may be a combination of dasatinib and bosutinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and saracatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and imatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and saracatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and imatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of imatinib and sunitinib According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib, bosutinib and saracatinib.

According to certain embodiments, the autoantigen is further used in combination with a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

According to particular embodiments, such compound is a cyclooxygenase inhibitor, such as a non-steroidal anti-inflammatory drug (NSAID). According to more particular embodiments, the NSAID is selected from the group consisting of Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac, Nabumetone, Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid, Salsalate (Disalcid), Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, and Nimesulide.

According to certain embodiments, the cyclooxygenase inhibitor is a propionic acid derivative, such as Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin or Loxoprofen.

According to certain embodiments, the cyclooxygenase inhibitor is a Acetic acid derivative, such as Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac or Nabumetone.

According to certain embodiments, the cyclooxygenase inhibitor is a Salicylate, such as Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid or Salsalate.

According to certain embodiments, the cyclooxygenase inhibitor is an enolic acid (Oxicam) derivative, such as Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam or Isoxicam.

According to certain embodiments, the cyclooxygenase inhibitor is an anthranilic acid derivative, such as Mefenamic acid, Meclofenamic acid, Flufenamic acid or Tolfenamic acid.

According to certain embodiments, the cyclooxygenase inhibitor is selective COX-2 inhibitor, such as Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib or Etoricoxib.

According to more particular embodiments, the cyclooxygenase inhibitor is Ibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Dexibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Naproxen.

According to more particular embodiments, the cyclooxygenase inhibitor is Fenoprofen

According to more particular embodiments, the cyclooxygenase inhibitor is Ketoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Dexketoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Flurbiprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Oxaprozin.

According to more particular embodiments, the cyclooxygenase inhibitor is Loxoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Indomethacin.

According to other particular embodiments, such compound is a CTLA-4 compound, such as a cytotoxic T-lymphocyte–associated antigen 4 immunoglobulin.

According to more particular embodiments, the CTLA-4 compound is abatacept.

According to other particular embodiments, such compound is a TNF alpha inhibitor, such as Adalimumab, Certolizumab, Etanercept, Golimumab or Infliximab. According to more particular embodiments, the TNF alpha inhibitor is Adalimumab. According to other more particular embodiments, the TNF alpha inhibitor is Certolizumab. According to other more particular embodiments, the TNF alpha inhibitor is Etanercept. According to other more particular embodiments, the TNF alpha inhibitor is Golimumab. According to other more particular embodiments, the TNF alpha inhibitor is Infliximab.

According to other particular embodiments, the autoantigen is for use in the prevention and/or treatment of latent autoimmune diabetes, such as latent autoimmune diabetes in a GAD-antibody positive individual.

According to particular embodiments, the autoimmune disease is type 1 diabetes.

5   According to other particular embodiments, the autoimmune disease is autoimmune diabetes.

According to other particular embodiments, the autoimmune disease is latent autoimmune diabetes.

According to other particular embodiments, the autoimmune disease is recurrence of autoimmune diabetes, such as recurrence of autoimmune diabetes in an individual (e.g. a human) with

10   autoimmune diabetes that has been subject to islet cell transplantation or other cell therapies including stem cell therapy.

Hence, the present invention provides a combination (e.g. a therapeutic combination) comprising at least one autoantigen and at least one IL-10 inducing compound.

More particularly, the present invention provides a combination (e.g. a therapeutic combination)

15   comprising i) at least one autoantigen selected from the group consisting of insulin, B chain insulin, proinsulin, and beta cell autoantigens, and ii) at least one IL-10 inducing compound selecting from the group consisting of vitamin-D, vitamin-D analogs and tyrosine kinase inhibitors.

According to certain embodiments, the at least one autoantigen is a beta cell autoantigen, such as, glutamic acid decarboxylase (GAD), insulinoma antigen-2, ZnT8, islet-specific glucose-6-phosphate

20   catalytic subunit-related protein (IGRP) or chromogranin A.

According to particular embodiments, the beta cell autoantigen is GAD, such as GAD-65 including fragments thereof, derivatives thereof, or a nucleotide coding therefor.

According to more particular embodiments, the beta cell autoantigen is GAD-65, a fragment thereof, derivative thereof, or a nucleotide coding therefor. According to more particular embodiments, the

25   beta cell autoantigen is GAD-65. According to other particular embodiments, the beta cell autoantigen is a fragment derived from GAD-65 (i.e. a GAD65 fragment.

According to other particular embodiments, the beta cell autoantigen is insulinoma antigen-2.

According to other particular embodiments, the beta cell autoantigen is ZnT8.

According to other particular embodiments, the beta cell autoantigen is islet-specific glucose-6-

30   phosphate catalytic subunit-related protein (IGRP).

According to other particular embodiments, the beta cell autoantigen is chromogranin A.

According to certain other embodiments, the at least one autoantigen is insulin.

According to certain other embodiments, the at least one autoantigen is B chain insulin.

According to certain other embodiments, the at least one autoantigen is proinsulin.

The combination according to the invention may comprise more than one autoantigen. Thus, according to certain embodiments, the combination comprises at least two autoantigens. According to certain other embodiments, the combination comprises at least three autoantigens. According to certain other embodiments, the combination comprises at least four autoantigens.

Therefore, according to particular embodiments, the combination comprises at least GAD, such as GAD-65, and insulin. According to other particular embodiments, the combination comprises at least insulinoma antigen-2 and insulin. According to other particular embodiments, the combination comprises at least ZnT8 and insulin. According to other particular embodiments, the combination comprises at least IGRP and insulin. According to other particular embodiments, the combination comprises at least chromogranin A and insulin.

According to other particular embodiments, the combination comprises at least GAD, such as GAD-65, and B chain insulin. According to other particular embodiments, the combination comprises at least insulinoma antigen-2 and B chain insulin. According to other particular embodiments, the combination comprises at least ZnT8 and B chain insulin. According to other particular embodiments, the combination comprises at least IGRP and B chain insulin. According to other particular embodiments, the combination comprises at least chromogranin A and B chain insulin.

According to other particular embodiments, the combination comprises at least GAD, such as GAD-65, and proinsulin. According to other particular embodiments, the combination comprises at least insulinoma antigen-2 and proinsulin. According to other particular embodiments, the combination comprises at least ZnT8 and proinsulin. According to other particular embodiments, the combination comprises at least IGRP and proinsulin. According to other particular embodiments, the combination comprises at least chromogranin A and proinsulin.

According to other particular embodiments, the combination comprises at least insulin and B chain insulin. According to other particular embodiments, the combination comprises at least insulin and proinsulin. According to other particular embodiments, the combination comprises at least B chain insulin and proinsulin.

According to particular embodiments, the combination comprises at least GAD, such as GAD-65, insulin and B chain insulin. According to other particular embodiment, the combination comprises at least GAD, such as GAD-65, insulin and proinsulin. According to yet other particular embodiments, the combination comprises at least GAD, such as GAD-65, B chain insulin and proinsulin. According to yet other particular embodiments, the combination comprises at least insulin, B chain insulin and proinsulin.

According to other particular embodiments, the combination comprises GAD, such as GAD-65, insulin, B chain insulin and B chain insulin.

According to certain embodiments, the at least one autoantigen, such as the at least two autoantigens or at least three autoantigens, is formulated in an adjuvant.

According to particular embodiments, the adjuvant is alum.

According to certain embodiments, the at least one IL-10 inducing compound is vitamin-D or a vitamin-D analog. According to particular embodiments, the at least one IL-10 inducing compound is vitamin-D.

According to more particular embodiments, the at least one IL-10 inducing compound is 1,25-
5   Dihydroxyvitamin D.

According to certain other embodiments, the at least one IL-10 inducing compound is a vitamin-D analog, such as TX527.

According to certain other embodiments, the at least one IL-10 inducing compound is a tyrosine kinase inhibitor, such as dasatinib, bosutinib, saracatinib, imatinib, sunitinib, or combinations
10   thereof.

According to particular embodiments, the tyrosine kinase inhibitor is dasatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is bosutinib.

According to other particular embodiments, the tyrosine kinase inhibitor is saracatinib.

According to other particular embodiments, the tyrosine kinase inhibitor is imatinib.

15   According to other particular embodiments, the tyrosine kinase inhibitor is sunitinib.

According to other particular embodiments, the tyrosine kinase inhibitor is a combination of at least two of dasatinib, bosutinib, saracatinib, imatinib and sunitinib. For example, the tyrosine kinase inhibitor may be a combination of dasatinib and bosutinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and saracatinib. According
20   to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and imatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and saracatinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and imatinib.
25   According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of bosutinib and sunitinib. According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of imatinib and sunitinib According to other more particular embodiments, the tyrosine kinase inhibitor is a combination of dasatinib, bosutinib and saracatinib.

The combination according to the invention may comprise more than one IL-10 inducing compound.
30   Thus, according to certain embodiments, the combination comprises at least two IL-10 inducing compounds. According to certain other embodiments, the combination comprises at least three IL-10 inducing compounds. According to certain other embodiments, the combination comprises at least four IL-10 inducing compounds.

Therefore, according to particular embodiments, the combination comprises at least Vitamin-D and
35   a tyrosine kinase inhibitor. According to other particular embodiments, the combination comprises at least a Vitamin-D analog and a tyrosine kinase inhibitor.

According to more particular embodiments, the combination comprises at least Vitamin-D and dasatinib. According to other more particular embodiments, the combination comprises at least Vitamin-D and bosutinib. According to other more particular embodiments, the combination comprises at least Vitamin-D and saracatinib. According to other more particular embodiments, the combination comprises at least Vitamin-D and imatinib. According to other more particular embodiments, the combination comprises at least Vitamin-D and sunitinib.

According to more particular embodiments, the combination comprises at least a Vitamin-D analog and dasatinib. According to other more particular embodiments, the combination comprises at least a Vitamin-D analog and bosutinib. According to other more particular embodiments, the combination comprises at least a Vitamin-D analog and saracatinib. According to other more particular embodiments, the combination comprises at least a Vitamin-D analog and imatinib. According to other more particular embodiments, the combination comprises at least a Vitamin-D analog and sunitinib.

According to certain embodiments, the combination further comprises iii) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a cyclooxygenase inhibitor, a CTLA-4 compound or a TNF alpha inhibitor.

According to particular embodiments, such compound is a cyclooxygenase inhibitor, such as a non-steroidal anti-inflammatory drug (NSAID). According to more particular embodiments, the NSAID is selected from the group consisting of Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac, Nabumetone, Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid, Salsalate (Disalcid), Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, and Nimesulide.

According to certain embodiments, the cyclooxygenase inhibitor is a propionic acid derivative, such as Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin or Loxoprofen.

According to certain embodiments, the cyclooxygenase inhibitor is a Acetic acid derivative, such as Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac or Nabumetone.

According to certain embodiments, the cyclooxygenase inhibitor is a Salicylate, such as Aspirin (acetylsalicylic acid), Diflunisal (Dolobid), Salicylic acid or Salsalate.

According to certain embodiments, the cyclooxygenase inhibitor is an enolic acid (Oxicam) derivative, such as Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam or Isoxicam.

According to certain embodiments, the cyclooxygenase inhibitor is an anthranilic acid derivative, such as Mefenamic acid, Meclofenamic acid, Flufenamic acid or Tolfenamic acid.

According to certain embodiments, the cyclooxygenase inhibitor is selective COX-2 inhibitor, such as Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib or Etoricoxib.

According to more particular embodiments, the cyclooxygenase inhibitor is Ibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Dexibuprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Naproxen.

According to more particular embodiments, the cyclooxygenase inhibitor is Fenoprofen

According to more particular embodiments, the cyclooxygenase inhibitor is Ketoprofen.

5   According to more particular embodiments, the cyclooxygenase inhibitor is Dexketoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Flurbiprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Oxaprozin.

According to more particular embodiments, the cyclooxygenase inhibitor is Loxoprofen.

According to more particular embodiments, the cyclooxygenase inhibitor is Indomethacin.

10   According to other particular embodiments, such compound is a CTLA-4 compound, such as a cytotoxic T-lymphocyte–associated antigen 4 immunoglobulin.

According to more particular embodiments, the CTLA-4 compound is abatacept.

According to other particular embodiments, such compound is a TNF alpha inhibitor, such as Adalimumab, Certolizumab, Etanercept, Golimumab or Infliximab. According to more particular
15   embodiments, the TNF alpha inhibitor is Adalimumab. According to other more particular embodiments, the TNF alpha inhibitor is Certolizumab. According to other more particular embodiments, the TNF alpha inhibitor is Etanercept. According to other more particular embodiments, the TNF alpha inhibitor is Golimumab. According to other more particular embodiments, the TNF alpha inhibitor is Infliximab.

20   The combination of the present invention may be used in therapy, in particular in the prevention and/or treatment of an autoimmune disease. Accordingly, the combination of the present invention may be a therapeutic combination.

According to certain embodiments, the combination is for use as medicament, such as for use in the prevention and/or treatment of an autoimmune disease.

25   According to particular embodiments, the combination is for use in the prevention and/or treatment of type 1 diabetes.

According to other particular embodiments, the combination is for use in the prevention and/or treatment of autoimmune diabetes.

According to other particular embodiments, the combination is for use in the prevention and/or
30   treatment of latent autoimmune diabetes.

According to yet other particular embodiments, the combination is for use in the prevention and/or treatment of recurrence of autoimmune diabetes, such as recurrence of autoimmune diabetes in an individual (e.g. a human) with autoimmune diabetes that has been subject to islet cell transplantation or other cell therapies including stem cell therapy.

The present invention provides in a further aspect the use of a composition or combination comprising i) at least one autoantigen, such as at least one autoantigen as detailed above, ii) at least one IL-10 inducing compound, such as at least one IL-10 as detailed above, and optionally iii) a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a compound

5   that reduces the dendritic cells' ability to activate naïve CD4+ Tcells as detailed above, in the manufacture of a medicament.

The present invention provides in a further aspect method(s) for the prevention and/or treatment of an autoimmune disease in an individual in need thereof, the method(s) comprises administering to said individual at least one autoantigen, such as at least one autoantigen as detailed above; and

10   administering to said individual at least one IL-10 inducing compound, such as at least one IL-10 as detailed above.

According to certain embodiments, the methods further comprise administering to said individual a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as a compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells as detailed above.

15   According to certain embodiments, the individual to be treated according to the present invention is a mammal.

According to particular embodiments, the individual to be treated according to the present invention is a human.

According to certain embodiments, the individual to be treated according to the present invention is

20   an adult.

According to particular embodiments, the individual to be treated according to the present invention is an adult human.

The medicaments, pharmaceutical compositions or therapeutic combinations according to the present invention may be in any form suitable for the application to humans and/or animals,

25   preferably humans including infants, children and adults and can be produced by standard procedures known to those skilled in the art. The medicament, (pharmaceutical) composition or therapeutic combination can be produced by standard procedures known to those skilled in the art, e.g. from the table of contents of "Pharmaceutics: The Science of Dosage Forms", Second Edition, Aulton, M.E. (ED. Churchill Livingstone, Edinburgh (2002); "Encyclopedia of Pharmaceutical

30   Technology", Second Edition, Swarbrick, J. and Boylan J.C. (Eds.), Marcel Dekker, Inc. New York (2002); "Modern Pharmaceutics", Fourth Edition, Banker G.S. and Rhodes C.T. (Eds.) Marcel Dekker, Inc. New York 2002 y "The Theory and Practice of Industrial Pharmacy", Lachman L., Lieberman H. And Kanig J. (Eds.), Lea & Febiger, Philadelphia (1986). The respective descriptions are hereby incorporated by reference and form part of the disclosure. The terms "medicament",

35   "pharmaceutical composition" and "pharmaceutical formulations" may be used interchangeably.

The pharmaceutical composition of the present invention may for example be administered parenterally, including intramuscular, intraperitoneal, or intravenous injection, transmucosal or sublingual application; or orally, including administration as tablets, pellets, granules, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions, sprays or as reconstituted dry

40   powdered form with a liquid medium.

Likewise, the at least one autoantigen used in accordance with the present invention may for example be administered parenterally, including intramuscular, intraperitoneal, or intravenous injection, transmucosal or sublingual application; or orally, including administration as tablets, pellets, granules, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions, sprays or as reconstituted dry powdered form with a liquid medium.

Likewise, the at least one IL-10 inducing compound used in accordance with the present invention may for example be administered parenterally, including intramuscular, intraperitoneal, or intravenous injection, transmucosal or sublingual application; or orally, including administration as tablets, pellets, granules, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions, sprays or as reconstituted dry powdered form with a liquid medium.

Likewise, the therapeutic combination of the present invention may for example be administered parenterally, including intramuscular, intraperitoneal, or intravenous injection, transmucosal or sublingual application; or orally, including administration as tablets, pellets, granules, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions, sprays or as reconstituted dry powdered form with a liquid medium.

According to certain embodiments, the at least one autoantigen is administered intrainguinal.

According to certain other embodiments, the at least one autoantigen is administered is intradermal or subcutaneous, such as in the stomach close to the pancreatic draining lymph nodes. According to particular embodiments, the at least one autoantigen is administered is intradermal. According to other particular embodiments, the at least one autoantigen is administered is subcutaneous.

The medicament, pharmaceutical composition or therapeutic combination according to the present invention may further comprise one or more pharmaceutically acceptable excipients.

Carriers, diluents and excipients which are suitable for the preparation of a medicament, pharmaceutical composition or therapeutic combination according to the present invention are well known to those skilled in the art, e.g. from the "Handbook of Pharmaceutical Excipients" Sixth Edition, Raymond C. Rowe, Paul J. Sheskey and Marian E Quinn (Eds.), American Pharmaceutical Association (July 2009), which is hereby incorporated by reference and forms part of the disclosure.

The at least one autoantigen and the at least one IL-10 inducing compound may be administered simultaneously or separately. According to certain embodiments, the at least one autoantigen and the at least one IL-10 inducing compound are administered simultaneously. According to certain embodiments, the at least one autoantigen and the at least one IL-10 inducing compound are administered separately.

According to certain embodiments, the at least one IL-10 inducing compound is administered prior to administration of the at least one autoantigen. According to particular embodiments, the at least one IL-10 inducing compound is administered at least one day, such as at least two days, at least three days, at least four days, at least five days, at least six days or at least one week, prior to the first administration of the at least one autoantigen. Hence, according to such embodiments, a course of IL-10 inducing compound(s) is commenced at least one day, such as at least two days, at least three days, at least four days, at least five days, at least six days or at least one week prior to first administration of the at least one autoantigen.

According to more particular embodiments, the at least one IL-10 inducing compound is administered at least one week, such as at least two weeks, prior to the first administration of the at least one autoantigen. Hence, according to such embodiments, a course of IL-10 inducing compound(s) is commenced at least one week, such as at least two weeks, prior to first
5   administration of the at least one autoantigen.

The compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells may be administered simultaneously or separately with the at least one autoantigen and/or the at least one IL-10 inducing compound. According to certain embodiments, the CTLA-4 compound is administered simultaneously with the at least one autoantigen. According to certain other embodiments, the
10   compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells is administered separately to the at least one autoantigen.

According to certain embodiments, the at least one IL-10 inducing compound and the compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells are administered simultaneously. According to certain embodiments, the at least one IL-10 inducing compound and
15   the compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells are administered separately.

According to particular embodiments, the compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells, such as for example 10mg/kg abatacept, is administered +/- 7 days around the time of first administration of the at least one autoantigen.

20   According to certain embodiments, administration of the at least one autoantigen and administration of the compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells is repeated on day 14, 28 and 45 +/- one week as the case may be to ensure blockage of CD28 on Tcells.

According to certain embodiments, at least one 2-20ug dose GAD, such as GAD-65, optionally
25   formulated in alum, is administered per treatment occasion.

According to certain embodiments, at least one 1000-2000 IU vitamin D, such as Vitamin D3, is administered per treatment occasion.

According to certain embodiments, APCs are made more tolerogenic by administering between 7,000 -70,000 IU of Vitamin D, such as Vitamin D3, per week for between 1-10 weeks prior to first
30   administration of the at least one autoantigen and for 4 weeks after last administration of the at least one autoantigen.

According to certain embodiments, administrations of GAD, such as GAD-65, optionally formulated in alum, are made approximately one week following each administration of the compound that reduces the dendritic cells' ability to activate naïve CD4+ Tcells.

35   According to certain embodiments, at least one 400 mg dose of NSAID, such as Ibuprofen, is administered per treatment occasion.

The invention assumes the understanding of conventional molecular biology methods that include techniques for manipulating polynucleotides that are well known to the person of ordinary skill in

the art of molecular biology. Examples of such well known techniques can be found in Molecular Cloning: A Laboratory Manual 2nd Edition, Sambrook et al., Cold Spring Harbor, N.Y. (1989). Examples of conventional molecular biology techniques include, but are not limited to, in vitro ligation, restriction endonuclease digestion, PCR, cellular transformation, hybridization,

5   electrophoresis, DNA sequencing, and the like.

The invention also assumes the understanding of conventional immunobiological methods that are well known to the person of ordinary skill in the art of immunology. Basic information and methods can be found in Current Protocols in Immunology, editors Bierer et al., 4 volumes, John Wiley &

10   Sons, Inc., which includes teachings regarding: Care and Handling of Laboratory Animals, Induction of Immune Responses, In Vitro Assays for Lymphocyte Function, In Vivo Assays for Lymphocyte Function, Immunofluorescence and Cell Sorting, Cytokines and Their Cellular Receptors, Immunologic Studies in Humans, Isolation and Analysis of Proteins, Peptides, Molecular Biology, Biochemistry of Cell Activation, Complement, Innate Immunity, Animal Models for Autoimmune and

15   Inflammatory Disease (which includes chapters on the NOD mouse model, the SLE mouse model (for lupus), and induction of autoimmune disease by depletion of regulatory T cells), Antigen Processing and Presentation, Engineering Immune Molecules and Receptors, Ligand-Receptor Interactions in the Immune System, Microscopy, and Abbreviations and Terminology for common immune system genes and proteins, including the CD system for Leukocyte Surface Molecules.

20

Examples

The following examples disclose studies that may be performed in order to establish the safety and efficacy of various aspects of the present invention. The beta cell autoantigen used in the examples is glutamic acid decarboxylase (GAD), and may be replaced with any other beta cell autoantigen as

25   described herein.

Example 1
Clinical Trial in Patients with Recent Onset Type 1-DiabetesStudy Design:
The study is a 4-arm, randomized, double-blind, placebo-controlled, multicenter, clinical trial. Patients in arm A received oral 400 mg Ibuprofen per day for 90 days every morning. From Day 1 the

30   patients also received oral 2000 IU vitamin D per day during 15 months (i.e. 25 drops per day), and 2 subcutaneous injections in the stomach area of 20 µg Diamyd® (a GAD-based diabetes therapy) in a prime-and-boost regimen Day 15 and 45.

Arm B received oral 2000 IU vitamin D per day during 15 months (i.e. 25 drops per day), and 2 subcutaneous injections of 20 µg Diamyd in a prime-and-boost regimen Day 15 and 45.

35   Arm C received oral 2000 IU vitamin D per day during 15 months (i.e. 25 drops per day), and receive 2 subcutaneous injections of 20 µg Diamyd at two different sites (which gives a total of 40 µg Diamyd per occasion) in a prime-and-boost regimen Day 15 and 45.

Arm D received placebo.

The patients will be followed for a total of 30 months with a blinded study period of 6 months.

40   *Description of Treatment Groups:*

42

The patients were assessed for eligibility at the screening visit (Visit 1) 2 to 4 weeks prior to the start of the treatment. On Visit 2 (Day 1, baseline), patients eligible for the study were randomized to 1 of 4 treatment groups:

5   ▪ 15 patients were assigned to receive Ibuprofen as oral suspension; 400 mg every morning from Day 1 through 90 and from Day 1 through 450 also oral drops of Vitamin D 2000 IU per day (i.e. 25 drops per day). In addition 1 subcutaneous injection with 20 µg Diamyd and one with placebo given at two different sites in the stomach area, each on Days 15 and 45, i.e., prime and booster dose (providing a total dose of 40 µg Diamyd).

10

▪ 15 patients were assigned to receive placebo as oral suspension every morning from Day 1 through 90 and from Day 1 through 450 also oral drops of Vitamin D 2000 IU per day (i.e. 25 drops per day). In addition 1 subcutaneous injection with 20 µg Diamyd and one with placebo given at two different sites in the stomach area, each on Days 15 and 45, i.e., prime and booster dose (providing a total

15   dose of 40 µg Diamyd).

▪ 15 patients were assigned to receive placebo as oral suspension every morning from Day 1 through 90 and from Day 1 through 450 also oral drops of Vitamin D 2000 IU per day (i.e. 25 drops per day). In addition 2 subcutaneous injections with 20 µg Diamyd given at two different sites in the stomach

20   area, each on Days 15 and 45, i.e., prime and booster doses (providing a total dose of 80µg Diamyd).

▪ 15 patients were assigned to receive placebo as oral suspension every morning from Day 1 through 90 and placebo oral drops from Day 1 through 450, and 2 subcutaneous injections with placebo (given at two different sites in the stomach area), each on Days 15 and 45.

25

Efficacy Variables:
The primary and secondary efficacy endpoints include
- •   Change in C-peptide (90 minute value and AUCmean 0-120 min) during an MMTT.
- •   Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L
30 •   Biomarkers, mechanistic data.
- •   Fasting C-peptide, change between baseline and 6 month.

Safety Variables:
The safety assessment includes observation of reactions at the injection site, GAD65Ab titer,
35   occurrence of adverse events (AEs), laboratory measurements, vital signs, neurological assessments, and limited physical examination.

Results from 20 patients that completed 6 months in the study show that improved beta cell function is seen for the patient group having received the diamyd involving treatment compared to the placebo treated group.

	GADA+		C-Peptide						Max (6 month) − Max (baseline)
			Visit 2			Visit 6			
PatientID	Visit 3	Visit 5	60 min	90 min	120 min	60 min	90 min	120 min	
Identified as Placebo group	1st inj	3mths							
3	678	228,5	0.23	0.28	0.27	0.13	0.11	0.11	-0.15
4	2053	2117	0.71	0.75	0.68	0.77	0.69	0.64	0.02
31	465,8	345,3	0.77	0.79	0.72	0.63	0.73	0.69	-0.06
63	1378	1187	0.86	1,00	1,01		0.60		-0.41
49	118,6	114	0.90	0.91	0.90	0.66	0.82	1,07	0.16
48	555	1187	1,29	1,40	1,88	1,40	1,55	1,58	-0.30
9	61,4	224	0.35	0.38	0.34	0.15	0.17	0.15	-0.21
Mean (max-max)									-0.14
Identified as active group	1st injection	3mths							
1	964	5460	0.91	0.96	1,03	0.95	1,44	1,47	0.44
6	992	2622	0.48	0.44	0.51	0.41	0.41	0.33	-0.10
17	266,7	2408	0.53	0.48	0.51	0.53	0.55	0.59	0.06
25	109,3	1823	0.61	0.70	0.70	0.77	0.81	0.70	0.11
28	393,7	1931	0.67	0.82	0.76	0.84	0.87	0.88	0.06
33	340.4	1095	0.58	0.64	0.64	0.61	0.62	0.66	0.02
35	125,8	4300	0.96	1,14	1,16	0.75	0.72	0.79	-0.37
40	604	3506	0.37	0.44	0.53	0.15	0.12	0.12	-0.38
41	2266	4230	0.52	0.42	0.40	0.20	0.17	0.13	-0.32
46	132,0	3485	0.65	0.82	0.88	0.32	0.49	0.51	-0.37
47	135,1	3574	1,49	1,71	1,58	1,08	1,30	1,66	-0.05
53	967	4104	0.90	0.84	0.85	0.97	0.94	0.78	0.07
54	162,4	2035	0.77	0.69	0.68	0.81	0.87	1,01	0.24
Mean (max-max)									0.04538

5

Example 2

Pilot Trial to preserve residual insulin secretion in adults with recent-onset Type 1 diabetes by giving GAD-antigen (Diamyd®) therapy into lymph nodes. (DIAGNODE)

1.1 Background and rationale

The incidence of Type 1 diabetes (T1D) in children is next to Finland highest in Sweden in the world, and is increasing rapidly. T1D is by far the most common

chronic, serious, life-threatening disease among children and adolescents in our country, and the incidence of Type 1 diabetes is high also in young adults. The disease tends to become an extremely serious global problem. The disease is characterized by lack of insulin. Even though several patients at diagnosis have rather impressive residual beta cell function (1) the deficiency becomes soon very pronounced and finally complete (2,3). Residual insulin secretion is of crucial importance. In rare cases the beta cell function improves so much shortly after diagnosis that glucose metabolism normalizes and no insulin is required for some time, that is the patient goes into so called complete remission (4). As long as the patient is in a complete remission there is no need of active treatment, more than perhaps some recommendation of sound life style regarding physical exercise and diet. There are no symptoms, no acute complications and if somebody stayed in complete remission it is unlikely that such an individual would ever develop late complications. Slight abnormality of glucose or lipid metabolism might increase the risk of macrovascular complications in the same way as for individuals with chemical diabetes or impaired glucose tolerance. Complete remission is rare, but partial remission it is not (4). During this period the patient usually has near normal blood glucose values, not even mild hypoglycemia and no episodes of ketoacidosis. The quality of life is very good as the patient feels well, children grow normally, few restrictions are needed, if any with regard to food, the patients can exercise with great variation without getting hypoglycemia, and experiences very good home blood glucose tests. Only some residual insulin secretion is enough to diminish the risk of ketoacidosis (5). Furthermore, it has been shown in the DCCT trial that even quite modest residual insulin secretion, a response to a beta cell stimulation with serum Cpeptide >0.20pmol/ml, plays an important role for prevention of complications (6). This effect may be due to the fact that residual insulin secretion should reasonably make it easier to reach good blood glucose balance, but it is also possible that Cpeptide per se has a physiological function. It has in fact been reported that Cpeptide influences vascular permeability, decreases leakage in retinal vessel, and not least has a positive effect on nerve function (7) although the effect of C-peptide per se still is under debate.

1.1.1 Factors influencing the natural course

At diagnosis of T1D it has been claimed that 80-90% of the beta cells in pancreas have been destroyed. However, the proof for this is scarce, and it may well be that the main problem is deterioration of function. Furthermore there is great difference between patients, as some have quite good residual insulin secretion and others have not. Shortly after diagnosis, especially when an active insulin treatment is given, there is an increase of C-peptide production, and at the same time an improvement of insulin sensitivity. Good metabolic control seems to improve the milieu and metabolism for the beta cells and the beta cell function is preserved, which in turn contributes to better metabolic control, and vice versa. The intensity of the autoimmune process plays a role, and it seems evident that children have a more aggressive immune process than adults with Type 1

diabetes, but it is still difficult to predict the course. Some studies have suggested that high concentrations of autoantibodies are followed by a more rapid loss of insulin secretion, while others have not found such a relationship, or even the opposite. No special signs of cell-mediated immunity have so far been proven to predict beta cell loss but our own studies have shown that disease

5   process is related to a T-helper-1 (Th-1) deviation of the immune system with increases of certain cytokines such as IFNg and decrease of IL-10, IL-13.

The effect of insulin treatment on beta cell function.

Active insulin treatment during the first period of the disease prolonged the partial remission long time ago, and this finding could be confirmed and validated by improved residual insulin secretion

10   (2). Intensified treatment seems to improve residual beta cell function at least for some time (8), but it may also have long-term positive effects (9). Active treatment has been shown not only to prevent or postpone diabetes in experimental animals, but studies have indicated that such treatment could prevent diabetes in high risk individuals (10). However, when tried at a larger scale in the Diabetes Prevention Trial, parenteral insulin treatment did not prevent diabetes (11). Oral treatment with

15   insulin might have an effect (12) and therefore further studies are needed.

1.1.2 Interventions

In the 1970ies it became clear that T1D is an autoimmune disease and therefore immune interventions were tried. We performed the first immune intervention studies in the world on diabetic children when we already 30 years ago used plasmapheresis in newly-diagnosed children

20   and adolescents with some positive effects (13). As a side effect of that treatment a new protein with the weight 64kD was found in plasma (14), which later showed to be Glutamic Acid Decarboxylase (GAD). The breakthrough, taken as a proof for the concept of immune intervention, was cyclosporin, which doubtless slowed down the autoimmune destructive process and gave improved residual insulin secretion, while other trials with immune suppression had minimal effect,

25   especially so in children (15, 16,17), or showed too serious adverse events or risks (18, 19). In an effort to modulate the immune system we used photopheresis. Although clear effects on the immune system were demonstrated in a double blind placebo-controlled trial (20), the clinical effect was minimal and almost no improvement of residual beta cell function could be seen (21). Thus, with no successful immune intervention, our interest was directed to protective agents such as

30   Nicotinamide and Diazoxide, with no or transient effect (22, 23, 24).

With increasing knowledge of the immune process leading to beta cell destruction, it has become possible to direct more precisely the immune intervention to target the important T-cells. Promising studies using anti-CD3antibodies in an attempt to block the destructive immune process have been performed. Results from both North- American and French trials with anti-CD3 have shown that it is

35   possible to block the destructive autoimmune process and thereby at least postpone the decline of the beta cell function (25,26). The decline of residual insulin secretion was significantly slowed down, but unfortunately it looks as if the decline was just delayed a year, and thereafter the declining C-peptide curve went parallel to the declining curve in the placebo group. Furthermore, a majority of the patients experience some Cytokine Release Syndrome (CRS), which may be quite

40   serious, and in addition a number of side effects were seen in most of the patients. We have participated in one of two recent Phase III trials (Protegé trial), which failed to reach the primary endpoint, although the arm with the most intense treatment indeed showed some preservation of

residual insulin secretion and lower insulin requirement to reach good HbA1c (27; Sherry, Hagopian, Ludvigsson et al Lancet 2011). New studies are needed but it is difficult to believe that this type of treatment alone will be the accepted solution for general clinical use. Even less likely is such a treatment accepted as a preventive treatment in otherwise healthy individuals of whom many never

5   would develop diabetes.

1.1.3 Immune therapy with auto-antigens

In the treatment of allergic diseases, immunotherapy with small amount of disease specific antigen has been efficiently used during many years.The mechanism for this treatment remains unclear, although immune modulation of the immune responses and induction of regulatory cells have been

10   suggested. In autoimmune diseases no such treatment has been successful, but should be tried (28). Experiments in diabetes prone animals have shown that treatment with a heat shock protein could delay or postpone development of diabetes. The use of Diapep277 peptide in a study in adults showed significant preservation of insulin secretion without almost any adverse events (29). Later trials in children and adolescents with T1D (30), however, have shown no effect. Studies with

15   Diapep277 treatment in so called LADA (Latent Autoimmune Diabetes in the Adult) are ongoing, and preliminary results (report at IDF, Dubai Dec 2011 and at ADA June 2012) suggests that treatment with Diapep277 may preserve beta cell function in adults with mild Type 1 diabetes. However, the results are a bit unclear, as there was a weak C-peptide preservation only seen after Glucagon stimulation, but no effect at all after Mixed Meal Tolerance Test, and there was no differences

20   whatsoever between the actively treated group and placebo in immune markers. Active treatment with insulin has been shown not only to prevent or postpone diabetes in experimental animals but preliminary open studies indicated that such treatment could prevent diabetes in high risk individuals (10). Insulin, clearly a beta cell specific auto-antigen, has been parentally administrated (DPT) to prevent diabetes in high risk individuals with no effect, while oral insulin administration

25   with the same purpose may have a slight effect (12).

1.1.4 Previous clinical studies with GAD-Alum

1.1.4.1 GAD-vaccination

GAD (Glutamic Acid Decarboxylase), can be regarded as an auto-antigen, as it is produced in the islets with increased release as response to beta cell stimulation. This protein has been shown to

30   deeply influence the autoimmune immune process (31,32,33,34). Several studies have shown that indeed GAD can prevent diabetes in experimental animals (35-42). The similarity of GAD with viral proteins may be important for the therapeutic action. The observed effect, even after the start of the immune process, suggests that it might be possible to expect the same effect in humans after the start of the immune process. In a phase II study in LADA patients the administration of one low

35   dose, Diamyd 20 µg, led to improved beta cell function for up to 2 years compared to the placebo treated group, with no side effects. Also other doses were tried: 4 µg showed no effect, 100 µg showed a similar effect as 20 µg, while 500 µg showed no effect. None of the doses showed any adverse events, still so after several years follow-up (43). Association with change in the ratio of CD4+CD25+/ CD4- CD25- cells was found, indicating a mechanism for the effect. With this promising

40   background we started a Phase II study in Type 1 diabetic patients 10-18 years with recent onset. Based on the idea that the treatment earlier had effect in slowly progressive LADA patients we included patients with up to 18 months duration of T1D diabetes at intervention. The patients were

randomized to either 20 µg GAD-alum (Diamyd) sc at Day 1 and 30, or placebo. The effect still after 30 months was remarkable, and clearly both statistically and clinically significant (44), with about half of the C-peptide decline in the GAD treated group compared with the placebo group. Patients with a diabetes duration < 3 months had a remarkably good effect with no or minimal decline of

5   beta cell function during the follow-up of the first 15 months. Almost all effect was seen in patients with < 6 months duration at vaccination. Even more, in contrast to other intervention treatments, this effect was gained with no adverse events at all, making the treatment very encouraging! Still after 48 months the patients treated with < 6 months duration hade significantly preserved C-peptide and still no adverse events (45). So far GAD-treatment looked very promising. Two Phase III

10   trials were performed, one European with Johnny Ludvigsson (JL) as PI, and one in USA with Jerry Palmer as PI and JL as coinvestigator. In the European trial 334 patients were recruited into three arms, one arm with GAD-alum (Diamyd) 20 µg at Day 1,30, 90 and 270 ,another arm with GADalum 20 µg at Day 1 and 30, and placebo at Day 90 and 270 and a third arm with Placebo at Day 1,30,90 and 270. Primary endpoint, serum C-peptide AUC after a Mixed Meal Tolerance Test (MMTT) at 15

15   months was not met! (C-peptide AUC p=0.1; Fasting C-peptide p=0.07) (46). This prompted the company (Diamyd Medical + Johnson&Johnson) to close the phase III trials early. However, the Phase III trial did show several positive effects. Thus statistically significant efficacy was seen in several pre-specified subgroups. Furthermore, 45 Swedish patients had passed the 30 month's visit when the study was stopped, and those 15 patients who had received two doses of GAD-alum

20   (Diamyd) 20 µg showed a significant preservation of C-peptide after 30 months compared with placebo! This is especially remarkable as the Swedish patients were the ones without efficacy after 15 months, while efficacy was found after 15 months in the non-Nordic countries.

1.1.4.2 Possible Reasons to the different results Phase II and Phase III

In Phase III randomization, patients receiving active drug were more often in the 10- 11 year age

25   group than in the 16-18 year age group whereas placebo was more frequent than active drug in the higher age group. This may have influenced the result. The Phase II patients were treated in March– April and those patients in Phase III who were treated in March-April had also significant effect of GAD-treatment. In the Phase II trial no vaccinations were accepted, but in Phase III Influenzavaccination was allowed. Unfortunately an epidemic of H1N1-flu lead to that almost all

30   patients were vaccinated, many of them in connection with the GAD-vaccinations.

In Sweden and Finland the vaccine contained squalen, suspected to influence the immune system towards auto-immunity, and in these two countries there was no efficacy of GAD-treatment, while the efficacy was significant in other European countries. Patients in Sweden who did not get the influenza vaccination close to the GAD-treatment, had better efficacy of GAD-treatment (46).

35   1.1.4.3 Ongoing DIABGAD-1 trial

Since Jan 2013 the Phase II DIABGAD-1 trial is ongoing in Sweden. This is a trial in 60 patients, 10-18 years old, with recent-onset Type 1 diabetes, who are treated in a double-blind placebo-controlled randomized study with GAD-alum 20 µg resp 40 µg given twice with 30 days interval, in combination with Vitamin D, 2000 units daily for 450 days, and Ibuprofen 400 mg daily for 90 days. Recruitment is

40   fulfilled and now closed. 60 patients are randomized and another 4 patients are screened, waiting for screening results.. There are so far no Serious Adverse Events judged as related to study medication, and very slight adverse events, not related to the treatment except for mild transient

reactions on the site of injection of GAD-alum. An interim analyses is planned already after 6 months follow-up of all patients while more extended analyses will be performed after 15 and 30 months.

1.1.5. Intra lymph-node immunotherapy

Antigen therapy aims at presenting the antigen to the T-cells in the lymph nodes to get a new
5   balance of the immune system and tolerance against the antigen. In the treatment of autoimmune diseases so far antigen has been given either orally, intranasally or subcutaneously, in order to present the antigen to antigen presenting/dendritic cells which in turn are expected to present the antigen to the Tcells of the immune system. However, animal studies have shown that intralymphatic injections induce a strong and relevant T-cell response (47,48) and in the allergy field
10   clinical studies have shown that presentation of the antigen/allergen directly into the lymph nodes seems to be more effective than traditional administration (49). Lower doses of the allergen can be used, the number of treatments can be radically reduced, and there have been no treatment-related adverse events. Inguinal lymphnodes are readily accessible in patients and the pain associated with the injection is rated as below that of venous puncture (50). With this background it is our aim to
15   study whether the same approach can be used in patients with the autoimmune form of Type 1 diabetes. For ethical reasons we will do the first pilot trial in adults.

1.2 Hypothesis

The encouraging results of the Phase II GAD trial and the partly positive results of the Phase III European Trial, support the concept that administration of GAD-alum (Diamyd) may decrease the
20   autoimmune process and contribute to preservation of residual insulin secretion ⬚ As previous studies have indicated that the dose should be somewhere between 20 and 100 μg Diamyd, a lower dose of 3 μg given three times should be adequate when administrated directly into lymph nodes. ⬚ Injection of GAD-alum (Diamyd) directly into lymph nodes will give no serious adverse events, have desired immunological effects and will (shown in future studies) improve efficacy

25   ### 2. Risk-Benefit Analyses

The incidence of Type 1 diabetes is next to Finland higher in Sweden than in any other country of the world. The incidence is continuously increasing for decades. The disease cannot be cured and cannot be prevented. In spite of a very heavy, intensive, expensive treatment many patients get life-threatening serious both acute and late complications, and the mortality is much increased. At
30   diagnosis many patients have some slight residual insulin secretion. As long as this is the case it is much easier to keep blood glucose stable, the incidence of hypoglycaemia decreases as well as the risk of ketoacidosis. Quality of life for the patient as well as for parents of children with diabetes is better as long as there is some residual insulin secretion.

Type 1 diabetes in adults differ from the disease in children and adolescents as the disease process
35   often is milder, the decline of residual insulin secretion slower, and it is easier to control blood glucose. However, there are still great similarities, similar treatment and complications, and preservation of beta cell function is also very important in adults.

It is evident that there is a great benefit of preservation of residual insulin secretion, and therefore therapies aiming at preservation of this function justifiy treatments that are quite heavy, even
40   dangerous and expensive. Thus it has been regarded as justified to treat Type 1 diabetes at onset

with drugs like monoclonal antibodies against the CD3-receptor, which causes adverse events in principally all patients, some even quite serious adverse events and risks. Even pure cytostatics have been used.

In our proposed study we use GAD-alum (Diamyd) 4µg x 3, a treatment which has been used in much larger doses given to children and adults with almost no adverse events seen during follow-up of thousands of patient years. In our study we plan to use a very low dose, which means that the general risk can be expected to be even lower, but the administration will be directly into a lymph node which might give local reactions. The effect on the immune system may become more pronounced but should not lead to any adverse effects. Previous studies in allergy (where one of the co-investigators, Helene Zachrisson has given the intra lymph node injections of alum-formulated allergens) have not shown any adverse effect neither generally nor locally). For safety reasons, as this is the first pilot trial ever with this type of autoantigen treatment into lymphnodes, we try first in adults who can give their free informed consent. Even though Type 1 diabetes in adults is somewhat milder than in younger patients, it is of great value to preserve beta cell function, and therefore the proposed treatment may be of great therapeutical value also for adult patients.

When summarizing the pros and cons, there is a clear possibility of therapeutic benefit of great importance, both for the participating patients, for patients in future studies, whereas the risk is very small. If these studies contribute to the development of a good treatment, this will be of enormous value for a great number of patients.

3. Aim of present study

Our aim of the present pilot study is to get information on whether GAD-alum can be given into lymph nodes without treatment related serious adverse events, to allow future Phase II-studies with the same technique to improve efficacy in preserving residual insulin secretion in Type 1 diabetes. Thus we want to see whether this treatment give any adverse events, and how treatment regimens influence the immune system, cause the desired Th-2 deviation, increase of T-regulatory cells, and hopefully indications of preservation of residual beta cell function. Based on the short-term results of this pilot study (6 month follow-up) we then may want to design a larger Phase II trial, to include younger patients and to get more robust information. The main long-term goal is then to find a treatment at onset of Type 1 diabetes in young patients which is tolerable for the patients, safe, and which can preserve residual insulin secretion and give the patients a better quality of life, with less acute complications and in the long run less risk of late complications.

4. Objectives

Objectives:

To evaluate the safety of giving Diamyd directly into lymph glands and to evaluate the immune response (51-54) and effect on preservation of endogenous insulin secretion, measured at baseline and after 6, 15 and 30 months.

5. Population

Adult patients with recent onset of Type 1 diabetes at Linköping university hospital are given information about the study and they are asked to participate in the trial.

5.1 Inclusion criteria

1. Informed consent given by patients and guardians/parents

2. Type 1 diabetes according to the ADA classification with < 6 months diabetes duration

3. Age 18.00-29.99 years at diagnosis of Type 1 diabetes

5   4. Fasting C-peptide ≥0.12 nmol/ml

5. Pos GADA but < 50 000 random units

6. Females must agree to avoid pregnancy and have a negative urine pregnancy test

7. Patients must agree to using adequate contraception, until 1 year after the last administration of GAD-Alum/Placebo

10   5.2 Exclusion criteria

1. Previous or current treatment with immunosuppressant therapy (although topical or inhaled steroids are accepted)

2. Continuous treatment with any inflammatory drug (sporadic treatment e.g. because of headache or in connection with fever a few days will be accepted)

15   3. Treatment with any oral or injected anti-diabetic medications other than insulin

4. A history of anaemia or significantly abnormal haematology results at screening

5. A history of epilepsy, head trauma or cerebro-vascular accident, or clinical features of continuous motor unit activity in proximal muscles

6. Clinically significant history of acute reaction to vaccines or other drugs in the past

20   7. Treatment with any vaccine, including influenza vaccine, within 4 months prior to planned first study drug dose or planned treatment with any vaccine up to 4 months after the last injection with study drug.

8. Participation in other clinical trials with a new chemical entity within the previous 3 months

25   9. Inability or unwillingness to comply with the provisions of this protocol

10. A history of alcohol or drug abuse

11. A significant illness other than diabetes within 2 weeks prior to first dosing

12. Known human immunodeficiency virus (HIV) or hepatitis

13. Females who are lactating or pregnant (the possibility of pregnancy must be excluded by
30   urine βHCG on-site within 24 hours prior to the GAD-alum treatment)

14. Males or females not willing to use adequate contraception until 1 year after the last GAD-alum treatment

15. Presence of associated serious disease or condition, including active skin infections that preclude subcutaneous injection, which in the opinion of the investigator makes the patient non-eligible for the study

16. Deemed by the investigator not being able to follow instructions and/or follow the study protocol

5.3 Recruitment and screening

Eligible subjects will have the study explained to them, and will receive the written patient information. After having had the time to review the nature of the study, they will have the opportunity to ask questions to the investigational team. If, after this, the subjects agree to participate, they will personally sign and date the written informed consent form. The patients will then receive a copy of the signed and dated patient information/informed consent form.

5.4 Patient withdrawal

In accordance with the Declaration of Helsinki, the investigator must explain to the patient that they have the right to withdraw from the study at any time, and that this will in no way prejudice their future treatment. However, unless safety issues occur, we plan to follow the patients for the entire duration of the study in order to analyse efficacy and safety variables also for those patients withdrawing from the study. The reason for any kind of withdrawal must be recorded on the appropriate CRF.

There will be different categories for withdrawals from the study: Complete withdrawal (i.e. stopping investigational product and also continued efficacy and safety evaluations)

Standard reasons for withdrawing from further participation in the study and from the follow-up visits (and <e.g. blood tests>) may be:

- Patient's decision (withdrawal of consent to participate)
- Patient lost to follow-up
- Standard reasons from withdrawing from taking further investigational product, but continuing follow-up visits and safety evaluations may be:
- Unacceptable adverse events
- Patient request
- Investigator's discretion
- Patient lost to follow-up/non-attendance
- Intercurrent illness
- The patient becomes pregnant

Thus intra lymph node GAD-alum should not be given to the patient if the patient after inclusion in the study has got

- brain damage, epilepsia, head trauma, neurological disease

- any active, serious hormonal disease other than Type 1 diabetes

- other severe autoimmune disease(except celiac disease which is accepted for inclusion)

- immune-suppressive treatment

- cancer, cancer treatment

5   - any other diabetes drugs other than insulin

- any vaccination

- drug/alcohol abuse

or if the patient has

- become pregnant or is no longer willing to use safe contraceptives during the study

10   However, whenever a patient is withdrawn from a study, or for whatever reason is not coming to any further visits, a final study evaluation must be completed for that patient (visit <>) - stating the reason(s) why the patient was withdrawn from the study. All documentation concerning the patient must be as complete as possible. Withdrawals due to non-attendance must be followed up by the investigator to obtain the reason for non-attendance. Withdrawals due to intercurrent illnesses or

15   adverse events must be fully documented in the case record form, with the addition of supplementary information if available and/or appropriate.

6. Treatment procedures

6.1 Study Design and Treatment

The trial is singlecenter, open-label, pilot study in GADA positive T1D patients of either gender, 18.00

20   to 29.99 years old, diagnosed with T1D within 6 months at time of screening (Visit 1) and fasting C-peptide levels equal to or above 0.12 nmol/L. In total, approximately 5 patients will be recruited at one site in Linköping, Sweden. The patients will be assessed for eligibility at the screening visit (Visit 1) 10 to 21 days prior to the start of treatment. The screened patients will be assigned a sequential screening number and this screening number will be used as patient identification throughout the

25   study.

Patients who qualify for inclusion in the study will then be enrolled in the study to receive investigational study drug at the subsequent visits according to table 1 below. The patients will be followed for a total study period of 30 months which includes 8 visits to the site.

See table 1 below for an overview of the study visits.

Table 1 Schedule of Patient Visits, Visit Windows and Study Drug Administration

Study	Screening	Intervention				Follow-up		
	Day -10 to - 21 Screening	Day 1 Baseline	Day 30 ± 3	Day 60 ± 3	Day 180 ± 14 Month 6	Day 450 ± 14 Month 15	Day 900 ± 30 Month 30
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 6	Visit 7	Visit 8
GAD-Alum/ Placebo		GAD-alum treatment	GAD-alum treatment	GAD-alum treatment			

6.2 Assessments and procedures

1. Standard insulin treatment, education and psychosocial support for newly diagnosed Type 1 diabetes patients.

2. Normalization of fluid, electrolyte and acid-base balance.

3. Thereafter information about the study.

4. When the patients have given their informed consent, at the latest 120 days after diagnosis, screening is performed with a fasting venous sample from patients who are eligible according to other criteria than C-peptide and GADA concentrations (Visit 1).

5. At Baseline (Visit 2), 6, 15 and 30 months, assessment of residual endogenous insulin secretion by MMTT. HbA1c, safety (haematology and chemistry), autoantibody titres (GAD65, IA-2), immunology, are followed by blood samples at every study visit. Exogenous insulin dose/24 hours, Aes and concomitant medication is registered at every study visit.

6. Self-reported hypoglycaemia (defined as needing help from others and/or seizures and/or unconscious) registered at every study visit.

7. Any symptoms or signs of other medical problem should be treated at the discretion of the clinical doctor.

Examinations will be performed according to Table 2 in Section 7 below, and in the order outlined in the case report form (CRF).

6.2.1 All Visits, Visit 1 through 7

Note that the patient should attend all study visits in the morning following an overnight fast (> 10 hours, water allowed). For patients with evidence of an infection (including fever), the complete visit should be postponed for 5 days or until the patient has recovered.

6.2.2 Administrations of GAD-Alum, Visits 2, 3 and 4

After administration, the patient shall remain in the vicinity of the study site for the next hour, and the administration site will be examined by investigator/study nurse 1 hour post injection.

6.2.3 Mixed Meal Tolerance Test (MMTT), Visits 2, 5, 6 and 7

- The MMTT must be performed according to the instructions in the CRF. The patient should:
- Come to the study site following an overnight fast (>10hr), i.e. the patient may not eat but is permitted to drink water
- Not take short acting/direct acting insulin within 6 hours before the MMTT. The patient is allowed to take base-insulin day/night before, but not in the morning before the MMTT.
- Patients with CSII (insulin pump) must continue with their basal dose insulin, but not add bolus dose during the last 6 hours before the MMTT
- Have a fasting plasma glucose level in the range defined by 4-12 mmol/L on the patient's home blood glucose meter in the morning of the test If the patient does not fulfill all of the above criteria, the MMTT should be rescheduled and the patient should return to the study site within 5 days if possible.

If for safety reasons, subjects need to eat or take insulin, the visit should also be rescheduled.

6.3 Laboratory tests and examinations:

1. Immunological tests:
   a. Autoantibodies (Anti-GAD65, Anti-Insulin, Anti-IA-2, ZnT8)
   b. Relevant cytokines and chemokines are determined (see below)
   c. T-cells are classified and studied (see below)
2. Genetics:
   a. HLA determinations is done and genes related to diabetes development
   b. Array studies to elucidate the importance of diabetes-related genes
3. Virus assays:
   a. Genetic, immunological and microbiological tests may be used.
4. Diabetes status:
   a. HbA1c
   b. Fasting glucose and fasting C-peptide
   c. Meal stimulated glucose and C-peptide
5. Blood sampling for safety:
   a. Hematology
   b. Chemistry

6.4 Medical history

A complete review of the subject's past medical history will be undertaken by the investigator and documented on the Medical History CRF.

All pre-existing conditions/diseases will be reported on the Medical History CRF page at the screening visit (Visit 1).

The subject's Type 1 diabetes diagnosis date and family history of Type 1 diabeteswill also be documented.

6.5 Physical Examination Including Neurological Examination

At the screening visit (Visit 1) the patient will undergo a general physical examination and a neurological examination and any findings will be reported as pre-existing conditions on the Medical History CRF page.

5 During the subsequent study visits the patient will be examined for any new medical conditions or worsening of the pre-existing ones. Any change in pre-existing conditions or new conditions must be entered on the AE page in the CRF and any medication given on the concomitant medication pages.

The patients will, in addition to the limited physical examination by the physician, undergo a standardized clinical neurological examination at screening, 0, 6, 15 and 30 months. The neurological tests are performed in order to detect possible mild signs of neuromuscular disease such as

10 disturbance of strength, balance, and coordination.

The neurological examination includes:

- Extremity reflexes
- Romberg (balance and coordination)
- Walk on a line, 2 meters (balance and coordination)
15 - Standing on 1 leg, left and right, 15 seconds per leg (balance and coordination)
- Finger-nose (coordination)
- Mimic (cranial nerves)
- Babinski reflex (central function)
- Muscle strength (shake hands) biceps, triceps, distal extensors, and flexors

20 These examinations may also be repeated between scheduled visits at the discretion of the investigator. Screening for neurological disease with electroencephalogram (EEG) is not included due to low sensitivity and specificity. However, if any signs of neurological dysfunction are detected, the patient should be referred to a neurologist for further evaluation.

6.7 Concomitant Medication

25 Any concomitant medication used during the study, whether considered relevant for the study or not by the investigator must be reported on the concomitant medication log of the CRF. Please also see section 8.5, below.

7. Scheduling of procedures

Table 1   Pilot DIAGNODE-1 Study, Schedule of Study Events, T1D patients

Study Period	Scree-ning	Intervention				Follow-up	
Visit number	1	2	3	4	5	6	7
Time (Day/Week/Month)	D -10 to - 21	D1 Base-line	D30 ± 3	D60 ± 3	M 6 (D180 ± 14)	M 15 (D450 ± 14)	M 30 (D900 ± 30)
Informed Consent	X						
GAD-alum (Diamyd) treatment		X	X	X			
Medical History	X						
Physical Examination	X	X	X	X	X	X	X
Neurological Assessment	X	X	X	X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X
Height	X						
Weight, BMI	X	X	X	X	X	X	X
Urine pregnancy test (females)		X	X	X			
Injection site inspection [a]		X	X	X			
Insulin dose	X	X	X	X	X	X	X
Self Reported Hypoglycemia [b]		X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X
Blood sampling for safety:							
* Hematology	X	X	X	X	X	X	X
* Chemistry	X	X	X	X	X	X	X
Auto-antibodies:							
* GADA	X	X	X	X	X	X	X
* diabetes-related autoantibodies	X	X	X	X	X	X	X
Blood sampling for Diabetes status:							
* HbA1c	X	X	X	X	X	X	X
* Fasting glucose/C-peptide	X	X	X	X	X	X	X
* MMTT glucose/ C-peptide		X			X	X	X
Blood sampling for , genetics, immunology		X	X	X	X	X	X
Vitamin D in serum	X						
Blood sampling for biobank	X	X	X	X	X	X	X

[a] Inspection of injection site before and 60 minutes after injection by investigator or nurse
[b] Hypoglycemia defined as needing help from others and/or seizures and/or unconscious

7.1 Visits

The first visit, the screening visit (Visit 1) should be performed 10 to 21 days before planned Visit 2 (Baseline). Visit 3 and 4 should then be scheduled with a visit window of ±3 days (second and third administration of GAD-Alum) and of ±14 days for Visit 5, 6 and ±30 days for Visit 7. Please note that all visits must be calculated from the baseline visit (Visit 2) according to the visit schedule. Please

5   also note that the visits must be performed within the visit windows to comply with the study protocol.

Please see table 1 and 2 above, for schedule of patient visits, visit windows and study drug administration.

8. Study medication

10   8.1 Study medication

The following medication supplies will be used in the study:
Study medication: GAD-Alum (Diamyd), 4 µg x 3 (given three times with one month interval)
IMP supplier: Diamyd Medical AB, Stockholm, Sweden.
8.2 Supply

15   GAD-alum (Diamyd) a formulation, supplied by Diamyd Medical. It will be supplied as pre-packed medication from Diamyd Medical to local pharmacy. All dosing will take place in the hospital, and handled only by trained and authorised study personnel. The study medication will be labelled with information according to local regulation. GAD-alum will be stored in a refrigerator at 2-8 ºC in a secure area (e.g. a locked cabinet or drug storage room), protected from unintended use. All study

20   medication will be labelled with information according to local regulations.

8.3 Dosage and administration

GAD-Alum: 4 µg given into lymph node in the inguinal area (by help of ultra sound technique) three times with one month interval

8.4 Duration of treatment

25   See 8.3

8.5 Concomitant medication

No systemic immune modulating medication, and no other diabetes medication other than insulin, whether marketed or not, are allowed.

9. Response variables and outcomes

30   9.1 Exploratory assessment of efficacy

9.1.1. Efficacy variables

As this is a pilot Phase I study there is no primary efficacy endpoint, but we will still follow

- Change in C-peptide fasting and C-peptide (90 minute value and AUCmean 0-120 min) during an MMTT from baseline to month 6, 15 resp to month 30.

- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5, 10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1beta, IL-17, and increase of T-regulatory cells. Change between baseline and subsequent visits.
- Inflammatory markers e.g. TNF-alfa, IL-1 beta, IL-2, IL-17. Change between baseline and subsequent visits.
- Hemoglobin A1c (HbA1c), change between baseline and subsequent visits
- Exogenous insulin dose per kg body weight and 24 hours, change between baseline and subsequent visits

10. Statistical methodology and data management

10.1 Study design

The DIAGNODE-1 study is an open-label pilot Phase I intervention study

Study Participants: Newly diagnosed classic type 1 diabetes patients: N=5. Age 18-29.99 years. Recruited from one Endocrinology clinic in Sweden

10.2 Estimation of sample size

Power analysis: No formal power analyses are done for this pilot study.

10.3 Statistical analysis plan

In brief the following analyses are planned:

All continuous variables will have the following descriptive statistics displayed: number of observations (n), mean value, standard deviation, minimum, median, and maximum. All variables of a categorical nature will be displayed with frequencies and percentages. The tabulation of the descriptive statistics will be split by visit. Where appropriate, baseline (screening) descriptive statistics will also be included.

Demographic and other baseline characteristics

Demographics and baseline characteristics will be presented using descriptive statistics (summary tables).

Safety variables and Efficacy data

The AE/SAE data will be presented using a standardized tabulation of the frequency and incidence rate of all observed AEs/SAEs. The frequencies and incidence rates are calculated on a per patient basis. Adverse events will be summarized by body system, causality, and severity. Other safety data will be presented by descriptive statistics.

Efficacy data regarding C-peptide and immune system as well as Adverse events and other safety data will be summarized descriptively.

After 6 months analysis of the safety data. (The results will be used for design of a Phase II DIAGNODE trial).

10.4 Study populations

Intention-to-treat population

Patients will be included in the primary intention-to-treat population for analysis of efficacy if they receive at least 1 dose of all study drugs in that arm, and are assessed at a later visit.

5   Per protocol population

In order to qualify for the stringent per protocol population, the subjects must have followed the study protocol without any major violations. Any examinations missed will be substituted with the last observation carried forward, but examinations from not more than 1 visit may be lost.

Total population

10   Any patient who withdraws after having received at least one treatment (visit 2) will be included in the safety analysis (adverse events and safety parameters). Data for all patients will be listed, and a list of withdrawn patients, with all reasons for withdrawal, will be given.

10.5 Data collection / case report forms

Case report forms (CRFs) will be supplied for recording data from each patient. Since it is important
15   to have proper data collection in a timely manner, the investigator or assigned designee shall complete the CRFs promptly. When the monitor requests additional data or clarification of data for the CRF, the request must be answered satisfactorily in due time.

It is the responsibility of the investigator to ensure that these case report forms are properly completed. The investigator will sign the designated signature pages to confirm that the case report
20   form is accurate and complete.

To ensure legibility the CRFs should be completed in block capitals with a black or blue ballpoint pen (not pencil, felt-tip or fountain pen). Any corrections to the CRFs must be carried out by the investigator or his designate. A single stroke must be drawn through the original entry. The correction has to be dated and initialled. Incorrect entries must not be covered with correcting fluid,
25   or obliterated, or made illegible in any way. Even if there are no changes from a previous examination, in the interests of completeness of data acquisition the questions, which are repeated in each section of the CRFs should be answered in full. A reasonable explanation must be given by the investigator for all missing data.

10.6 Data management

30   Data will be coded and entered into a computer database. The handling of data, including data quality control, will comply with regulatory guidelines (e.g., International Conference on Harmonization [ICH] and Good Clinical Practice [GCP]).

11. Regulatory and administrative procedures

Any regulatory requirements must have been met before starting the study. The Sponsor will apply
35   for the regulatory approval to the appropriate authorities. Study sites, facilities, laboratories and all

data (including source data) and documentation must be made available for inspection by the authorities.

11.2 Patient Information / Informed Consent

The investigator is responsible for giving the patients full and adequate verbal and written

5   information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patients should have reasonable time to read and understand the information before signing. The investigator is responsible for obtaining signed informed consent from all patients before including the patient in any study related procedures. A copy of the patient information and of the Informed Consent Form

10   will be given to the patients.

11.4 Patient treatment plan

All patients will continue to receive standard care for Type 1 diabetes during the study. After the individual completion of the study, the patient will return to the standard treatment received prior to study participation.

15

Example 3

The therapy regime of this example has an "orthogonal" action and mitigates T1D autoimmunity in the long term. The immune system is downregulated by etanercept, which in turn downregulates the inflammation around the beta cells, at the same time as a beta cell autoantigen (GAD) is

20   presented by dendritic cells, whose tolerance inducing capability has been enhanced by treatment with vitamin D.

Study: Open Label trial to evaluate the tolerability of a combination therapy consisting of GAD-alum (Diamyd®), etanercept and vitamin D in children and adolescents newly diagnosed with type 1 diabetes

25   Active Ingredients: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65), Calciferol (Vitamin D), Etanercept.

Phase of Development: Phase IIa

Objectives:

•   Evaluate the tolerability of a combination therapy with rhGAD65, vitamin D and etanercept

30   •   Evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion

Study Design:

The study is a multicenter, open-label, pilot clinical trial. All patients will from Day 1 receive 2 000 IU

35   vitamin D per os per day during 15 months, and from Days 1-90 receive etanercept (Enbrel) injected subcutaneously 0.8 mg/kg body weight (max 50 mg) once a week, and receive 2 subcutaneous

injections of 20 μg Diamyd in a prime-and-boost regimen on Days 30 and 60. The patients will be evaluated for tolerability for 6 months (main study period, 6 visits) and followed for additionally 24 months (extension study period, 3 visits). The total study period is 30 months.

Selection of Subjects: Patients must be age 8.00 to 17.99 years old, and diagnosed with type 1 diabetes (T1D) within the previous 100 days at the time of screening. Patients will be eligible for enrolment if fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and elevated levels of GAD65 antibodies are present.

Number of Subjects Planned: Approximately 20 patients will be enrolled.

Description of Treatment Groups:

There is one single treatment group. The patients will be assessed for eligibility at the screening visit (Visit 1) 2 to 4 weeks prior to the start of the treatment. On Visit 2 (Day 1), patients eligible for the study will start the treatment as mentioned above.

Endpoints

Primary endpoints:

To Evaluate the tolerability of a combination therapy with Diamyd, Vitamin D and etanercept at Month 6 (main study Period), 9, 15 and 30 (extension study period)

Variables to evaluate tolerability:

- Reactions at the injection site
- Infections
- Occurrence of Adverse Events (AEs)
- Occurrence of Serious Adverse Events
- Physiological and Neurology assessments
- Laboratory measurements (biochemistry and haematology), including Calcium and Vitamin D in serum
- GAD65AB titer (GADA)

Secondary endpoints:

To evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion at Month 6 (main study Period), 9, 15 and 30 (extension study period)

Variables to evaluate the influence on the immune system:

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17
- Increase of T-regulatory cells

Variables to evaluate the effect of endogenous insulin secretion:

- C-peptide (90 minute value and AUCmean 0-120 min) during an MMTT
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours

Sample size:

No real sample size calculation is done as this is an open-label pilot study just to see if the treatment is tolerable and does not cause negative effects on beta cell function and/or immune system.

All variables will be summarized descriptively

Example 4

Title of Study: Effect of GABA or Combination GABA/GAD on the Progression of Type1 Diabetes Mellitus in Children

Phase of Development: Pilot study in humans

Objectives:

- Evaluate the safety and influence of treatment with GABA on preservation of residual insulin secretion in recent-onset type 1 diabetes.
- Evaluate the safety and influence of treatment with two doses of GAD-alum (Diamyd®) plus GABA on preservation of residual insulin secretion in recent-onset type 1 diabetes.

Study Design: The study is a 3-arm, randomized, double-blind, placebo-controlled, clinical trial. Patients will receive either

i)   oral GABA, dosed per kg, twice daily for 12 months plus 2 subcutaneous injections of 20 μg Diamyd in a prime-and-boost regimen over a period of 30 days
ii)   oral GABA, dosed per kg, twice daily for 12 months
iii)   placebo.

The patients will be followed for a total of 12 months.

Selection of Subjects: Patients must be age 4 to 17 years old, and diagnosed with type 1 diabetes (T1D) within the previous 4 weeks of randomization. Patients will be eligible for enrollment if elevated levels of GAD65 antibodies are present.

Number of Subjects Planned: Approximately 75 patients will be enrolled.

Description of Treatment Groups:

The patients will be assessed for eligibility prior to randomization. On Visit 1 (Day 1, baseline), patients eligible for the study will be randomized to 1 of 3 treatment groups:

- 25 patients will be assigned to receive oral GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injections with 20 µg Diamyd (GAD-alum) will be given at Day 1 and Month 1, i.e., 1 prime and 1 booster dose (providing a total dose of 40 µg Diamyd).
- 25 patients will be assigned to receive oral GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injections with Placebo Diamyd will be given at Day 1 and Month 1
- 25 patients will be assigned to receive oral Placebo GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injections with Placebo Diamyd will be given at Day 1 and Month 1

Primary Endpoint:

- Evaluate the effect of GABA and GABA + GAD-alum combination on pancreatic beta cell function, as measured by meal stimulated C-peptide secretion levels compared to age-matched placebo controls, before and after one year of treatment.

Secondary Endpoints:

- Evaluate the effect of GABA and GABA + GAD-alum combination on autoimmune diabetes autoantibodies: GAD-65, ICA512, and Zinc Transporter 8(ZnT8A) and the effect on HbA1c, fasting and stimulated glucose & glucagon levels, fasting C-peptide and the amount of daily insulin usage by participants, from baseline through subsequent visits
- Evaluate the safety of GABA and GABA/GAD-alum combination

Safety

The safety assessment includes observation of reactions at the injection site, occurrence of adverse events (AEs), laboratory measurements, neurological assessments, and limited physical examinations.

Sample size:

The sample size for the proposed study is 75 children; 50 in the treatment groups and 25 in the placebo group. For the primary comparison of the 12-month post-baseline C-peptide measurements between these groups, assuming an α of 0.05 and a mean (SD) C-peptide AUC of 1.0 (0.4) this sample size yields a ~40% power to detect a 25% difference and ~97% power to detect a 50% difference. Adverse events and other data will be summarized descriptively.

Example 5

Name of Active Ingredient: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)

Title of Study: A double-blind, randomized investigator-initiated study to determine the safety and the effect of Diamyd® on the progression to type 1 diabetes in children with multiple islet cell autoantibodies.

Objectives:

5   • The primary objective is to demonstrate that Diamyd® is safe in children at risk for type 1 diabetes. The subjects will be followed for 5 years.
        • The secondary objective is to evaluate if Diamyd® may delay or stop the autoimmune process leading to clinical type 1 diabetes in children with ongoing persistent beta-cell autoimmunity as indicated by multiple positive islet cell autoantibodies.
10

Study Design:

The study is a two-arm, randomized, double-blind, placebo-controlled, single center, clinical trial. The study participants will receive 2 subcutaneous injections of either Diamyd® 20 µg or placebo in a prime-and-boost regimen over a period of 30 days. The study participants will be followed for 5
15   years.

Post diagnosis intervention protocol (PDIP)

Pending study drug shelf life, children diagnosed with clinical type 1 diabetes within the study period may be offered to continue in the trial in a post diagnosis intervention protocol (PDIP) to receive additionally 2 injections of Diamyd® regardless of which treatment group they were randomized to
20   in the prevention part of the study.

Selection of Subjects:

Subjects must be above 4.00 years old and positive for GADA and at least one additional type 1 diabetes-associated autoantibody (IA-2Ab >5, ZnT8R/W/Q/A Ab>72 or IAA>0.8).

Number of Subjects Planned: 50 patients will be enrolled.

25   Description of Treatment Groups:

The patients will be assessed for eligibility at the screening visit (Visit 0) approximately 30 days prior to the first injection. On Visit 1 (Day 1), patients eligible for the study will be randomized to 1 of 2 treatment groups:

        • 25 patients will be assigned to receive 2 subcutaneous injections with 20 µg Diamyd® on
30   Days 1 and 30, i.e., 1 prime and 1 booster dose.
        • 25 patients will be assigned to receive 2 subcutaneous injections of placebo, 1 each on Days 1 and 30.

Post diagnosis intervention protocol (PDIP)

35   Within 4 months since diabetes diagnosis, participants will receive one injection of Diamyd® 20 µg on Day 1 in the Post Diagnosis Follow-up, followed by a second injection of Diamyd® on Day 30, in a

65

prime and boost fashion (a total received dose of 40 µg Diamyd® for participants who pre-diagnosis was randomized to receive placebo and a total received dose of 80 µg Diamyd® for participants who pre-diagnosis was randomized to receive Diamyd®).

Sample size:

Up to 50 children will be asked to participate in DIAPREV-IT 2. It is expected that 50 % of children with more than one positive islet cell autoantibody will develop type 1 diabetes within 5 years.

Analyses:

Analyses of study data will be conducted to address both safety and efficacy of the treatment.

Example 6

Title of Study: A double-blind, randomized investigator-initiated study to determine the safety and the effect of Diamyd® in combination with Vitamin D on the progression to type 1 diabetes in children with multiple islet cell autoantibodies

Name of Active Ingredient: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65), Calciferol (Vitamin D3)

Objectives:

• The primary objective is to evaluate if Diamyd®, in children treated with relatively high dose vitamin D, may delay or stop the autoimmune process leading to clinical type 1 diabetes in children with ongoing persistent beta cell autoimmunity as indicated by multiple positive islet cell autoantibodies.
• The secondary objective is to demonstrate that Diamyd® is safe in children at risk for type 1 diabetes.

Study Design:

The study is a two-arm, randomized, double-blind, placebo-controlled, single center, clinical trial. The study participants will receive 2 subcutaneous injections of either Diamyd® 20 µg or placebo in a prime-and-boost regimen over a period of 30 days. All study participants will be supplemented with Vitamin D at a daily dose of 2000 IE during the total study period (regardless of which treatment group they are randomized to). The study participants will be followed for 5 years.

Post diagnosis intervention protocol (PDIP)

Pending study drug shelf life, children diagnosed with clinical type 1 diabetes within the study period may be offered to continue in the trial in a post diagnosis intervention protocol (PDIP) to receive additionally 2 injections of Diamyd® regardless of which treatment group they were randomized to in the prevention part of the study. All children that are enrolled in the PDIP will be discontinued from the original prevention protocol to be followed thoroughly for safety and efficacy according to the PDIP or 15 months following the first injection of Diamyd® in the PDIP

Selection of Subjects:

Subjects must be age 4.00 to 17.99 years old and positive GADA and at least one additional type 1 diabetes-associated autoantibody (IA-2A, ZnT8R/W/QA or IAA).

Number of Subjects Planned: Approximately 80 patients will be enrolled.

5   Description of Treatment Groups:

The patients will be assessed for eligibility at the screening visit (Visit 0) approximately 30 days prior to the first injection. On Visit 1 (Day 1), patients eligible for the study will be randomized to 1 of 2 treatment groups:

- Approximately 40 patients will be assigned to receive 2 subcutaneous injections with 20 μg
10   Diamyd® on Days 1 and 30, i.e., 1 prime and 1 booster dose.
- Approximately 40 patients will be assigned to receive 2 subcutaneous injections of placebo, 1 each on Days 1 and 30.

Both treatment groups will be supplemented with Vitamin D3 at a daily dose of 2000 IE during the total study period of 5 years.

15   Post diagnosis intervention protocol (PDIP)

- Within 4 months since clinical type 1 diabetes diagnosis, participants will receive one injection of Diamyd® 20 μg on Day 1 in the PDIP, followed by a second injection of Diamyd® on Day 30, in a prime and boost fashion, regardless of which treatment group they were randomized to in the prevention part of the study (prior to diagnosis)

20   Endpoints:

Primary endpoint:

The proportion of subjects diagnosed with clinical type 1 diabetes in the Diamyd® treated group, compared to the placebo treated group at five years after the first injection.

Secondary endpoints:

25   To evaluate safety and the change in metabolic status from normal to impaired glucose metabolism in the group of children with normal glucose metabolism at baseline as well as the progression in metabolic status in the children with impaired glucose metabolism at baseline screening, in the non-diabetic children with multiple islet autoantibodies treated with Diamyd® compared to those treated with placebo.

30   Variables to evaluate safety:

- Injection site reactions
- Occurrence of adverse events (AEs)
- Laboratory measurements (biochemistry and haematology including complete blood count (CBC)), including Calcium and Vitamin D3 in serum
35   - Urine analysis
- Physical examinations, including neurological assessments

- Epitope-specific GADA titer, isotypes and subtypes as well as antiidiotypic autoantibodies to GADA

Metabolic status:

- Change from normal to impaired glucose metabolism, defined as any of
  a) F-glucose $\geq$ 6.1 mmol/L
  b) Maximum p-glucose at 30, 60, 90 minutes $\geq$ 11.1 mmol/L in the OGTT
  c) 120 min p-glucose $\geq$ 7.8 mmol/L on OGTT
  d) HbA1c $\geq$ 39 mmol/mol

Impaired glucose metabolism has to be confirmed at a second visit. This endpoint will be used in the group of children with normal glucose metabolism at baseline screening

- Progression of impaired glucose metabolism from one or several of the above variables to additional signs of reduced glucose metabolism, confirmed at a second visit. This endpoint will be used in the group of children with impaired glucose metabolism at baseline.

Exploratory endpoints:

- Proportion of subjects diagnosed with clinical type 1 diabetes at 1, 2, 3 and 4 years of follow-up.
- Time from baseline visit to clinical type 1 diabetes diagnosis
- Change from baseline in the following key metabolic variables at various time points: HbA1c, First phase insulin response and K-value from IvGTT, AUC p-glucose and C-peptide from OGTT, 120 minutes glucose and C-peptide after OGTT, fasting C-peptide, insulin and glucose
- Change in other metabolic variables from baseline: AUC C-peptide, glucose and insulin from IvGTT, AUC insulin from OGTT, change in max p-glucose on OGTT.

Sample size:

Up to 80 children will be asked to participate in DIAPREV-IT 2. It is expected that 50 % of the untreated children with multiple autoantibodies will develop type 1 diabetes within 5 years. This frequency has previously been reported equal among relatives to diabetes patients and in the general population. If 20 % of the treated children will develop type 1 diabetes within the same period of time, we will have a power of 82 % with alfa=5 % with a group of 40+40=80 children. P value <0.05 will be used as significance level.

Analyses:

Analyses of study data will be conducted to address both safety and efficacy of the treatment. A Statistical Analytic Plan (SAP) will be developed before the statistical analyses.

Analyses will be performed by using parametric statistics. If criteria of normality of variables are not met (e.g. Kolmogorov-Smirnov tests) non-parametric statistics of Wilcoxon type will be used. Four-fields tables will be analysed using the Fisher´s exact test. Time to diabetes will be analysed using life-table analyses, such as Kaplan-Meier and Cox regression. Analyses of efficacy will be performed

as well on per-protocol (PP) as on intention-to-treat (ITT) basis. In ITT the last observation carried forward will be applied.

Adverse events and other safety data will be summarized descriptively.

5   *Example 7*

Title of Study: A Phase II, 2-Arm, Randomized, Double-Blind, Placebo-Controlled, Multicenter Study of Multiple Increasing Doses of Diamyd® , in Combination with Vitamin D, to Evaluate the Safety, Immune Response and Diabetes Status in Young Adults Newly Diagnosed with Type 1 Diabetes.

Name of Active Ingredients: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65), and
10   Calciferol (Vitamin D)

Objectives:

Primary objective

- •   To evaluate the safety of administering Diamyd® of multiple increasing doses in combination with vitamin D

15   Secondary objectives

- •   To evaluate the influence of the immune system of administering Diamyd® of multiple increasing doses in combination with vitamin D
- •   To compare diabetes status variables between Diamyd and Placebo before and after administering Diamyd® of multiple increasing doses in combination with vitamin D

20   Study Design:

The study is a 2-arm, randomized, double-blind, placebo-controlled, multicenter, clinical trial. Eligible patients will receive vitamin D per os per day during 1 month prior to first Diamyd® injection. From Month 1 (Baseline) all patients will receive a maintenance dose of vitamin D during additionally 5 months.

25   Weekly subcutaneous injections of Diamyd® of increasing doses (or Placebo) will be administered from Month 1 (Baseline) over a period of 13 weeks (dose escalation) where after the maximum dose will be administered at 2, 4 and 8 weeks interval (from week 15 through week 27) (maintenance dose). The maintenance dose will then be administered every 8-12 weeks for 1 year. The patients will be evaluated for safety and immunological parameters at Month 6 and will then be evaluated
30   for diabetes status variables at Months 15 and 30.The total study period is 30 months.

Selection of Subjects: Patients must be age 18 to 30 years old, and diagnosed with type 1 diabetes (T1D) within the previous 6 months at the time of screening. Patients will be eligible for enrollment if fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and elevated levels of GAD65 antibodies are present.

35   Number of Subjects Planned: Approximately 40 patients will be enrolled.

Description of Treatment Groups:

The patients will be assessed for eligibility at the screening visit (Day -14-28) 2 to 4 weeks prior to randomization. All eligible patients will from Day 1 receive 7000 IU vitamin D per os per day during 1 month days to ensure vitamin D levels are above > 70nM/L prior to first Diamyd® / Placebo

5   injection. From Month 1 (Baseline) all patients will receive a maintenance dose of vitamin D of 2000 IU per day during additionally 5 months.

On Day 1, patients eligible for the study will be randomized to 1 of 2 treatment groups:

- Approximately 20 patients will be assigned to receive subcutaneous injections of Diamyd® of increasing doses, starting at Baseline (Month 1) as follows: 0.4; 0.8; 2; 3.2; 4; 6.4; 8; 12; 16;

10   20; 24, 32, 40 µg, Diamyd® weekly, where after 40 µg Diamyd® will be administered from week 15 through 27 week at 2, 4 and 8 weeks interval. 40 µg Diamyd® will thereafter be administered every 8-12 weeks for 1 year. Vitamin D will be administered as described above (from Day 1).

- Approximately 20 patients will be assigned to receive subcutaneous injections of Placebo on

15   the same time schedule as above. Vitamin D will be administered as described above (no Placebo for vitamin D).

The patients will be followed for three follow-up visits at Month 6, Month 15, and Month 30

Endpoints:

Primary endpoint:

20   To evaluate the safety of administering Diamyd® of multiple increasing doses in combination with vitamin D treatment at Months 6, 15 and 30.

Variables to evaluate safety:

- Reactions of the injection site
- Occurrence of adverse events (AEs)

25   - Laboratory measurements (biochemistry and haematology)
- Urinalysis (microalbuminuria, creatinine)
- Physical examinations, including neurological assessments
- GAD65AB titer (GADA)

Secondary endpoints:

30   To evaluate the influence of the immune system and diabetes status variables between Diamyd and Placebo before and after administering Diamyd® of multiple increasing doses in combination with vitamin D treatment at Month 15 and 30.

Variables to evaluate the influence on the immune system:

- Inflammatory marker (TNF-alfa, IL-1 beta, IL-2, IL-17)

35   - Th2-deviation of cell-mediated immune response (seen as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17)

- Increase of T-regulatory cells

Variables to evaluate the effect of endogenous insulin secretion:

- Hemoglobin A1c (HbA1c), change between baseline and subsequent visits
- Exogenous insulin dose per kg body weight and 24 hours, change between baseline and subsequent visits
- Number of self-reported episodes of hypoglycemia
- Fasting C-peptide, change between baseline and subsequent visits
- C-peptide AUCmean 0-120 min during MMTT, change between baseline and subsequent visits
- C-peptide measured at 30, 60, 90, and 120 minutes during MMTT
- Maximum C-peptide during MMTT, change between baseline and subsequent visits
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L

Sample size:

No real sample size calculation is done as this is a study just to see if the different treatments are safe and do not cause negative effects on beta cell function and/or immune system. Adverse events and other safety data will be summarized descriptively. Immunological parameters and diabetes status variables will be summarized descriptively.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1.   A method for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent autoimmune diabetes in a subject, comprising administering to the subject a composition comprising glutamic acid decarboxylase (GAD), by injection directly into a lymph node.

2.   Use of a composition comprising glutamic acid decarboxylase (GAD) in preparation of a medicament for prevention and/or treatment of an autoimmune disease selected from type 1 diabetes, autoimmune diabetes, and latent autoimmune diabetes in a subject, wherein said composition is administered to the subject by injection directly into a lymph node.

3.   The method according to claim 1 or the use according to claim 2, wherein the GAD is administered in an amount of 1-15 µg per injection.

4.   The method or use according to any one of claims 1 to 3, wherein the composition comprising GAD is administered at least 2 times, with each administration being at least 14 days apart.

5.   The method or use according to any one of claims 1 to 4 wherein the GAD is administered in an amount of 4 µg per injection at least 3 times, with each administration being a month apart.

6.   The method or use according to any one of claims 1 to 5, further comprising administering a cyclooxygenase inhibitor to the subject.

7.   The method or use according to claim 6, wherein the cyclooxygenase inhibitor is selected from the group consisting of Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Aceclofenac, Nabumetone, acetylsalicylic acid, Diflunisal (Dolobid), Salicylic acid, Salsalate (Disalcid), Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, and Nimesulide.

8.   The method or use according to any one of claims 1 to 7, further comprising administering a CTLA4 compound to the subject.

9.   The method or use according to any one of claims 1 to 8, further comprising administering a TNF-alpha inhibitor to the subject.

10.   The method or use according to claim 9, wherein the TNF-alpha inhibitor is selected from the group consisting of Adalimumab, Certolizumab, Etanercept, Golimumab and Infliximab.

11.   The method or use according to any one of claims 1 to 10, further comprising administering vitamin-D, vitamin-D analogs, tyrosine kinase inhibitors, gamma-amino butyric acid or a gamma-amino butyric acid analog to the subject, and/or exposing the subject to UVB-radiation.

12.   The method or use according to claim 11, wherein administration of vitamin-D and/or vitamin-D analogs and/or exposure to UVB-light, is performed for between 7 to 90 days before administration of the composition to said subject.

13.   The method or use according to claim 11 or 12, wherein the method comprises administration of vitamin-D and/or vitamin-D analogs in an amount of 7000-70000 IU/week for 3-48 months.

14.   The method or use according to any one of claims 1-13, wherein the glutamic acid decarboxylase is formulated in aluminium hydroxide (alum).

15.   The method or use according to any one of claims 1 to 13, comprising administering to the subject a composition comprising a plurality of particles, each having immobilised on its surface at least one first and at least one second antigen, wherein the first antigen is GAD, and the second antigen is either a tolerogen or a beta cell autoantigen, the composition further comprising pharmaceutically acceptable adjuvants, excipients, solvents, and/or buffers.

16.   The method or use according to claim 15, wherein the second beta cell autoantigen is glutamic acid decarboxylase (GAD).

17.   The method or use according to claim 15 or claim 16, wherein at least one GAD is GAD-65.

18.   The method or use according to claim 15, wherein the second antigen is a tolerogen.

19.   The method or use according to claim 18, wherein the tolerogen is a native human protein.

20.   The method or use according to any one of claims 15 to 19, wherein the particle is an aluminium hydroxide (alum) particle, a liposome, a nanoparticle, a gold particle, or a biodegradable particle.

21.   The method or use according to any one of claims 15 to 20, wherein each particle has immobilised on its surface 2, 3, 4, 5, 6, 7, 8, 9, or 10 different antigens selected from the group consisting of tolerogens and GAD.