IMPROVED METHODS OF TREATMENT USING IMMUNOGENIC PEPTIDES

BACKGROUND OF THE INVENTION

Several strategies have been described to prevent the generation of an unwanted immune response against an antigen. WO2008/017517 describes a strategy using peptides comprising an MHC class II T cell epitope of a given antigenic protein and an oxidoreductase motif. These peptides convert CD4+ T cells into a cell type with cytolytic properties called cytolytic CD4+ T cells. These cells are capable to kill via triggering apoptosis those antigen presenting cells (APC), which present the antigen from which the peptide is derived. WO2008/017517 demonstrates this concept for allergies and auto-immune diseases such as type 1 diabetes. Herein insulin can act as an auto-antigen.

WO2009101207 and Carlier et al. (2012) *Plos one* **7,10** e45366 further describe the antigen specific cytolytic cells in more detail.

WO2016059236 discloses further modified peptides wherein an additional histidine is present in the proximity of the oxidoreductase motif.

WO2018162498 further discloses a peptide comprising an oxidoreductase motif with an additional histidine and a MHCII T cell epitope from insulin and its use in the treatment of type 1 diabetes (T1D).

However, even when taking into account the above, little is known regarding the actual effect of such peptides in patients during treatment and there remains a need for improving the therapeutic effect of such immunogenic peptides.

SUMMARY

The present disclosure provides improved methods for treating auto-immune diseases with immunogenic peptides comprising an epitope of an auto-antigen and an oxidoreductase motif.

The inventors have found that in patients, the level of responsiveness can be increased by adjusting the treatment and dosage scheme of administering said immunogenic peptides.

The disclosure hence provides the following aspects:

1. An immunogenic peptide with a length of between 9 and 50 amino acids for use in preventing or treating a disease or disorder selected from: an auto-immune

disorder, a demyelinating disorder, allograft or transplant rejection, a tumor or cancer, an infection with an intracellular pathogen, an immune response to a soluble allofactor, an immune response to an allergen exposure, or an immune response to a viral vector used for gene therapy or gene vaccination in a subject, said peptide comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, a T cell epitope sequence of an antigen involved in said disease or disorder,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- (SEQ ID NO: 12 to 36) or Z_m-C-X_n-[CST]- (SEQ ID NO: 37 to 61),
wherein n is an integer from 0 to 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2,
in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid,
wherein said immunogenic peptide is administered in at least 5 doses of from 300 to 1500 µg of said immunogenic peptide with an interval of from about 12 days to about 28 days between two doses.

In a preferred example said administration is done through intramuscular or subcutaneous injection.

In said general formula of the oxidoreductase motif, the hyphen (-) indicates the point of attachment of the oxidoreductase motif to the N-terminal end of the linker or the T-cell epitope, or to the C-terminal end of the linker or the T cell epitope.

In a preferred example, the T cell epitope in said immunogenic peptide is not, or does not comprise, an amino acid sequence selected from the group consisting of: MHC class II T cell epitopes FLRVPCWKI (SEQ ID NO: 4), and FLRVPSWKI (SEQ ID NO: 5), or NKT cell epitopes FLRVPCW (SEQ ID NO: 10), and FLRVPSW (SEQ ID NO: 11).

In a preferred example, said oxidoreductase motif is not part of a repeat of the standard C-XX-[CST] (SEQ ID NO: 1) or [CST]-XX-C (SEQ ID NO 2) oxidoreductase motifs such as repeats of said motif which can be spaced from each other by one or more amino acids (e.g. CXXC X CXXC X CXXC (SEQ ID NO: 6)), as repeats which are adjacent to each other (CXXCCXXCCXXC (SEQ ID NO: 7)) or as repeats which overlap with each other CXXCXXCXXC (SEQ ID NO: 8) or CXCCXCCXCC (SEQ ID NO: 9)), especially when n is 0 or 1 and m is 0 in the general formula as defined in aspect 1.

In a preferred example, the antigen is an autoantigen.

2. The immunogenic peptide for use according to aspect 1, wherein said immunogenic peptide is administered through intramuscular or subcutaneous injection of 6 doses of from 300 to 1500 µg of said immunogenic peptide with an interval of about 12 to 28 days between two doses.

3. The immunogenic peptide for use according to aspect 1 or 2, wherein each of said doses of from 300 to 1500 µg of said immunogenic peptide is administered with an interval of about 12 to about 16 days, or about 2 weeks between two doses.

4. The immunogenic peptide for use according to any one of aspects 1 to 3, wherein each dose contains:

- from 300 to 600 µg of said immunogenic peptide;
- from 600 to 800 µg of said immunogenic peptide;
- from 800 to 1000 µg of said immunogenic peptide;
- from 1000 to 1200 µg of said immunogenic peptide; or
- from 1200 to 1500 µg of said immunogenic peptide.

In a preferred example of said aspect, said dose contains 450 or 1350 µg of said immunogenic peptide.
In an even more preferred example, said dose is administered 6 times, with an interval of about 12 to about 16 days, or about 2 weeks between doses.

5. The immunogenic peptide for use according to any one of aspects 1 to 4, wherein a boost administration is performed of a dose of from 300 to 1500 µg of said immunogenic peptide at about week 22 to 30, counted from the start of the treatment.

6. The immunogenic peptide for use according to aspect 5, wherein said boost administration is performed at about week 22 to 26 counted from the start of the treatment.

7. The immunogenic peptide for use according to aspect 5 or 6, wherein said boost contains:

- from 300 to 600 µg of said immunogenic peptide;
- from 600 to 800 µg of said immunogenic peptide;
- from 800 to 1000 µg of said immunogenic peptide;
- from 1000 to 1200 µg of said immunogenic peptide; or
- from 1200 to 1500 µg of said immunogenic peptide.

In a preferred example of said aspect, said dose contains 450 or 1350 µg of said immunogenic peptide, preferably at about week 23 to 25 of the treatment, more preferably around week 24 of the treatment.

8. The immunogenic peptide for use according to any one of aspects 1 to 7, wherein half of the dose is to be administered concomitantly in two sites (both upper arms, preferably in the region of the lateral part of the arms, more preferably midway between the elbow and the shoulder).

9. An *in vitro* method for analysing the response of a patient to the treatment of a disease or disorder selected from: an auto-immune disorder, a demyelinating disorder, allograft or transplant rejection, a tumor or cancer, an infection with an intracellular pathogen, an immune response to a soluble allofactor, an immune response to an allergen exposure, or an immune response to a viral vector used for gene therapy or gene vaccination in a subject, with an immunogenic peptide with a length of between 9 and 50 amino acids, said peptide comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, an MHC class II T cell epitope sequence of an (auto)-antigen of involved in said disease or disorder,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- (SEQ ID NO: 12 to 36) or Z_m-C-X_n-[CST]- (SEQ ID NO: 37 to 61),
wherein n is an integer from 0 to 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2, in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid,
wherein said method comprises the analysis of samples taken from a patient being treated with said immunogenic peptide at the following time points:
- Day 0 of the treatment,
- In week 11 to 13, such as in week 12 of the treatment,
- In week 23 to 25, such as in week 24 of the treatment, and
- In week 47 to 49, such as in week 48 of the treatment.

wherein m is an integer selected from 0, 1, or 2, wherein Z is any amino acid, preferably a basic amino acid selected from: H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, preferably K or H, most preferably K ;
(f) Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-, wherein n is 5, thereby creating an internal $X^1X^2X^3X^4X^5$ (SEQ ID NO: 65) amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0, 1, or 2, wherein Z is any amino acid, preferably a basic amino acid selected from: H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, preferably K or H, most preferably K;
(g) Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-, wherein n is 6, thereby creating an internal $X^1X^2X^3X^4X^5X^6$ (SEQ ID NO: 66) amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0, 1, or 2, wherein Z is any amino acid, preferably a basic amino acid selected from: H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, preferably K or H, most preferably K; or
(h) Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-, wherein n is 0 to 6 and wherein m is 0, and wherein one of the C or [CST] residues has been modified so as to carry an acetyl, methyl, ethyl or propionyl group, either on the N-terminal amide of the amino acid residue of the motif or on the C-terminal carboxy group.
In preferred examples of such a motif, n is 2, and m is 1 or 2, wherein the internal X^1X^2, each individually, can be any amino acid selected from the group consisting of: G, A, V, L, I, M, F, W, P, S, T, C, Y, N, Q, D, E, K, R, and H, or non-natural amino acids. Preferably, X^1 and X^2 in said motif is any amino acid except for C, S, or T. In a further example, at least one of X^1or X^2 in said motif is a basic amino acid selected from: H, K, or R, or a non-natural basic amino acid as defined herein, such as L-ornithine. In another example of the motif, at least one of X^1or X^2 in said motif is P or Y. Specific non-limiting examples of the internal X^1X^2 amino acid couple within the oxidoreductase motif: PY, HY, KY, RY, PH, PK, PR, HG, KG, RG, HH, HK, HR, GP, HP, KP, RP, GH, GK, GR, GH, KH, and RH. Preferably said modification results in an N-terminal acetylation of the first cysteine in the motif (N-acetyl-cysteine).

20. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 19, wherein said immunogenic peptide has an oxidoreductase motif which comprises the sequence CC, KCC, RCC, CRC, CKC, KCRC (SEQ ID NO: 154), KCKC (SEQ ID NO: 152), KCHC (SEQ ID NO: 225), RCRC (SEQ ID NO: 156), RCKC (SEQ ID NO: 226), CPYC (SEQ ID NO: 227), HCPYC (SEQ ID NO: 228), KCPYC (SEQ ID NO: 229), RCPYC (SEQ ID NO: 230), CRPYC (SEQ ID NO: 231), CPRYC (SEQ ID NO: 232), CPYRC (SEQ ID NO: 233),

from proteins encoded by the HY chromosome (H-Y antigens), such as Dby. Other examples can be found in, for instance, Goulmy E, Current Opinion in Immunology, vol 8, 75-81, 1996 (see Table 3 therein in particular). It has to be noted that many minor histocompatibility antigens in man have been detected via their presentation into MHC class I determinants by use of cytolytic CD8+ T cells. However, such peptides are derived by the processing of proteins that also contain MHC class II restricted T cell epitopes, thereby providing the possibility of designing peptides of the present disclosure. Tissue-specific alloantigens can be identified using the same procedure. One example of this is the MHC class I restricted epitope derived from a protein expressed in kidneys but not in spleen and capable of eliciting CD8+ T cells with cytotoxic activity on kidney cells (Poindexter et al, Journal of Immunology, 154: 3880- 3887, 1995).

More preferably, said antigenic protein is an autoantigen involved in type-1 diabetes (T1D), demyelinating disorders such as multiple sclerosis (MS) or neuromyelitis optica (NMO), or in rheumatoid arthritis (RA).

Non-limiting examples of autoantigens involved in T1D.

Source: Mallone R et al., Clin Dev Immunol. 2011:513210.

Auto-antigen	**UniProtKB identifier**	**Examples of T-cell epitopes**
Insulin	P01308	LALEGSLQK (SEQ ID NO: 3)
GAD65	Q9UGI5	
GAD67	Q99259	
IA-2 (ICA512)	Q16849	
IA-2 (beta/phogrin)	Q92932	
IGRP	Q9NQR9	
Chromogranin	P10645	
ZnT8	Q8IWU4	
HSP-60	P10809	

Example of autoantigens involved in NMO

Auto-antigen	**UniProtKB identifier**	**Comments**	**Examples of T-cell epitopes**
Myelin oligodendrocyte glycoprotein (MOG)	Q16653	Immunodominant epitopes in the following regions : amino acids 1-22, 35-55 and 92-106.	FLRVPCWKI (SEQ ID NO: 4) or FLRVPSWKI (SEQ ID NO: 5)

In some examples, in any one of the aspects defined herein, the epitope in said peptide is not a sequence derived from the MOG antigen amino acid sequence.

In some examples, in any one of the aspects defined herein, said disease or disorder is not MS.

In some examples, in any one of the aspects defined herein, said disease or disorder is not a disease that is known to be treated by fumarate, or is not a fumarate-related disease or disorder as defined herein elsewhere.

25. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20, wherein the auto-immune disease is MS and wherein the T-cell epitope in said peptide is an MHC class II T cell or NKT cell epitope from an antigenic protein selected from the group comprising: Myelin Oligodendrocyte Glycoprotein (MOG), Myelin basic protein (MBP), Proteolipid protein (PLP), myelin-associated antigen (MAG), Oligodendrocyte-specific protein (OSP), myelin-associated oligodendrocyte basic protein (MOBP), 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase), S100β protein and transaldolase H, preferably MOG.

In an alternative example, in the immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20, the auto-immune disease is MS and the T-cell epitope in said peptide is an MHC class II T cell or NKT cell epitope from an antigenic protein selected from the group comprising: Myelin basic protein (MBP), Proteolipid protein (PLP), myelin-associated antigen (MAG), Oligodendrocyte-specific protein (OSP), myelin-associated oligodendrocyte basic protein (MOBP), 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase), S100β protein and transaldolase H.

26. The immunogenic peptide for use according to any one of aspects 1 to 8, or the method according to any one of aspects 9 to 20, wherein the auto-immune disease is NMO and wherein the T-cell epitope in said peptide is an MHC class II T cell or NKT cell epitope from Myelin Oligodendrocyte Glycoprotein (MOG).

27. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20, wherein the auto-immune disease is RA and wherein the T-cell epitope in said peptide is an MHC class II T cell or NKT cell epitope from an antigenic protein selected from the group comprising: GRP78, HSP60, 60 kDa chaperonin 2, Gelsolin, Chitinase-3-like protein 1, Cathepsin S, Serum albumin, vinculin, and Cathepsin D.

28. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20, wherein the auto-immune disease is Psoriasis and wherein said antigenic protein is selected from the group consisting of: ADAMTSL5, PLA2G4D, Keratin, such as Keratin 14 or Keratin 17, an antigen from *Triticum aestivum*, Pso p27, cathelicidin antimicrobial peptide, ceutrophil defensin 1 and LL37, preferably LL37.

29. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 28, wherein when said auto-immune disease is MS, NMO or psoriasis, said treatment does not include treatment with a fumarate compound as defined herein or a derivative thereof.

In a preferred example, the T cell epitope of said peptide is not a T-cell epitope of an antigenic protein or antigen involved in a fumarate-related or fumarate-treated disease or disorder, more preferably an MHC class I or II molecule or an NKT cell epitope.

26. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20 and 24 or 25, wherein said epitope is derived from the Myelin-oligodendrocyte glycoprotein (MOG) antigen amino acid sequence. More preferably said epitope is selected from the group comprising amino acid residues: 40-60, 41-55, 43-57, 44-58, 45-59, and 35-55 of the mature MOG amino acid sequence defined by SEQ ID NO: 74:

GQFRVIGPRHPIRALVGDEVELPCRISPGKNATGMEVGWYRPPFSRVVHLYRNGKDQDGDQA
PEYRGRTELLKDAIGEGKVTLRIRNVRFSDEGGFTCFFRDHSYQEEAAMELKVEDPFYWVSPG

VLVLLAVLPVLLLQITVGLIFLCLQYRLRGKLRAEIENLHRTFDPHFLRVPCWKITLFVIVPVLGPL VALIICYNWLHRRLAGQFLEELRNPF,

Such as those selected from the group comprising:

YRPPFSRVVHLYRNGKDQDGD (SEQ ID NO: 75)

RPPFSRVVHLYRNGK (SEQ ID NO: 76)

PFSRVVHLYRNGKDQ (SEQ ID NO: 77)

FSRVVHLYRNGKDQD (SEQ ID NO: 78)

SRVVHLYRNGKDQDG (SEQ ID NO: 79)

VVHLYRNGK (SEQ ID NO: 80)

MEVGWYRSPFSRVVHLYRNGK (mouse SEQ ID NO: 81),

MEVGWYRPPFSRVVHLYRNGK (human SEQ ID NO: 82),

YRSPFSRVV (mouse SEQ ID NO: 83), ands

YRPPFSRVV (human SEQ ID NO: 84),

or combinations thereof.

In a preferred example of aspect 6 or 7, said immunogenic peptide comprises a T-cell epitope having or comprising the T-cell epitope defined by the following sequence: FLRVPCWKI (SEQ ID NO: 4) or FLRVPSWKI (SEQ ID NO:5),

or said immunogenic peptide comprises or consists essentially of the amino sequence:

HCPYCVRYFLRVPSWKITLF (SEQ ID NO: 85),

HCPYCVRYFLRVPCWKITLF (SEQ ID NO: 86),

KHCPYCVRYFLRVPSWKITLFKK (SEQ ID NO: 87), or

KHCPYCVRYFLRVPCWKITLFKK (SEQ ID NO: 88).

27. The immunogenic peptide for use according to any one of aspects 1 to 8 or the method according to any one of aspects 9 to 20 and 24, wherein the epitope in said immunogenic or tolerogenic peptide is derived from the myelin proteolipid protein (also called proteolipid protein (PLP) or lipohilin) antigen amino acid

HKGFKGVDAQGTLSK (SEQ ID NO: 128)

KGFKGVDAQGTLSKI (SEQ ID NO: 129)

GFKGVDAQGTLSKIF (SEQ ID NO: 130)

FKGVDAQGTLSKIFK (SEQ ID NO: 131)

KGVDAQGTLSKIFKL (SEQ ID NO: 132)

GVDAQGTLSKIFKLG (SEQ ID NO: 133)

VDAQGTLSKIFKLGG (SEQ ID NO: 134), and

LSRFSWGAEGQRPG (SEQ ID NO: 135),

or combinations thereof,

or any one or more of the fragments defined by amino acid residues 30-44, 80-94, 83-99, 81-95, 82-96, 83-97, 84-98, 110-124, 130-144, 131-158, 131-145, 140-148, 142-152, 132-146, 134-148,135-149, 136-150,137-151, 138-152,139-153, 140-154 and 133-147 of the MBP amino acid sequence defined by SEQ ID NO: 136 (UniProtKB - P02686-5 (MBP_HUMAN)):

MASQKRPSQRHGSKYLATASTMDHARHGFLPRHRDTGILDSIGRFFGGDR

GAPKRGSGKDSHHPARTAHYGSLPQKSHGRTQDENPVVHFFKNIVTPRTP

PPSQGKGRGLSLSRFSWGAEGQRPGFGYGGRASDYKSAHKGFKGVDAQGT

LSKIFKLGGRDSRSGSPMARR.

29. In any one of the aspects disclosed herein relating to methods of treatment or medical uses of the immunogenic peptide, said peptides can also be administered as a nucleic acid encoding said respective peptide. A nucleic acid encoding the immunogenic or tolerogenic peptide according to any one of the aspects or examples disclosed herein, preferably selected from isolated desoxyribonucleic acid (DNA), plasmid DNA (pDNA), coding DNA (cDNA), ribonucleic acid (RNA), messenger RNA (mRNA) or modified versions thereof, such as non-immunogenic mRNA comprising N(1)-methyl-pseudouridine (m1ψ). In some examples, said nucleic acid can be part of an expression cassette, optionally incorporated in a (viral) vector or plasmid that can be used for gene-therapy or can be present in the form of encapsulated or naked DNA or RNA to be administered according to techniques known in the pharmaceutical and gene therapeutic field.

30. In any one of the aspects disclosed herein relating to methods of treatment or medical uses of the immunogenic peptide, said peptide can be recognized by specific HLA types:

- when said disease is T1D, said antigen is preferably recognized in the context of HLA-DRB1*03 and DRB1*04 haplotype groups. In the DRB1*03 group, two alleles are common, namely DRB1*0301 and DRB1*0302, but other alleles have been reported, such as DRB1*0303, DRB1*0304 and DRB1*0307. In the DRB1*04 group, ten major alleles can be found, namely: DRB1*0401, DRB1*0402, DRB1*0403, DRB1*0404, DRB1*0405, DRB1*0406, DRB1*0407, DRB1*0408, DRB1*0410 and DRB1*0411.

- when said disease or disorder is MS, said antigen is preferably recognized in the context of HLA-DRB1*15:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA DRB5*0101, or DQ6 type of HLA. More preferred are patients having a HLA-DRB1* type 15:01;

- when said disease or disorder is NMO, said antigen is preferably recognized in the context of HLA-DRB1*03:01 or HLA-DPB1*05:01 (for Asia); or

- when said disease or disorder is RA, said antigen is preferably recognized in the context of HLA-DRB1*01:01, 04:01 or 04:04.

31. In a preferred example, the T1D patients treated with the immunogenic peptide are DR4 positive (i.e. positive for one of the DRB1*04 haplotypes), more preferably the T1D patients treated with the immunogenic peptide are DR4 positive (i.e. positive for one of the DRB1*04 haplotypes) and DR3 negative (i.e. negative for all DRB1*03 haplotypes). Alternatively, the T1D patients treated with the immunogenic peptide are DR3 positive (i.e. positive for one of the DRB1*03 haplotypes). The term "HLA DR4 positive" encompasses both patients being heterozygous or homozygous HLA-DR4 positive. In one example, said patients are also HLA-DR3 negative (HLA-DR3-). The term "HLA-DR3 negative" refers to patients being homozygous HLA-DR3 negative.

32. In any one of the aspects defined herein, said peptide can be administered as a pharmaceutical composition comprising said peptide and a pharmaceutically acceptable carrier and/ or an adjuvant.

33. The disclosure also provides for methods of preventing or treating an auto-immune disorder, a demyelinating disorder, transplant rejection or cancer, comprising administering an immunogenic peptide as defined in any one of the aspects above, using the administration scheme of any one of the aspects above.

34. The disclosure also provides for the use of an immunogenic peptide as defined in any one of the aspects above for the manufacture of a medicament for preventing or treating an auto-immune disorder, a demyelinating disorder, transplant rejection or cancer, wherein said immunogenic peptide is administered according to the administration scheme of any one of the aspects above.

35. A method of preventing or treating an auto-immune disorder, a demyelinating disorder, transplant rejection or cancer, comprising the steps of administering to a subject in need thereof the immunogenic peptide as defined in any one of the preceding aspects or nucleic acid encoding such, wherein said immunogenic peptide or nucleic acid is administered according to the administration scheme of any one of the preceding aspects.

In a first aspect, there is provided an immunogenic peptide with a length of between 9 and 50 amino acids for use in preventing or treating type-1-diabetes (T1D) in a subject, said peptide comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, an MHC class II T cell epitope sequence of an antigen involved in T1D,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-,
wherein n is an integer selected from 2, 1, 0, 3, 4, 5, or 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2,
in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid,
wherein said immunogenic peptide is to be administered in at least 5 doses of from 300 to 1500 µg of said immunogenic peptide with an interval of about 12 days to about 28 days between two doses.

In a second aspect, there is provided an *in vitro* method for analysing the response of a patient to the treatment of type-1-diabetes (T1D) in a subject, with an immunogenic peptide with a length of between 9 and 50 amino acids, said peptide

comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, an MHC class II T cell epitope sequence of an antigen involved in T1D,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-,
wherein n is an integer from 0 to 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2,
in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid
wherein said method comprises the analysis of samples taken from a patient being treated with said immunogenic peptide at the following time points:
Day 0 of the treatment,
In about week 11 to 13, such as in week 12 of the treatment,
In about week 23 to 25, such as in week 24 of the treatment, and
In about week 47 to 49, such as in week 48 of the treatment.

In a third aspect, there is provided use of an immunogenic peptide as defined herein or the nucleic acid encoding such, in the manufacture of a medicament for preventing or treating type-1-diabetes (T1D), wherein said immunogenic peptide or nucleic acid is to be administered according to the administration scheme as defined herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: schematic representation of the main clinical study and the sub clinical study performed with the investigational medicinal product (IMP). See text for details.

Figure 2: represent the patient's journey from ≤9 weeks post diagnosis to week 48. DBS: Dry Blood Spot analysis. FUp: follow up. See text for details.

Figure 3: represents net % of CD4+ T cells responding to proinsulin epitope C20-A1 stimulations in IMP (continuous line) and Placebo (dotted line) treated patient groups at various time points. The straight lines connect the mean values while the error bars show the standard errors at each time point. The p-values at individual time points compare the IMP and Placebo treated patient groups using Mann-Whitney-Wilcoxon U test. The p-values displayed on the right hand side of the line plots signify the dependence of mean response on time using repeated measures ANOVA.

CLAIMS

1. An immunogenic peptide with a length of between 9 and 50 amino acids for use in preventing or treating type-1-diabetes (T1D) in a subject, said peptide comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, an MHC class II T cell epitope sequence of an antigen involved in T1D,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-,
wherein n is an integer selected from 2, 1, 0, 3, 4, 5, or 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2,
in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid,
wherein said immunogenic peptide is to be administered in at least 5 doses of from 300 to 1500 µg of said immunogenic peptide with an interval of about 12 days to about 28 days between two doses.

2. The immunogenic peptide for use according to claim 1, wherein said immunogenic peptide is to be administered via intramuscular or subcutaneous injection.

3. The immunogenic peptide for use according to claim 1 or 2, wherein said immunogenic peptide is to be administered through intramuscular or subcutaneous injection of 6 doses of from 300 to 1500 µg of said immunogenic peptide with an interval of about 2 to 3 weeks between two doses.

4. The immunogenic peptide for use according to any one of claims 1 to 3, wherein each of said doses of from 300 to 1500 µg of said immunogenic peptide is to be administered with an interval of about 12 to about 16 days, or about 2 weeks between two doses.

5. The immunogenic peptide for use according to any one of claims 1 to 4, wherein each dose contains:

from 300 to 600 µg of said immunogenic peptide;
from 600 to 800 µg of said immunogenic peptide;
from 800 to 1000 µg of said immunogenic peptide;
from 1000 to 1200 µg of said immunogenic peptide; or

from 1200 to 1500 µg of said immunogenic peptide.

6. The immunogenic peptide for use according to any one of claims 1 to 5, wherein said dose contains 450 or 1350 µg of said immunogenic peptide.

7. The immunogenic peptide for use according to any one of claims 1 to 6, wherein said dose is to be administered 6 times, with an interval of about 12 to about 16 days, or about 2 weeks between doses.

8. The immunogenic peptide for use according to any one of claims 1 to 7, wherein a boost administration is to be performed of a dose of from 300 to 1500 µg of said immunogenic peptide at about week 22 to 30, counted from the start of the treatment.

9. The immunogenic peptide for use according to claim 8, wherein said boost administration is to be performed at about week 22 to 26 counted from the start of the treatment.

10. The immunogenic peptide for use according to claim 8 or 9, wherein said boost contains:

from 300 to 600 µg of said immunogenic peptide;
from 600 to 800 µg of said immunogenic peptide;
from 800 to 1000 µg of said immunogenic peptide;
from 1000 to 1200 µg of said immunogenic peptide; or
from 1200 to 1500 µg of said immunogenic peptide, preferably wherein said boost contains 450 or 1350 µg of said immunogenic peptide.

11. The immunogenic peptide for use according to claim 10, wherein said boost is to be administered at about week 23 to 25 of the treatment, more preferably around week 24 of the treatment.

12. The immunogenic peptide for use according to any one of claims 1 to 11, wherein half of the dose is to be administered concomitantly in two sites (both upper arms, preferably in the region of the lateral part of the arms, more preferably midway between the elbow and the shoulder.

13. An *in vitro* method for analysing the response of a patient to the treatment of type-1-diabetes (T1D) in a subject, with an immunogenic peptide with a length of between 9 and 50 amino acids, said peptide comprising an oxidoreductase motif and, separated from this motif by 0 to 7 amino acids, an MHC class II T cell epitope sequence of an antigen involved in T1D,
wherein said oxidoreductase motif comprises the motif:
Z_m-[CST]-X_n-C- or Z_m-C-X_n-[CST]-,
wherein n is an integer from 0 to 6, preferably 2, 1, 0, or 3.
wherein m is for an integer from 0 to 2,
in which C stands for cysteine, S for serine, T for threonine, X for any amino acid and Z for any amino acid, preferably a basic amino acid
wherein said method comprises the analysis of samples taken from a patient being treated with said immunogenic peptide at the following time points:
Day 0 of the treatment,
In about week 11 to 13, such as in week 12 of the treatment,
In about week 23 to 25, such as in week 24 of the treatment, and
In about week 47 to 49, such as in week 48 of the treatment.

14. The *in vitro* method according to claim 13, wherein a sample of said patient is analysed about 8 to 10 weeks prior to the start of the treatment, preferably about 9 weeks prior to the start of said treatment.

15. The immunogenic peptide for use according to any one of claims 1 to 12 or the method according to claim 13 or 14, wherein the auto-immune disease is T1D and wherein the T-cell epitope in said peptide is an MHC class II T cell epitope from (pro-)insulin.

16. The immunogenic peptide for use according to any one of claims 1 to 12 or the method according to any one of claims 13 or 14, wherein the amino acid sequence of said epitope is defined by the amino acid sequence LALEGSLQK [SEQ ID NO: 3].

17. The immunogenic peptide for use according to any one of claims 1 to 12 or the method according to any one of claims 13 to 16, wherein said patients are homozygous or heterozygous HLA type DR3 or DR4 positive.

18. The immunogenic peptide for use according to any one of claims 1 to 12 or the method according to any one of claims 13 to 17, wherein said peptide comprises a sequence selected from the group consisting of: Cxx[CST]SLQPLALEGSLQK [SEQ ID NO: 67], [CST]xxCSLQPLALEGSLQK [SEQ ID NO:68], CxxCSLQPLALEGSLQK [SEQ ID NO: 69], HCxx[CST]SLQPLALEGSLQK [SEQ ID NO:70], H[CST]xxCSLQPLALEGSLQK [SEQ ID NO:71], HCxxCSLQPLALEGSLQK [SEQ ID NO: 72], and HCPYCSLQPLALEGSLQKRG [SEQ ID NO: 73].

19. The immunogenic peptide for use according to any one of claims 1 to 12 or 15 to 18, wherein said peptide is to be administered as a nucleic acid encoding said respective peptide, preferably wherein said nucleic acid encoding the immunogenic peptide is selected from isolated desoxyribonucleic acid (DNA), plasmid DNA (pDNA), coding DNA (cDNA), ribonucleic acid (RNA), messenger RNA (mRNA) or modified versions thereof, such as non-immunogenic mRNA comprising N(1)-methyl-pseudouridine (m1ψ).

20. The use of an immunogenic peptide as defined in any one of claims 1 to 12 or 15 to 19 or the nucleic acid encoding such, in the manufacture of a medicament for preventing or treating type-1-diabetes (T1D), wherein said immunogenic peptide or nucleic acid is to be administered according to the administration scheme of any one of the preceding claims.