

## PEPTIDES AND METHODS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

### FIELD OF THE INVENTION

The present invention relates to immunogenic peptides. In particular, the invention relates to immunogenic peptides comprising an oxidoreductase motif linked to a T cell epitope derived from Myelin Oligodendrocyte Glycoprotein (MOG) and cytolytic CD4+ T cells generated by these peptides for use in the treatment of demyelinating disorders, such as Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO).

### BACKGROUND

Several strategies have been described to prevent the generation of an unwanted immune response against an antigen. WO2008/017517 describes a new 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 I diabetes.

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. WO2012069568 further discloses peptides comprising an NKT cell epitope of an antigenic protein and an oxidoreductase motif. These peptides are capable of eliciting activation of NKT cells, which represent a valuable approach for the treatment of many diseases such as infectious and autoimmune diseases or cancer. WO2017182528 describes the use of an immunogenic peptide comprising a MOG epitope for use in treating Multiple Sclerosis.

Multiple Sclerosis (MS) is the most common autoimmune disorder of the central nervous system and its prevalence has increased substantially in most regions the past two decades. It was estimated that in 2016, over 2.2 million people worldwide had MS. The global prevalence of MS is substantially different between men and women. In preteen children, the prevalence is roughly equal. However, during adolescence and older population groups with about twice as women developing the disease (GDB 2016

Motor Neuron Disease Collaborators. 2018. *Lancet Neurol.* 17(12), 1083-1097). MS is most commonly diagnosed by assessing clinical symptoms, combined with medical imaging and/or laboratory testing.

Clinical symptoms that may be manifested in a subject are diverse and include numerous neurological symptoms. However, autonomic, visual, motor, and sensory problems appear to be most common (Compston and Coles. 2008. *Lancet.* 372(9648):1502-17.

Myelin Oligodendrocyte Glycoprotein (MOG) is a glycoprotein solely expressed at the outermost surface of myelin sheaths and oligodendrocyte membranes. The exact molecular function of MOG is still debated, however there appears to be a consensus in the art that it is involved in the completion and/or maintenance of the myelin sheath and hereby likely acts as an adhesion molecule on the myelin sheath to provide structural integrity (Peschl et al. *Front. Immunol.* (2017). 8, 529). The hypothesized importance of MOG is substantiated by highly homogenous coding regions of MOG in mammals (Pham-Dinh et al. (1994) *J. Neurochem.* 63(6), 2353-2356) and observations that MOG may act as an autoantigen for T and B cell responses in experimental models and inflammatory demyelinating diseases (Peschl et al. *Front. Immunol.* (2017). 8, 529). A role for antibodies against MOG (anti-MOG Abs) in MS pathogenesis has been reported and may be considered as a biomarker in the diagnosis of MS, although the exact pathological effect and immunopathological role of human MOG Abs remains to be determined (Peschl et al. *Front. Immunol.* (2017). 8, 529). Further, anti-MOG Abs have been shown to be involved in the Neuromyelitis Optica (NMO).

The average age of persons being diagnosed with MS is approximately 30 years. This, combined with a progressive phase of the disease that often manifests itself one or two decades after diagnosis, thus contributes to a significant amount of disability-adjusted life years (DALYs) within the global population. While several therapies have proven to mitigate certain aspects of the disease (progression), no known cure is available for MS. For NMO, which in some cases has a substantial overlap in clinical symptoms with (certain) MS subtypes, no cure is available either.

Hence, novel and/or improved treatment strategies for MOG autoantigen-induced or anti-MOG antibody induced diseases such as MS and NMO, which are demyelinating diseases are needed.

## SUMMARY

The present invention provides novel peptides derived from Myelin Oligodendrocyte Glycoprotein (MOG) for the treatment of demyelinating disorders such as but not limited to Multiple Sclerosis and Neuromyelitis Optica (NMO). The peptides of the present invention have the advantage that they bind to HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 with much higher affinity than prior art peptides disclosed in WO2017182528. Stimulation of MS patients cells with the peptides of the invention induced specific CD4+ T cells with lytic properties.

10 The invention therefore provides the following aspects:

Aspect 1. An isolated immunogenic peptide comprising:

a1) an oxidoreductase motif with the sequence  $Z_m$ -[CST]- $X_n$ -C- (SEQ ID NO: 66 to 90) or  $Z_m$ -C- $X_n$ -[CST]- (SEQ ID NO: 91 to 115), wherein n is an integer chosen from 0 to 6, preferably 0, 1, 2, or 3, wherein m is an integer selected from 0 to 3, wherein X is any amino acid, wherein Z is any amino acid, in which C stands for cysteine, S for serine, T for threonine;

a2) a T-cell epitope with an amino acid sequence selected from the group consisting of: MHC class II T cell epitopes FLRVPCWKI (SEQ ID NO: 1), and FLRVPSWKI (SEQ ID NO: 2), or NKT cell epitopes FLRVPCW (SEQ ID NO: 63), and FLRVPSW (SEQ ID NO: 64),

wherein said oxidoreductase motif and said epitope are separated by a linker sequence of between 3 to 7 amino acids and comprising the sequence VRY, leading to the following linker-epitope sequences: VRYFLRVPCWKI (SEQ ID NO: 241), VRYFLRVPSWKI (SEQ ID NO: 242), VRYFLRVPCW (SEQ ID NO: 243), and VRYFLRVPSW (SEQ ID NO: 244).

Aspect 2. The peptide according to aspect 1, wherein said T-cell epitope is flanked at its C-terminus by the amino acid sequence TLF leading to the following T-cell epitope-flanker sequence: FLRVPCWKITLF (SEQ ID NO: 3), FLRVPSWKITLF (SEQ ID NO: 4), FLRVPCWTLF (SEQ ID NO: 245) or FLRVPSWTLF (SEQ ID NO: 246).

Aspect 3. The peptide according to aspect 1 or 2, wherein said immunogenic peptide additionally comprises one or more K amino acid residue(s) flanking the epitope at the C-terminus, leading for example to any one of the following sequences of linker-T-cell epitope-flanker:

- FLRVPCWKITLFLK (SEQ ID NO: 5), FLRVPSWKITLFLK (SEQ ID NO: 6),  
 FLRVPCWKITLFLKK (SEQ ID NO: 7), FLRVPSWKITLFLKK (SEQ ID NO: 8),  
 FLRVPCWKITLFLKKK (SEQ ID NO: 9), or FLRVPSWKITLFLKKK (SEQ ID NO: 10),  
 Alternatively, said immunogenic peptide additionally comprises one or more H amino  
 5 acid residue(s) flanking the epitope at the C-terminus, leading for example to any one  
 of the following sequences of linker-T-cell epitope-flanker: FLRVPCWKITLFLH (SEQ ID  
 NO: 11), FLRVPSWKITLFLH (SEQ ID NO: 12), FLRVPCWKITLFLHH (SEQ ID NO: 13),  
 FLRVPSWKITLFLHH (SEQ ID NO: 14), FLRVPCWKITLFLHHH (SEQ ID NO: 15), or  
 FLRVPSWKITLFLHHH (SEQ ID NO: 16),  
 10 Alternatively, said immunogenic peptide additionally comprises one or more R amino  
 acid residue(s) flanking the epitope at the C-terminus, leading for example to any one  
 of the following sequences of linker-T-cell epitope-flanker:  
 FLRVPCWKITLFLR (SEQ ID NO: 17), FLRVPSWKITLFLR (SEQ ID NO: 18),  
 FLRVPCWKITLFLRR (SEQ ID NO: 19), FLRVPSWKITLFLRR (SEQ ID NO: 20), or  
 15 FLRVPCWKITLFLRRR (SEQ ID NO: 21), or FLRVPSWKITLFLRRR (SEQ ID NO: 22).  
 Aspect 4. The peptide according to any one of aspects 1 to 3, wherein the  
 oxidoreductase motif has a sequence of  $Z_m-C-X_n-C-$  (SEQ ID NO: 116 to 140).  
 Aspect 5. The peptide according to any one of aspects 1 to 3, wherein the  
 oxidoreductase motif has a sequence of  $Z_m-[CST]-XX-C-$  or  $Z_m-C-XX-[CST]-$ .  
 20 Aspect 6. The peptide according to any one of aspects 1 to 5, wherein said  
 oxidoreductase motif is selected from the following amino acid motifs:  
 (a)  $Z_m-[CST]-X_n-C-$  or  $Z_m-C-X_n-[CST]-$ ,  
 wherein n is 0, and wherein m is an integer selected from 0, 1, or 2,  
 wherein Z is any amino acid, preferably a basic amino acid preferably selected from:  
 25 H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine,  
 more preferably K or H, most preferably K;  
 (b)  $Z_m-[CST]-X_n-C-$  or  $Z_m-C-X_n-[CST]-$ ,  
 wherein n is 1, wherein X is any amino acid, preferably a basic amino acid selected  
 from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably  
 30 K or R,  
 wherein m is an integer selected from 0, 1, or 2,  
 wherein Z is any amino acid, preferably a basic amino acid preferably selected from:  
 H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine,  
 more preferably K or H, most preferably K;

- (c)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]- as defined in aspect 1, wherein n is 2, thereby creating an internal  $X^1X^2$  amino acid couple within the oxidoreductase motif, wherein X is any amino acid, preferably wherein at least one X is a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or R,  
5 wherein m is an integer selected from 0, 1, or 2,  
wherein Z is any amino acid, preferably a basic amino acid preferably selected from: H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, more preferably K or H, most preferably K;
- 10 (d)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]- as defined in aspect 1, wherein n is 3, thereby creating an internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif, wherein X is any amino acid, preferably wherein at least one X is a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or R,  
15 wherein m is an integer selected from 0, 1, or 2,  
wherein Z is any amino acid, preferably a basic amino acid preferably selected from: H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, more preferably K or H;
- 20 (e)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]- as defined in aspect 1, wherein n is 4, thereby creating an internal  $X^1X^2X^3X^4$  (SEQ ID NO: 154) 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 ;
- 25 (f)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]- as defined in aspect 1, wherein n is 5, thereby creating an internal  $X^1X^2X^3X^4X^5$  (SEQ ID NO: 166) 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;  
30
- (g)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]- as defined in aspect 1, wherein n is 6, thereby creating an internal  $X^1X^2X^3X^4X^5X^6$  (SEQ ID NO: 177) 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)  $Z_m\text{[CST]}-X_n\text{-C-}$  or  $Z_m\text{-C-}X_n\text{[CST]-}$ , 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 (SEQ ID NO: 184 to 203).

Aspect 7. The peptide according to any one of aspects 1 to 6, wherein at least one X is a Proline (P) or a Tyrosine (Y), preferably wherein each X is a Proline or a Tyrosine, more preferably wherein the  $X_n$  or the XX portion of said oxidoreductase motif comprises the sequence PY, preferably wherein the oxidoreductase motif comprises the sequence CPYC (SEQ ID NO: 23).

Aspect 8. The peptide according to any one of aspects 1 to 7, wherein amino acid Z of the oxidoreductase motif is a basic amino acid, preferably a basic amino acid selected from the group of amino acids consisting of: H, K, R, and any non-natural basic amino acid, more preferably a basic amino acid selected from: H, K, and R, most preferably wherein Z is H or K.

Aspect 9. The peptide according to any one of aspects 1 to 8, wherein the oxidoreductase motif b1) has a sequence of HCPYC (SEQ ID NO: 24).

Aspect 10. The peptide according to any one of aspects 1 to 9, wherein said peptide comprises or consists of: the amino sequence HCPYCVRYFLRVPSWKITLF (SEQ ID NO: 25), HCPYCVRYFLRVPCWKITLF (SEQ ID NO: 26), KHCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 27), KHCPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 28), KHCPYCVRYFLRVPSWKITLF (SEQ ID NO: 247), or KHCPYCVRYFLRVPCWKITLF (SEQ ID NO: 248), preferably comprises or consists of the amino sequence KHCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 27).

Aspect 11. The immunogenic peptide according to any one of aspects 1 to 10, wherein said T cell epitope is an NKT cell epitope and the peptide has a length of between 12 and 50 amino acids, preferably of between 12 and 30 amino acids; or wherein said T-cell epitope is an MHC class II T cell epitope and the peptide has a length of between 12 and 50 amino acids, preferably of between 12 and 30 amino acids.

Aspect 12. A polynucleotide (nucleic acid molecule) encoding the immunogenic peptide according to any one of aspects 1 to 11, preferably selected from isolated

desoxyribonucleic acid (DNA), plasmid DNA (pDNA), coding DNA (cDNA), ribonucleic acid (RNA), messenger RNA (mRNA) or modified versions thereof. In some embodiments, 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.

Aspect 13. The peptide according to any one of aspects 1 to 11, or the polynucleotide according to aspect 12, for use as a medicament.

Aspect 14. The peptide or polynucleotide according to aspect 13 for use in treating of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder.

Demyelinating disorders include but are not limited to: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

Preferred are demyelinating disorders caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies such as Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Transverse Myelitis, Adrenoleukodystrophy, Vanishing White Matter Disease, and Rubella induced mental retardation. More preferred demyelinating disorders are Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO). In certain embodiments, said MS is selected from Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and MS-suspected radiology isolated syndrome (RIS).

Aspect 15. An in vitro method for the generation of a population of cytolytic CD4+ T cells, against APC presenting MOG epitopes, comprising the steps of:

- providing peripheral blood cells;
- contacting said cells in vitro with the peptide of any one of aspects 1 to 11, or the polynucleotide according to aspect 12; and
- expanding said cells in the presence of IL-2.

Aspect 16. A method for the generation of a population of cytolytic CD4+ T cells, against APC presenting MOG epitopes, comprising the steps of:

- administering to a subject an effective amount of the peptide of any one of aspects 1 to 11, or the polynucleotide according to aspect 12;
- 5 - obtaining said cytolytic CD4+ T cells from a peripheral blood cell population of said subject.

Aspect 17. A method for the generation of a population of NKT cells, against APC presenting MOG epitopes, comprising the steps of:

- administering to a subject an effective amount of the peptide of any one of aspects 1 to 11, or the polynucleotide according to aspect 12;
- 10 - obtaining said NKT cells from a peripheral blood cell population of said subject.

Aspect 18. A population of cytolytic CD4+ T cells or NKT cells, against APC presenting MOG epitopes, obtainable by the method of aspect 15, 16 or 17.

Aspect 19. A population of cytolytic CD4+ T cells or NKT cells, against APC presenting MOG epitopes, obtainable by the method of aspect 15, 16 or 17, for use as a medicament.

Aspect 20. A population of cytolytic CD4+ T cells or NKT cells for use according to aspect 19, for use in the treatment of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder or reducing the symptoms of a demyelinating disorder.

20 Demyelinating disorders include but are not limited to: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including  
25 tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

Preferred are demyelinating disorders caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies such as Multiple Sclerosis (MS), Neuromyelitis Optica  
30 (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Transverse Myelitis, Adrenoleukodystrophy, Vanishing White Matter Disease, and Rubella induced mental retardation. More preferred demyelinating disorders are Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO). In certain embodiments, said MS is selected from Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary

progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and MS-suspected radiology isolated syndrome (RIS).

Aspect 21. A pharmaceutical composition comprising the peptide of any one of aspects 1 to 11, the polynucleotide according to aspect 12, or the CD4+ T cells or NKT  
5 cells of any one of aspects 18 to 20, or any mixture thereof.

Aspect 22. The pharmaceutical composition of aspect 21, optionally further comprising a pharmaceutically acceptable carrier, and optionally further comprising an additional active ingredient suitable for treatment of a demyelinating disorder, or reducing the symptoms of a demyelinating disorder or preventing a demyelinating  
10 disorder.

Demyelinating disorders include but are not limited to: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central  
15 nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

Preferred are demyelinating disorders caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies such as Multiple Sclerosis (MS), Neuromyelitis Optica  
20 (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Transverse Myelitis, Adrenoleukodystrophy, Vanishing White Matter Disease, and Rubella induced mental retardation. More preferred demyelinating disorders are Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO). In certain embodiments, said MS is selected from  
25 Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and MS-suspected radiology isolated syndrome (RIS).

Aspect 23. The pharmaceutical composition of aspect 21 or 22, for use as a medicament.

Aspect 24. The pharmaceutical composition for use according to aspect 23, for use  
30 in treating of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder.

Demyelinating disorders include but are not limited to: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis,

Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, 5 Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

Preferred are demyelinating disorders caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies such as Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Transverse Myelitis, 10 Adrenoleukodystrophy, Vanishing White Matter Disease, and Rubella induced mental retardation. More preferred demyelinating disorders are Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO). In certain embodiments, said MS is selected from Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple 15 Sclerosis and MS-suspected radiology isolated syndrome (RIS).

Aspect 25. The peptide, polynucleotide, CD4+ T cells, NKT cells, or pharmaceutical composition for use in treating of, ameliorating the symptoms of, and/or preventing of MS according to any one of the previous aspects, wherein the subject is diagnosed with relapse-remitting MS (RRMS).

20 Aspect 26. The peptide, polynucleotide, CD4+ T cells, NKT cells, or pharmaceutical composition for use in treating of, ameliorating the symptoms of, and/or preventing of MS according to any one of the previous aspects, wherein the subject has an HLA-DRB1\* type selected from the group consisting of: HLA-DRB1\*15:01, HLA-DRB1\*03:01, HLA-DRB1\*04:01, and HLA-DRB1\*07:01, preferably wherein the subject 25 has HLA-DRB1\* 15:01.

Aspect 27. The peptide, polynucleotide, CD4+ T cells, NKT cells, or pharmaceutical composition for use in treating of, ameliorating the symptoms of, and/or preventing NMO, according to any one of the previous aspects wherein the subject has an HLA type selected from the group consisting of: HLA-DRB1\*03:01 and HLA-DPB1\*05:01.

30 Aspect 28. Use of an immunogenic peptide according to any one of aspects 1 to 11, the polynucleotide according to aspect 12, or the CD4+ T cells or NKT cells of any one of aspects 18 to 20, or any mixture thereof, for the manufacture of a medicament for treating of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder, preferably a demyelinating disorder caused or aggravated by MOG auto-

antigens and/or anti-MOG antibodies, most preferably Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO).

Aspect 29. A method for treating of, ameliorating the symptoms of, and/or preventing a demyelinating disorder in a subject, comprising the step of providing the peptide according to aspects 1 to 11, the polynucleotide according to aspect 12, or the CD4+ T cells or NKT cells of any one of aspects 18 to 20, or any mixture thereof, to a subject.

Aspect 30. The method according to aspect 29, wherein said demyelinating disorder is selected from: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

In preferred embodiments, the demyelinating disorder is caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies and hence selected from the group consisting of: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Transverse Myelitis, Adrenoleukodystrophy, Vanishing White Matter Disease, and Rubella induced mental retardation. In more preferred embodiments the demyelinating disorder is Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO). In certain embodiments, said MS is selected from Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and MS-suspected radiology isolated syndrome (RIS).

Aspect 31. The method according to aspect 29 or 30, further comprising a step of administering a fumarate compound to said subject. Examples of fumarate compounds are: monomethyl fumarate (MMF), dimethyl fumarate (DMF), compounds that can be metabolized into MMF *in vivo*, monomethyl fumarate prodrugs such as diroximel fumarate or tepilamide fumarate, or a combination of any one or more thereof, or a deuterated form, a clathrate, a solvate, a tautomer, a stereoisomer, or a pharmaceutically acceptable salt of any one or more thereof, or a combination of any one of the foregoing.

Aspect 32. An *in vitro* method for detecting MHC class II restricted CD4+ T cells specific for a MOG antigen in a sample comprising the steps of;

- contacting a subject sample with a complex of an isolated MHC class II molecules and a peptide according to aspects 1 to 11, or the polynucleotide according to aspect 12;

- detecting CD4+ T cells by measuring the binding of said complex with cells in said sample, wherein the binding of the complex to a cell is indicative for the presence of CD4+ T cells in said sample.

The above and further aspects and preferred embodiments of the invention are described in the following sections and in the appended claims. The subject matter of the appended claims is hereby specifically incorporated in this specification.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1:** Kinetics of the redox activities of P1 to P5 immunogenic peptides. DTT is used as a positive control, while Blank represents the assay buffer. The results are expressed in Relative Fluorescent Units (RFU). The assay is described in detail in the Examples section.

**Figure 2:** Binding of the P1 to P5 peptides to HLA-DR3 (DRB1\*03:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

**Figure 3:** Binding of the P1 to P5 peptides to HLA-DR4 (DRB1\*04:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

**Figure 4:** Binding of the P1 to P5 peptides to HLA-DR15 (DRB1\*15:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

**Figure 5:** Binding of the P1, P6 and P7 peptides to HLA-DR3 (DRB1\*03:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

**Figure 6:** Binding of the P1, P6 and P7 peptides to HLA-DR4 (DRB1\*04:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

- 5 **Figure 7:** Binding of the P1, P6 and P7 peptides to HLA-DR15 (DRB1\*15:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

- 10 **Figure 8:** Binding of the P4 and P8 to P11 peptides to HLA-DR3 (DRB1\*03:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

- 15 **Figure 9:** Binding of the P4 and P8 to P11 peptides to HLA-DR4 (DRB1\*04:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

- 20 **Figure 10:** Binding of the P4 and P8 to P11 peptides to HLA-DR15 (DRB1\*15:01 MHC II) protein. The decreasing fluorescence signal (RFU) demonstrates the dose-dependent relationship produced following the competition with biotin-tagged high-affinity control peptide and revealed by Eu<sup>3+</sup> streptavidin interaction.

**Figure 11:** Frequency of effector cells (CD154+) specific for the P2 peptide on the CD4+ cell lines of patients MS017 (S9), MS022 (S10) and MS027 (S12) (S, stimulation).

- 25 **Figure 12:** Specific secretion of cytokines (IL-5 and IL-13) induced by P2 in culture supernatant of MS026 CD4+ cell line (S11).

**Figure 13:** Frequency of effector cells (CD154+) specific for the P4 peptide on the CD4+ cell lines of patients MS017 (S12), MS020 (S7), MS021 (S9), MS024 (S7), MS026 (S12), MS027 (S12), MS028 (S11) and MS029 (S9) (S, stimulation).

- 30 **Figure 14:** Frequency of effector cells (CD154+) and effector cells expressing Fas ligand (CD154+/FasL+) specific for P4 peptide on the CD4+ cell lines of patients MS017 (S9) and MS020 (S10) (S, stimulation).

**Figure 15:** Specific secretion of cytokine (IL-5) induced by P4 in culture supernatant of MS017 (S15), MS024 (S20) and MS026 (S14) CD4+ cell lines (S, stimulation).

**Figure 16:** Frequency of effector cells (CD154+) specific for the P4 peptide and its corresponding short C-WT epitope (DPHFLRVPCWKITLFFK, SEQ ID NO: 29) on the CD4+ cell lines of patients MS017 (S14) and MS026 (S13) (S, stimulation).

**Figure 17:** Frequency of effector cells (CD154+) specific for the P4 peptide and its corresponding short S-WT epitope (KLHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253) on the CD4+ cell lines of patients MS024 (S20), MS017 (S9), MS026 (S13), MS028 (S11) and MS029 (S9) (S, stimulation).

**Figure 18:** Specific secretion of cytokine (IL-5) induced by P4 peptide and its corresponding short C-WT epitope (DPHFLRVPCWKITLFFK, SEQ ID NO: 29) and long C-WT epitope (QYRLRGKLRAEIEENLHRTFDPHFLRVPCWKITLFFVIVPVLGP, SEQ ID NO: 30) in culture supernatant of MS017 (S15) CD4+ cell line (S, stimulation).

**Figure 19:** Specific secretion of cytokine (IL-5) induced by P4 peptide and its corresponding short S-WT epitope (KLHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253) in culture supernatant of MS017 (S12) and MS024 (S20) CD4+ cell lines (S, stimulation).

**Figure 20:** Percentage of specific LCL apoptosis when labelled autologous LCL, loaded with P4 peptide or its corresponding short S-WT epitope (KLHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253), are co-cultured with the P4-specific CD4+ cell lines of patients MS017 (S7), MS026 (S12), MS028 (S11) and MS029 (S9) (S, stimulation).

**Figure 21:** Blinded evaluation of clinical EAE scoring (0-5) performed daily from day 7 to day 28. Mice were injected with MOG<sub>35-55</sub> to induce EAE at day 0, and were left untreated or therapeutically treated with IMCY-0189 or P4 (see table 2 for details). The mean clinical score was determined each day for each group of mice.

**Figure 22:** AUC calculated from EAE scores displayed in figure 21 for each group of mice. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 23:** MMS calculated from EAE scores displayed in figure 21 for each group of mice. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 24:** Inflammation levels for each group of mice presented in table 2. Inflammatory foci of approximately 20 cells were counted in each H&E stained section. When inflammatory infiltrates consisted of more than 20 cells, an estimate was made

of how many foci of 20 cells were present. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 25:** Demyelination levels for each group of mice presented in table 2. Demyelination was scored in each anti-MBP (using immunohistochemistry) stained section. The demyelination score represents an estimate of demyelinated area for each section. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 26:** Serum neurofilaments levels for each group of mice presented in table 2. Neurofilament light (NF-L) protein levels were quantified at Quanterix™ through the NF-light Simoa® assay advantage kit. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 27:** Blinded evaluation of clinical EAE scoring (0-5) performed daily from day 4 to day 43. Mice were prophylactically immunized or not with IMCY-0189, then injected with MOG<sub>35-55</sub> to induce EAE at day 0, and immunized again or not with IMCY-0189 (see table 4 for details). The mean clinical score was determined each day for each group of mice.

**Figure 28:** AUC calculated from EAE scores displayed in figure 27 for each group of mice. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 29:** MMS calculated from EAE scores displayed in figure 27 for each group of mice. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 30:** Inflammation levels for each group of mice presented in table 4. Inflammatory foci of approximately 20 cells were counted in each H&E stained section. When inflammatory infiltrates consisted of more than 20 cells, an estimate was made of how many foci of 20 cells were present. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 31:** Demyelination levels for each group of mice presented in table 4. Demyelination was scored in each anti-MBP (using immunohistochemistry) stained section. The demyelination score represents an estimate of demyelinated area for each section. Significant differences are referred as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure 32:** Plasma neurofilaments levels for each group of mice presented in table 4. Neurofilament light (NF-L) protein levels were quantified at Quanterix™ through the NF-light Simoa® assay advantage kit. Significant differences are referred as follows: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001.

5

## DETAILED DESCRIPTION OF THE INVENTION

The present invention will be described with respect to particular embodiments, but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The following terms or definitions are

10 provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. The definitions provided herein should not be construed to have a scope less than the one understood by a person of ordinary skill in the art.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are

15 not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks, to the general background art referred to above and to the further references cited therein.

As used herein, the singular forms 'a', 'an', and 'the' include both singular and plural

20 referents unless the context clearly dictates otherwise. The term "any" when used in relation to aspects, claims or embodiments as used herein refers to any single one (i.e. anyone) as well as to all combinations of said aspects, claims or embodiments referred to.

The terms 'comprising', 'comprises' and 'comprised of' as used herein are synonymous

25 with 'including', 'includes' or 'containing', 'contains', and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. Said terms also encompass the embodiments "consisting essentially of" and "consisting of". The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

30 The term 'about' as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less,

and still more preferably  $\pm 0.1\%$  or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier 'about' refers is itself also specifically, and preferably, disclosed.

- 5 As used herein, the term "for use" as used in "composition for use in treatment of a disease" shall disclose also the corresponding method of treatment and the corresponding use of a preparation for the manufacture of a medicament for the treatment of a disease".

The term "**peptide**" as used herein refers to a molecule comprising an amino acid  
10 sequence of between 9 and 200 amino acids, connected by peptide bonds, but which can comprise non-amino acid structures, synthetic amino acids or modified amino acids.

Peptides according to the invention can contain proteinogenic and/or non-proteinogenic amino acids. Said peptides can contain any of the conventional 20 amino  
15 acids or modified versions thereof, or can contain non-naturally occurring amino-acids incorporated by chemical peptide synthesis or by chemical or enzymatic modification.

The term "**antigen**" as used herein refers to a structure of a macromolecule, typically protein (with or without polysaccharides) or made of proteic composition comprising one or more hapten(s) and comprising T cell epitopes.

- 20 The term "**antigenic protein**" as used herein refers to a protein comprising one or more T cell epitopes. An auto-antigen or auto-antigenic protein as used herein refers to a human or animal protein present in the body, which elicits an immune response within the same human or animal body.

The term "**epitope**" refers to one or several portions (which may define a  
25 conformational epitope) of an antigenic protein which is/are specifically recognised and bound by an antibody or a portion thereof (Fab', Fab2', etc.) or a receptor presented at the cell surface of a B or T cell lymphocyte, and which is able, by said binding, to induce an immune response.

- The term "**T cell epitope**" in the context of the present invention refers to a dominant,  
30 sub-dominant or minor T cell epitope, i.e. a part of an antigenic protein that is specifically recognised and bound by a receptor at the cell surface of a T or NKT cell. Whether an epitope is dominant, sub-dominant or minor depends on the immune reaction elicited against the epitope. Dominance depends on the frequency at which

such epitopes are recognised by T or NKT cells and able to activate them, among all the possible T cell epitopes of a protein.

In the context of the present invention, the T cell epitope can be an epitope recognized by MHC class II molecules and presented to CD4+ T cells, or can be an epitope  
5 recognized by CD1d molecules and presented to NKT cells.

An **epitope recognised by MHC class II molecules** typically comprises or consists of a sequence of +/- 9 amino acids which fit in the groove of the MHC II molecule. Within a peptide sequence representing a T cell epitope, the amino acids in the epitope are numbered P1 to P9, amino acids N-terminal of the epitope are numbered P-1, P-2  
10 and so on, amino acids C terminal of the epitope are numbered P+1, P+2 and so on. Peptides recognised by MHC class II molecules and not by MHC class I molecules are referred to as MHC class II restricted T cell epitopes.

The term "**MHC**" refers to "**major histocompatibility antigen**". In humans, the MHC genes are known as HLA ("human leukocyte antigen") genes. Although there is no  
15 consistently followed convention, some literature uses HLA to refer to HLA protein molecules, and MHC to refer to the genes encoding the HLA proteins. As such the terms "MHC" and "HLA" are equivalents when used herein. The HLA system in man has its equivalent in the mouse, i.e., the H2 system. The most intensely-studied HLA genes are the nine so-called classical MHC genes: HLA-A, HLA-B, HLA-C, HLA-DPA1,  
20 HLA-DPB1, HLA-DQA1, HLAs DQB1, HLA-DRA, and HLA-DRB1. In humans, the MHC is divided into three regions: Class I, II, and III. The A, B, and C genes belong to MHC class I, whereas the six D genes belong to class II. MHC class I molecules are made of a single polymorphic chain containing 3 domains (alpha 1, 2 and 3), which associates with beta-2-microglobulin at cell surface. Class II molecules are made of 2  
25 polymorphic chains, each containing 2 chains (alpha 1 and 2, and beta 1 and 2). Class I MHC molecules are expressed on virtually all nucleated cells. Since the HLA system is inherited in a Mendelian manner, HLA series of genes, or haplotypes, can be distinguished in subjects of a given population.

In general, the peptides of the current disclosure have improved binding to HLA-  
30 DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 with much higher affinity than prior art peptide disclosed in WO2017182528. Hence, a preferred HLA type of a patient suffering from a demyelinating disorder is selected from the group consisting of HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01. In the global MS patient population, about 50% to 60% have HLA-DRB1\* type 15:01. Further, over 75% of the

MS patient population has a HLA-DRB1\*15:01, HLA-DRB1\*03:01, HLA-DRB1\*04:01, or HLA-DRB1\*07:01 type of HLA. A preferred HLA type of an MS patient in view of the current invention is therefore selected from the group consisting of: DRB1\*15:01, HLA-DRB1\*03:01, HLA-DRB1\*04:01, and HLA-DRB1\*07:01. More preferred are MS patients having a HLA-DRB1\* type 15:01. Further preferred are RRMS diagnosed MS patients having an HLA type selected from the group consisting of: DRB1\*15:01, HLA-DRB1\*03:01, HLA-DRB1\*04:01, and HLA-DRB1\*07:01. Further preferred are RRMS diagnosed MS patients having an HLA type HLA-DRB1\*15:01. Preferred HLA haplotypes of NMO patients in the current invention are therefore HLA-DRB1\*03:01 and HLA-DPB1\*05:01, more preferably HLA-DRB1\*03:01. Peptide fragments presented in the context of class I MHC molecules are recognised by CD8+ T lymphocytes (cytolytic T lymphocytes or CTLs). CD8+ T lymphocytes frequently mature into cytolytic effectors which can lyse cells bearing the stimulating antigen. Class II MHC molecules are expressed primarily on activated lymphocytes and antigen-presenting cells. CD4+ T lymphocytes (helper T lymphocytes or Th) are activated with recognition of a unique peptide fragment presented by a class II MHC molecule, usually found on an antigen-presenting cell like a macrophage or dendritic cell. CD4+ T lymphocytes proliferate and secrete cytokines such as IL-2, IFN-gamma and IL-4 that support antibody-mediated and cell mediated responses.

Functional HLAs are characterised by a deep binding groove to which endogenous as well as foreign, potentially antigenic peptides bind. The groove is further characterised by a well-defined shape and physico-chemical properties. HLA class I binding sites are closed, in that the peptide termini are pinned down into the ends of the groove. They are also involved in a network of hydrogen bonds with conserved HLA residues. In view of these restraints, the length of bound peptides is limited to 8, 9 or 10 residues. However, it has been demonstrated that peptides of up to 12 amino acid residues are also capable of binding HLA class I. Comparison of the structures of different HLA complexes confirmed a general mode of binding wherein peptides adopt a relatively linear, extended conformation, or can involve central residues to bulge out of the groove.

In contrast to HLA class I binding sites, class II sites are open at both ends. This allows peptides to extend from the actual region of binding, thereby "hanging out" at both ends. Class II HLAs can therefore bind peptide ligands of variable length, ranging from 7, 8 or 9 to more than 25 amino acid residues. Similar to HLA class I, the affinity of a

class II ligand is determined by a "constant" and a "variable" component. The constant part again results from a network of hydrogen bonds formed between conserved residues in the HLA class II groove and the main chain of a bound peptide. However, this hydrogen bond pattern is not confined to the N-and C-terminal residues of the peptide but distributed over the whole chain. The latter is important because it restricts the conformation of complexed peptides to a strictly linear mode of binding. This is common for all class II allotypes. The second component determining the binding affinity of a peptide is variable due to certain positions of polymorphism within class II binding sites. Different allotypes form different complementary pockets within the groove, thereby accounting for subtype-dependent selection of peptides, or specificity. Importantly, the constraints on the amino acid residues held within class II pockets are in general "softer" than for class I. There is much more cross reactivity of peptides among different HLA class II allotypes. The sequence of the +/- 9 amino acids (i.e. 8, 9 or 10) of an MHC class II T cell epitope that fit in the groove of the MHC II molecule are usually numbered P1 to P9. Additional amino acids N-terminal of the epitope are numbered P-1, P-2 and so on, amino acids C-terminal of the epitope are numbered P+1, P+2 and so on.

An **epitope recognised by CD1d molecules** refers to a part of an antigenic protein that is specifically recognized and bound by a receptor at the cell surface of a T lymphocyte, in particular NKT cells. An epitope recognised by CD1d molecules typically comprises or consists of a sequence of +/- 7 amino acids which fit in the groove of the CD1d molecules. Typically, NKT cell epitopes are hydrophobic. The structure of the CD1d molecule indicates that hydrophobic amino acid residues are required to occupy the two hydrophobic pockets located at the extremities of the CD1d cleft and that an aliphatic residue should occupy the position in the middle of the cleft. Therefore, as a general but not limiting example of CD1d binding sequence, the motif [FWHY]-xx-[ILMV]-xx-[FWHY] (SEQ ID NO: 147) can be used in which [FWHY] indicates that either F, W, H or Y can occupy the first anchoring residue (P1), that the P4 position can be occupied by either I, L, M or V and that P7 can be occupied by F, W, H or Y. "x" in this general model motif stands for any amino acid. In a particular embodiment, the general model motif can be defined by the sequence [FW]-xx-[ILM]-xx-[FW] (SEQ ID NO: 148), preferably by the sequence [FW]-xx-[ILM]-xx-[W] (SEQ ID NO: 149).

The term "**NKT cells**" refers to cells of the innate immune system characterized by the fact that they carry receptors such as NK1.1 and NKG2D, and recognize epitopes presented by the CD1d molecule. In the context of the present invention, NKT cells can belong to either the type 1 (invariant) or the type 2 subset, or to any of the less characterized NKT cells with more polymorphic T cell receptors than type 1 or type 2 NKT cells. The participation of NKT cells in the control of immune responses in autoimmune diseases, or against allofactors or allergens has been reported on a number of occasions (Jahng et al Journal of experimental Medicine 199: 947-957, 2004; Van Belle and von Herrath, Molecular Immunology 47: 8-11, 2009) but is relatively difficult to describe. In the context of the present invention, we made the unexpected observation that peptides can be presented by the CD1d molecule. A characteristic of the CD1d molecule is to be made of 2 anti-parallel alpha chains forming a cleft sitting atop of a platform made of two anti-parallel beta chains. The cleft is narrow and deep and accept only hydrophobic residues, classically deemed to be only lipids. In fact, peptides with hydrophobic residues have the capacity to bind to the CD1d cleft. Besides, as the cleft is open both sides, peptides longer than 7 aminoacids can be accommodated. Hydrophobic peptides carrying the CD1d motif are found in autoantigens, allofactors and allergens, thereby endowing said autoantigen, allofactor or allergen with the capacity to activate CD4+ NKT cells. Direct elimination by killing of cells presenting said autoantigen, allofactor or allergen eliminates the capacity to mount an immune response against these antigens/factors.

The term "**CD1d molecule**" refers to a non-MHC derived molecule made of 3 alpha chains and an anti-parallel set of beta chains arranged into a deep hydrophobic groove opened on both sides and capable of presenting lipids, glycolipids or hydrophobic peptides to NKT cells. The term "immune disorders" or "immune diseases" refers to diseases wherein a reaction of the immune system is responsible for or sustains a malfunction or non-physiological situation in an organism.

The term "**homologue**" as used herein with reference to the epitopes used in the context of the invention, refers to molecules having at least 50%, at least 70%, at least 80%, at least 90%, at least 95% or at least 98% amino acid sequence identity with the naturally occurring epitope, thereby maintaining the ability of the epitope to bind an antibody or cell surface receptor of a B and/or T cell. Particular homologues of an epitope correspond to the natural epitope modified in at most three, more particularly in at most 2, most particularly in one amino acid.

The term "**derivative**" as used herein with reference to the peptides of the invention refers to molecules which contain at least the peptide active portion (i.e. the redox motif and the MHC class II epitope capable of eliciting cytolytic CD4+ T cell activity) and, in addition thereto comprises a complementary portion which can have different purposes  
5 such as stabilising the peptides or altering the pharmacokinetic or pharmacodynamic properties of the peptide.

The term "**sequence identity**" of two sequences as used herein relates to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the sequences, when the two sequences  
10 are aligned. In particular, the sequence identity is from 70% to 80%, from 81% to 85%, from 86% to 90%, from 91% to 95%, from 96% to 100%, or 100%.

The terms "**peptide-encoding polynucleotide (or nucleic acid)**" and "**polynucleotide (or nucleic acid) encoding peptide**" as used herein refer to a nucleotide sequence, which, when expressed in an appropriate environment, results  
15 in the generation of the relevant peptide sequence or a derivative or homologue thereof. Such polynucleotides or nucleic acids include the normal sequences encoding the peptide, as well as derivatives and fragments of these nucleic acids capable of expressing a peptide with the required activity. The nucleic acid encoding a peptide according to the invention or fragment thereof is a sequence encoding the peptide or  
20 fragment thereof originating from a mammal or corresponding to a mammalian, most particularly a human peptide fragment. Such polynucleotides or nucleic acids molecules may be readily prepared using an automated synthesiser and the well-known codon-amino acid relationship of the genetic code. Such polynucleotides or nucleic acids may be incorporated into expression vectors, including plasmids, which  
25 are adapted for the expression of the polynucleotide or nucleic acid and production of the polypeptide in a suitable host such as bacterium, e.g. Escherichia coli, yeast cell, human cell, animal cell or plant cell. For therapeutic means, polynucleotides encoding the immunogenic peptides disclosed herein can be part of an expression system, cassette, plasmid or vector system such as viral and non-viral expression systems.  
30 Viral vectors known for therapeutic purposes are adenoviruses, adeno-associated viruses (AAVs), lentiviruses, and retroviruses. Non-viral vectors can be used as well and non-limiting examples include: transposon-based vector systems such as those derived from Sleeping Beauty (SB) or PiggyBac (PB). Nucleic acids can also be

delivered through other carriers such as but not limited to nanoparticles, cationic lipids, liposomes etc.

The term "**immune disorders**" or "**immune diseases**" refers to diseases wherein a reaction of the immune system is responsible for or sustains a malfunction or non-physiological situation in an organism. Included in immune disorders are, inter alia,  
5 allergic disorders and autoimmune diseases.

The terms "**autoimmune disease**" or "**autoimmune disorder**" refer to diseases that result from an aberrant immune response of an organism against its own cells and tissues due to a failure of the organism to recognise its own constituent parts (down to  
10 the sub-molecular level) as "self". The group of diseases can be divided in two categories, organ-specific and systemic diseases. An "allergen" is defined as a substance, usually a macromolecule or a proteic composition which elicits the production of IgE antibodies in predisposed, particularly genetically disposed, individuals (atopics) patients. Similar definitions are presented in Liebers et al. (1996)  
15 Clin. Exp. Allergy 26, 494-516.

The term "**demyelination**" as used herein refers to damaging and/or degradation of myelin sheaths that surround axons of neurons which has as a consequence the formation of lesions or plaques. It is understood that the myelin acts as a protective covering surrounding nerve fibers in brain, optic nerves, and spinal cord. Due to  
20 demyelination, the signal conduction along the affected nerves is impaired (i.e. slowed or stopped), and may cause neurological symptoms such as deficiencies in sensation, movement, cognition, and/or other neurological function. The concrete symptoms a patient suffering from a demyelinating disease will vary depending on the disease and disease progression state. These may include a blurred and/or double vision, ataxia,  
25 clonus, dysarthria, fatigue, clumsiness, hand paralysis, hemiparesis, genital anaesthesia, incoordination, paraesthesia, ocular paralysis, impaired muscle coordination, muscle weakness, loss of sensation, impaired vision, neurological symptoms, unsteady way of walking (gait), spastic paraparesis, incontinence, hearing problems, speech problems, and others.

Therefore, "**demyelinating diseases**" or "**demyelinating disorders**" as used herein and commonly used in the art is indicative for any pathologic condition of the nervous system which involves impairment, for example damaging, or the myelin sheath of neurons. Demyelinating diseases may be stratified into central nervous system demyelinating diseases and peripheral nervous system. Alternatively, demyelinating

diseases may be classified according to the cause of demyelination: destruction of myelin (demyelinating myelinoclastic), or abnormal and degenerative myelin (dysmyelinating leukodystrophic). Non-limiting examples of demyelinating diseases are Multiple Sclerosis (MS) - (e.g. Relapsing/Remitting Multiple Sclerosis, Secondary Progressive Multiple Sclerosis, Progressive Relapsing Multiple Sclerosis, Primary Progressive Multiple Sclerosis, and Acute Fulminant Multiple Sclerosis), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), and Rubella induced mental retardation. It is appreciated by a skilled person that several of the above mentioned annotations are general classification names indicative of a group of diseases characterized by an identical or similar set of aberrant processes at the molecular level and/or an identical or similar set of (clinical) symptoms. A human patient having a demyelinating disorder can have one or more symptoms of a demyelinating disorder such as, but not limited to, impaired vision, numbness, weakness in extremities, tremors or spasticity, heat intolerance, speech impairment, incontinence, dizziness, or impaired proprioception (e.g., balance, coordination, sense of limb position). A human (e.g., a human patient) with a family history of a demyelinating disorder (e.g., a genetic predisposition for a demyelinating disorder), or who exhibits mild or infrequent symptoms of a demyelinating disorder described above can be, for the purposes of the method, considered at risk of developing a demyelinating disorder (e.g., Multiple Sclerosis). Preferred demyelinating diseases in the context of the current disclosure are those caused by MOG autoantigens or involving anti-MOG antibodies, including but not limited to Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO).

The term "**Multiple Sclerosis**", abbreviated herein and in the art as "**MS**", indicates an autoimmune disorder affecting the central nervous system. MS is considered the most common non-traumatic disabling disease in young adults (Dobson and Giovannoni, (2019) Eur. J. Neurol. 26(1), 27-40), and the most common autoimmune disorder affecting the central nervous system (Berer and Krishnamoorthy (2014) FEBS Lett. 588(22), 4207-4213). MS is considered in the art a demyelinating disorder of the central nervous system (Lubetzki and Stankoff. (2014). Handb Clin Neurol. 122, 89-

99. MS may manifest itself in a subject by a large number of different symptoms ranging from physical over mental to psychiatric problems. Typical symptoms include blurred or double vision, muscle weakness, blindness in one eye, and difficulties in coordination and sensation. In most cases, MS may be viewed as a two-stage disease, with early inflammation responsible for relapsing–remitting disease and delayed neurodegeneration causing non-relapsing progression, i.e. secondary and primary progressive MS. Although progress is being made in the field, a conclusive underlying cause of the disease remains hitherto elusive and over 150 single nucleotide polymorphisms have been associated with MS susceptibility (International Multiple Sclerosis Genetics Consortium Nat Genet. (2013). 45(11):1353-60). Vitamin D deficiency, smoking, ultraviolet B (UVB) exposure, childhood obesity and infection by Epstein-Barr virus have been reported to contribute to disease development (Ascherio (2013) Expert Rev Neurother. 13(12 Suppl), 3-9).

Hence, MS can be regarded as a single diseases existing within a spectrum extending from relapsing (wherein inflammation is the dominant feature) to progressive (neurodegeneration dominant). Therefore it is evident that the term Multiple sclerosis as used herein encompasses any type of Multiple Sclerosis belonging to any kind of disease course classification. In particular the invention is envisaged to be a potent treatment strategy patient diagnosed with, or suspected of having clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and even MS-suspected radiology isolated syndrome (RIS). While strictly not considered a disease course of MS, RIS is used to classify subjects showing abnormalities on the Magnetic Resonance Imaging (MRI) of brain and/or spinal cord that correspond to MS lesions and cannot be prima facie explained by other diagnoses. CIS is a first episode (by definition lasting for over 24 hours) of neurologic symptoms caused by inflammation and demyelination in the central nervous system. In accordance with RIS, CIS classified subjects may or may not continue to develop MS, with subjects showing MS-like lesions on a brain MRI more likely to develop MS. RRMS is the most common disease course of MS with 85% of subjects having MS being diagnosed with RRMS. RRMS diagnosed patients are a preferred group of patients in view of the current invention. RRMS is characterized by attacks of new or increasing neurologic symptoms, alternatively worded relapses or exacerbations. In RRMS, said relapses are followed by periods or partial or complete remission of the symptoms, and no

disease progression is experienced and/or observed in these periods of remission. RRMS may be further classified as active RRMS (relapses and/or evidence of new MRI activity), non-active RRMS, worsening RRMS (increasing disability over a specified period of time after a relapse, or not worsening RRMS. A portion of RRMS  
5 diagnosed subject will progress to the SPMS disease course, which is characterized by a progressive worsening of neurologic function, i.e. an accumulation of disability, over time. SPMS subclassifications can be made such as active (relapses and/or new MRI activity), not active, progressive (disease worsening over time), or non-progressive SPMS. Finally, PPMS is an MS disease course characterized by  
10 worsening of neurologic function and hence an accumulation of disability from the onset of symptoms, without early relapse or remission. Further PPMS subgroups can be formed such as active PPMS (occasional relapse and/or new MRI activity), non-active PPMS, progressive PPMS (evidence of disease worsening over time, regardless of new MRI activity) and non-progressive PPMS. In general, MS disease courses are  
15 characterized by substantial intersubject variability in terms of relapse and remission periods, both in severity (in case of relapse) and duration.

Several disease modifying therapies are available for MS, and therefore the current invention may be used as alternative treatment strategy, or in combination with these existing therapies. Non-limiting examples of active pharmaceutical ingredients include  
20 fumarate compounds, interferon beta-1a, interferon beta-1b, glatiramer acetate, glatiramer acetate, peginterferon beta-1a, teriflunomide, fingolimod, cladribine, siponimod, ozanimod, alemtuzumab, mitoxantrone, ocrelizumab, and natalizumab. Examples of fumarate compounds are: monomethyl fumarate (MMF), dimethyl fumarate (DMF), compounds that can be metabolized into MMF *in vivo*, monomethyl  
25 fumarate prodrugs such as diroximel fumarate or tepilamide fumarate, or a combination of any one or more thereof, or a deuterated form, a clathrate, a solvate, a tautomer, a stereoisomer, or a pharmaceutically acceptable salt of any one or more thereof, or a combination of any one of the foregoing. Alternatively, the invention may be used in combination with a treatment or medication aiming to relapse management,  
30 such as but not limited to methylprednisolone, prednisone, and adrenocorticotrophic hormone(s) (ACTH). Further, the invention may be used in combination with a therapy aiming to alleviate specific symptoms. Non-limiting examples include medications aiming to improve or avoid symptoms selected from the group consisting of: bladder

problems, bowel dysfunction, depression, dizziness, vertigo, emotional changes, fatigue, itching, pain, sexual problems, spasticity, tremors, and walking difficulties.

MS is characterized by three intertwined hallmark characteristics: 1) lesion formation in the central nervous system, 2) inflammation, and 3) degradation of myelin sheaths

5 of neurons. Despite traditionally being considered a demyelinating disease of the central nervous system and white matter, more recently reports have surfaced that demyelination of the cortical and deep gray matter may exceed white matter demyelination (Kutzelnigg et al. (2005). Brain. 128(11), 2705-2712). Two main hypotheses have been postulated as to how MS is caused at the molecular level. The

10 commonly accepted "outside-in hypothesis" is based on the activation of peripheral autoreactive effector CD4+ T cells which migrate to the central nervous system and initiate the disease process. Once in the central nervous system, said T cells are locally reactivated by APCs and recruit additional T cells and macrophages to establish inflammatory lesions. Noteworthy, MS lesions have been shown to contain CD8+ T  
15 cells predominantly found at the lesion edges, and CD4+ T cells found more central in the lesions. These cells are thought to cause demyelination, oligodendrocyte destruction, and axonal damage, leading to neurologic dysfunction. Additionally, immune-modulatory networks are triggered to limit inflammation and to initiate repair, which results in at least partial remyelination reflected by clinical remission.  
20 Nonetheless, without adequate treatment, further attacks often lead to progression of the disease.

MS onset is believed to originate well before the first clinical symptoms are detected, as evidenced by the typical occurrence of apparent older and inactive lesions on the MRI of patients. Due to advances in the development of diagnostic methods, MS can  
25 now be detected even before a clinical manifestation of the disease (i.e. pre-symptomatic MS). In the context of the invention, "treatment of MS" and similar expressions envisage treatment of, and treatment strategies for, both symptomatic and pre-symptomatic MS. In particular, when the immunogenic peptides and/or resulting cytolytic CD4+ T cells are used for treating a pre-symptomatic MS patient, the disease  
30 is halted at such an early stage that clinical manifestations may be partially, or even completely avoided.

The term "**Neuromyelitis Optica**" or "NMO" and "NMO Spectrum Disorder (NMOSD)", also known as "Devic's disease", refers to an autoimmune disorder in which white blood cells and antibodies primarily attack the optic nerves and the spinal cord, but

may also attack the brain (reviewed in Wingerchuk 2006, Int MS J. 2006 May;13(2):42-50). The damage to the optic nerves produces swelling and inflammation that cause pain and loss of vision; the damage to the spinal cord causes weakness or paralysis in the legs or arms, loss of sensation, and problems with bladder and bowel function.

5 NMO is a relapsing-remitting disease. During a relapse, new damage to the optic nerves and/or spinal cord can lead to accumulating disability. Unlike MS, there is no progressive phase of this disease. Therefore, preventing attacks is critical to a good long-term outcome. In cases associated with anti-MOG antibodies, it is considered that anti-MOG antibodies may trigger an attack on the myelin sheath resulting in

10 demyelination. The cause of NMO in the majority of cases is due to a specific attack on auto-antigens. Up to a third of subjects may be positive for auto-antibodies directed against a component of myelin called myelin oligodendrocyte glycoprotein (MOG). People with anti-MOG related NMO similarly have episodes of transverse myelitis and optic neuritis.

15 The term "**therapeutically effective amount**" refers to an amount of the peptide of the invention or derivative thereof, which produces the desired therapeutic or preventive effect in a patient. For example, in reference to a disease or disorder, it is the amount which reduces to some extent one or more symptoms of the disease or disorder, and more particularly returns to normal, either partially or completely, the physiological or

20 biochemical parameters associated with or causative of the disease or disorder. Typically, the therapeutically effective amount is the amount of the peptide of the invention or derivative thereof, which will lead to an improvement or restoration of the normal physiological situation. For instance, when used to therapeutically treat a mammal affected by an immune disorder, it is a daily amount peptide/kg body weight

25 of the said mammal. Alternatively, where the administration is through gene-therapy, the amount of naked DNA or viral vectors is adjusted to ensure the local production of the relevant dosage of the peptide of the invention, derivative or homologue thereof.

The term "**natural**" when referring to a peptide relates to the fact that the sequence is identical to a fragment of a naturally occurring protein (wild type or mutant). In contrast

30 therewith the term "**artificial**" refers to a sequence which as such does not occur in nature. An artificial sequence is obtained from a natural sequence by limited modifications such as changing/deleting/inserting one or more amino acids within the naturally occurring sequence or by adding/removing amino acids N- or C-terminally of a naturally occurring sequence.

The term "**oxidoreductase motif**", "**thiol-oxidoreductase motif**", "**thioreductase motif**", "**thioredox motif**" or "**redox motif**" are used here as synonymous terms and refers to a motif of general sequence thioreductase sequence motif C-X<sub>n</sub>-[CST]- (SEQ ID NO: 91 to 95) or [CST]-X<sub>n</sub>-C- (SEQ ID NO: 66 to 70), with n being an integer from 0 to 6. Such peptide motives exert reducing activity for disulfide bonds on proteins (such as enzymes) through redox active cysteines within conserved active domain consensus sequences: C-X<sub>n</sub>-[CST]- or [CST]-X<sub>n</sub>-C-, such as for example in C-XX-C (SEQ ID NO: 116), C-XX-S (SEQ ID NO: 150), C-XX-T (SEQ ID NO: 151), S-XX-C (SEQ ID NO: 152), T-XX-C (SEQ ID NO: 153) (Fomenko et al. (2003) Biochemistry 42, 1 1214-1 1225), in which "X" stands for any amino acid, in which C stands for cysteine, S for serine, T for threonine and X for any amino acid except tyrosine, phenylalanine or tryptophan.

The terms "cysteine", "C", "serine", "S", and "threonine", "T", when used in the light of the amino acid residues present in the oxidoreductase motifs disclosed herein respectively refer to naturally occurring cysteine, serine or threonine amino acids. Unless explicitly stated differently, said terms hence exclude chemically modified cysteines, serines and threonines such as those 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 a further embodiment thereto, said oxidoreductase motif is positioned N-terminally of the T-cell epitope.

Alternatively, the immunogenic peptides may contain an oxidoreductase motif in the form of the following general amino acid formula: Z<sub>m</sub>-[CST]-X<sub>n</sub>-C- (SEQ ID NO: 66 to 90) or Z<sub>m</sub>-C-X<sub>n</sub>-[CST]- (SEQ ID NO: 91 to 115),

wherein n is an integer chosen from 0 to 6, wherein m is an integer selected from 0 to 3, wherein X is any amino acid, wherein Z is any amino acid, in which C stands for cysteine, S for serine, T for threonine.

Preferably said oxidoreductase motif is not part of a repeat of the standard C-XX-[CST] or [CST]-XX-C 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: 249)), as repeats which are adjacent to each other (CXXCCXXCCXXC (SEQ ID NO: 250)) or as repeats which overlap with each other CXXCXXCXXC (SEQ ID NO: 251) or CXCCXCCXCC (SEQ ID NO: 252)), especially when n is 0 or 1 and m is 0.

Hence, envisaged are thus motifs of the form  $Z_m$ -[CST]-C- or  $Z_m$ -C-[CST]-, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m is 1 or 2  
 5 and Z is 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. Non-limiting preferred examples of such motifs are KCC, KKCC (SEQ ID NO: 31), RCC, RRCC (SEQ ID NO: 32), RKCC (SEQ ID NO: 33), or KRCC (SEQ ID NO: 34).

Further envisaged are motifs of the form  $Z_m$ -[CST]-X-C- or  $Z_m$ -C-X-[CST]-, wherein X  
 10 is any amino acid, preferably a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, preferably K or R, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m is 1 or 2 and Z is a basic  
 15 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. Non-limiting preferred examples of such motifs are KCXC, KKCXC, RCXC, RRCXC, RKCXC, KRCXC, KCKC, KKCKC, KCRC, KKCRC, RCRC, RRCRC, RKCKC, KRCKC (corresponding to SEQ ID NOs: 35 to 48), or RCKC (SEQ ID NO: 240).

Further envisaged are motifs of the form  $Z_m$ -[CST]-XX-C- or  $Z_m$ -C-XX-[CST]-. In these motifs, an internal  $X^1X^2$  amino acid couple is situated within the oxidoreductase motif, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m  
 25 is 1 or 2 and Z 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, preferably K or H.  $X^1$  and  $X^2$ , 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  
 30 least one of  $X^1$  or  $X^2$  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  $X^1$  or  $X^2$  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. Particularly preferred motifs of this form are HCPYC, KHCPYC, KCPYC, RCPYC, HCGHC, KCGHC, and RCGHC (corresponding to SEQ ID NOs: 49 to 55). Alternative preferred motifs of this form are KKCPYC, KRCPYC, KHCGHC, KKCGHC, and KRCGHC (SEQ ID NOs: 210 to 214).

- 5 Further envisaged are motifs of the form  $Z_m$ -[CST]-XXX-C- or  $Z_m$ -C-XXX-[CST]-, thereby creating an internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m
- 10 is 1 or 2 and Z 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, preferably K or H. In certain examples,  $X^1$ ,  $X^2$ , and  $X^3$ , 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,  $X^1$ ,  $X^2$ , and  $X^3$  in said motif is any amino acid
- 15 except for C, S, or T. In a specific embodiment, at least one of  $X^1$ ,  $X^2$ , or  $X^3$  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. Specific examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are: XPY, PXY, and PYX, wherein X can be any amino acid, preferably a basic amino acid such as K, R, or H, or a non-
- 20 natural basic amino acid such as L-ornithine. Non-limiting examples include KPY, RPY, HPY, GPY, APY, VPY, LPY, IPY, MPY, FPY, WPY, PPY, SPY, TPY, CPY, YPY, NPY, QPY, DPY, EPY, KPY, PKY, PRY, PHY, PGY, PAY, PVY, PLY, PIY, PMY, PFY, PWY, PPY, PSY, PTY, PCY, PYY, PNY, PQY, PDY, PEY, PLY, PYK, PYR, PYH, PYG, PYA, PYV, PYL, PYI, PYM, PYF, PYW, PYP, PYS, PYT, PYC, PYY, PYN, PYQ, PYD, or
- 25 PYE. Alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are XHG, HXG, and HGX, wherein X can be any amino acid, such as in KHG, RHG, HHG, GHG, AHG, VHG, LHG, IHG, MHG, FHG, WHG, PHG, SHG, THG, CHG, YHG, NHG, QHG, DHG, EHG, and KHG, HKG, HRG, HHG, HGG, HAG, HVG, HLG, HIG, HMG, HFG, HWG, HPG, HSG, HTG, HCG, HYG, HNG, HQG, HDG, HEG, HLG, HGK, HGR, HGH, HGG, HGA, HGV, HGL, HGI, HGM, HGF, HGW, HGP, HGS, HGT, HGC, HGY, HGN, HGQ, HGD, or HGE. Yet alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are XGP, GXP, and GPX, wherein X can be any amino acid, such as in KGP, RGP, HGP, GGP, AGP, VGP, LGP, IGP, MGP, FGP, WGP, PGP, SGP, TGP, CGP, YGP, NGP, QGP, DGP,
- 30

EGP, KGP, GKP, GRP, GHP, GGP, GAP, GVP, GLP, GIP, GMP, GFP, GWP, GPP, GSP, GTP, GCP, GYP, GNP, GQP, GDP, GEP, GLP, GPK, GPR, GPH, GPG, GPA, GPV, GPL, GPI, GPM, GPF, GPW, GPP, GPS, GPT, GPC, GPY, GPN, GPQ, GPD, or GPE. Yet alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the

5 oxidoreductase motif are XGH, GXH, and GHX, wherein X can be any amino acid, such as in KGH, RGH, HGH, GGH, AGH, VGH, LGH, IGH, MGH, FGH, WGH, PGH, SGH, TGH, CGH, YGH, NGH, QGH, DGH, EGH, KGH, GKH, GRH, GHH, GGH, GAH, GVH, GLH, GIH, GMH, GFH, GWH, GPH, GSH, GTH, GCH, GYH, GNH, GQH, GDH, GEH, GLH, GHK, GHR, GHH, GHG, GHA, GHV, GHL, GHI, GHM, GHF, GHW, GHP,

10 GHS, GHT, GHC, GHY, GHN, GHQ, GHD, or GHE. Yet alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are XGF, GXF, and GFX, wherein X can be any amino acid, such as in KGF, RGF, HGF, GGF, AGF, VGF, LGF, IGF, MGF, FGF, WGF, PGF, SGF, TGF, CGF, YGF, NGF, QGF, DGF, EGF, and KGF, GKF, GRF, GHF, GGF, GAF, GVF, GLF, GIF, GMF, GFF, GWF, GPF, GSF,

15 GTF, GCF, GYF, GNF, GQF, GDF, GEF, GLF, GFK, GFR, GFH, GFG, GFA, GFV, GFL, GFI, GFM, GFF, GFW, GFP, GFS, GFT, GFC, GFY, GFN, GFQ, GFD, or GFE. Yet alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are XRL, RXL, and RLX, wherein X can be any amino acid, such as in KRL, RRL, HRL, GRL, ARL, VRL, LRL, IRL, MRL, FRL, WRL, PRL, SRL, TRL,

20 CRL, YRL, NRL, QRLRL, DRL, ERL, KRL, GKF, GRF, GHF, GGF, GAF, GVF, GLF, GIF, GMF, GFF, GWF, GPF, GSF, GTF, GCF, GYF, GNF, GQF, GDF, GEF, and GLF, RLK, RLR, RLH, RLG, RLA, RLV, RLL, RLI, RLM, RLF, RLW, RLP, RLS, RLT, RLC, RLY, RLN, RLQ, RLD, or RLE. Yet alternative examples of the internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif are XHP, HXP, and HPX, wherein X can be any amino acid, such as in KHP, RHP, HHP, GHP, AHP, VHP, LHP, IHP, MHP, FHP, WHP, PHP, SHP, THP, CHP, YHP, NHP, QHP, DHP, EHP, KHP, HKP, HRP, HHP, HGP, HAF, HVF, HLF, HIF, HMF, HFF, HWF, HPF, HSF, HTF, HCF, HYP, HNF, HQF, HDF, HEF, HLP, HPK, HPR, HPH, HPG, HPA, HPV, HPL, HPI, HPM, HPF, HPW, HPP, HPS, HPT, HPC, HPY, HPN, HPQ, HPD, or HPE.

25 Particularly preferred examples are: CRPYC, KCRPYC, KHCRPYC, RCRPYC, HCRPYC, CPRYC, KCPRYC, RCPRYC, HCPRYC, CPYRC, KCPYRC, RCPYRC, HCPYRC, CKPYC, KCKPYC, RCKPYC, HCKPYC, CPKYC, KCPKYC, RCPKYC, HCPKYC, CPYKC, KCPYKC, RCPYKC, and HCPYKC (corresponding to SEQ ID NOs: 215 to 239).

Further envisaged are motifs of the form  $Z_m$ -[CST]-XXXX-C- or  $Z_m$ -C-XXXX-[CST]-, thereby creating an internal  $X^1X^2X^3X^4$  (SEQ ID NO: 154) amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m is 1 or 2 and Z 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, preferably K or H.  $X^1$ ,  $X^2$ ,  $X^3$  and  $X^4$  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 as defined herein. Preferably,  $X^1$ ,  $X^2$ ,  $X^3$  and  $X^4$  in said motif is any amino acid except for C, S, or T. In certain non-limiting examples, at least one of  $X^1$ ,  $X^2$ ,  $X^3$  or  $X^4$  in said motif is a basic amino acid selected from: H, K, or R, or a non-natural basic amino acid as defined herein. Specific examples include LAVL (SEQ ID NO: 56), TVQA (SEQ ID NO: 57) or GAVH (SEQ ID NO: 58) and their variants such as:  $X^1$ AVL,  $LX^2$ VL,  $LAX^3$ L, or  $LAVX^4$ ;  $X^1$ VQA,  $TX^2$ QA,  $TVX^3$ A, or  $TVQX^4$ ;  $X^1$ AVH,  $GX^2$ VH,  $GAX^3$ H, or  $GAVX^4$  (corresponding to SEQ ID NO: 155 to 165); wherein  $X^1$ ,  $X^2$ ,  $X^3$  and  $X^4$  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 basic amino acids as defined herein.

Further envisaged are motifs of the form  $Z_m$ -[CST]-XXXXX-C- or  $Z_m$ -C-XXXXX-[CST]-, thereby creating an internal  $X^1X^2X^3X^4X^5$  (SEQ ID NO: 166) amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m is 1 or 2 and Z 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, preferably K or H.  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$  and  $X^5$  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,  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$  and  $X^5$  in said motif is any amino acid except for C, S, or T. In certain examples, at least one of  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$  or  $X^5$  in said motif is a basic amino acid selected from: H, K, or R, or a non-natural basic amino acid as defined herein. Specific examples include PAFPL (SEQ ID NO: 59) or DQGGE (SEQ ID NO: 60) and their variants such as:  $X^1$ AFPL,  $PX^2$ FPL,  $PAX^3$ PL,  $PAFX^4$ L, or  $PAFPX^5$ ;  $X^1$ QGGE,  $DX^2$ GGE,  $DQX^3$ GE,  $DQGX^4$ E, or  $DQGGX^5$  (corresponding to SEQ

ID NO: 167 to 176), wherein  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$ , and  $X^5$  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 as defined herein.

Further envisaged are motifs of the form  $Z_m$ -[CST]-XXXXXX-C- or  $Z_m$ -C-XXXXXX-[CST]- as defined in aspect 1, wherein n is 6, thereby creating an internal  $X^1X^2X^3X^4X^5X^6$  (SEQ ID NO: 177) amino acid stretch within the oxidoreductase motif, wherein m is an integer selected from 0 to 3, 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. Preferred are motifs wherein m is 1 or 2 and Z 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, preferably K or H.  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$ ,  $X^5$  and  $X^6$  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 acid. Preferably,  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$ ,  $X^5$  and  $X^6$  in said motif is any amino acid except for C, S, or T. In certain examples, at least one of  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$ ,  $X^5$  or  $X^6$  in said motif is a basic amino acid selected from: H, K, or R, or a non-natural basic amino acid as defined herein. Specific examples include DIADKY (SEQ ID NO: 61) or variants thereof such as:  $X^1$ IADKY,  $DX^2$ ADKY,  $DIX^3$ DKY,  $DIAX^4$ KY,  $DIADX^5$ Y, or  $DIADKX^6$  (corresponding to SEQ ID NO: 178 to 183), wherein  $X^1$ ,  $X^2$ ,  $X^3$ ,  $X^4$ ,  $X^5$  and  $X^6$  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 basic amino acids as defined herein.

Further envisaged are motifs of the form  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]-, wherein n is 0 to 6 and wherein m is 0 (i.e. [CST]- $X_n$ -C- or C- $X_n$ -[CST]-), 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 embodiments of such a motif, n is 2 and m is 0, 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  $X^1$  or  $X^2$  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  $X^1$  or  $X^2$  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).

The redox motif in the above immunogenic peptides is placed either immediately adjacent to the T cell epitope sequence within the immunogenic peptide, or is separated from the T cell epitope by a **linker**. More particularly, the linker comprises an amino acid sequence of 7 amino acids or less. Most particularly, the linker comprises 1, 2, 3, or 4, 5, 6, or 7 amino acids. Said linker can be encompassing amino acids that are flanking the epitope in the natural MOG amino acid sequence or can be different from these amino acids.

In addition, the immunogenic peptides can have a **flanking sequence** ("flanker") following the epitope sequence. Said flanker can be encompassing amino acids that are flanking the epitope in the natural MOG amino acid sequence such as TLF or can be different from these amino acids. Preferred flankers in the present invention are TLF, TLFK (SEQ ID NO: 264) and TLFKK (SEQ ID NO: 263).

The sequence of the linker and/or flanking sequence can have an influence on the immunogenicity of the immunogenic peptide as a whole.

The term Myelin Oligodendrocyte Glycoprotein refers to the human protein encoded by the MOG gene. The terms MOG (protein) or Myelin Oligodendrocyte Glycoprotein as used herein are defined by the amino acid sequence corresponding to NCBI Gene 4340, and UniProtKB identifier Q16653 (MOG\_HUMAN) (SEQ ID NO: 62):

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MASLSRPSLPSCLCSFLLLLLLQVSSSYAGQFRVIGPRHPIRALVGDEVELPCRISPGKNAT
GMEVGWYRPPFSRVVHLYRNGKDQDQDAPEYRGRTELLKDAIGEGKVTLRIRNVRFSDGG
FTCFFRDHSYQEEAAMELKVEDPFYWVSPGVLVLLAVLPVLLLQITVGLIFLCLQYRLRGKL
RAEIENLHRTFDPHFLRVPCWKITLFVIVPVLGPLVALIICYNWLHRRLAGQFLEELRNPF
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Myelin Oligodendrocyte Glycoprotein is a membrane protein expressed on the oligodendrocyte cell surface and the outermost surface of myelin sheaths and is a primary target antigen involved in immune-mediated demyelination. The protein may be involved in completion and maintenance of the myelin sheath and in cell-cell communication. Alternatively spliced transcript variants encoding different isoforms have been identified. The MOG epitopes envisaged for incorporation in the immunogenic peptides of the invention may thus be epitopes that are present in the canonical MOG amino acid sequence (SEQ ID NO: 62), and/or one or more MOG

protein isoforms. A suitable MOG epitope in the context of the invention is a MOG epitope comprising, or consisting of, FLRVPCWKI (SEQ ID NO: 1). The SEQ ID NO: 1 portion of the human and mouse MOG protein is characterized by 100% sequence identity. Alternatively worded, SEQ ID NO: 1 can be retrieved in both the human and mouse MOG protein. Alternatively a point mutation may be introduced in the MOG epitope SEQ ID NO: 1 to form the amino acid sequence FLRVPSWKI (SEQ ID NO: 2), which is a preferred MOG epitope in the context of the invention. The FLRVPCWKI (SEQ ID NO: 1) and FLRVPSWKI (SEQ ID NO: 2) T cell epitopes are MHC class II epitopes having a length of 9 amino acids that also respectively comprise the 7 amino acids long NKT cell epitopes FLRVPCW (SEQ ID NO: 63) and FLRVPSW (SEQ ID NO: 64).

**Amino acids** are referred to herein with their full name, their three-letter abbreviation or their one letter abbreviation.

Motifs of amino acid sequences are written herein according to the format of Prosite. Motifs are used to describe a certain sequence variety at specific parts of a sequence. The symbol X is used for a position where any amino acid is accepted. Alternatives are indicated by listing the acceptable amino acids for a given position, between square brackets ('[]'). For example: [CST] stands for an amino acid selected from Cys, Ser or Thr. Amino acids which are excluded as alternatives are indicated by listing them between curly brackets ('{ }'). For example: {AM} stands for any amino acid except Ala and Met. The different elements in a motif are optionally separated from each other by a hyphen (-). In the context of the motifs disclosed in this specification, the disclosed general oxidoreductase motifs are typically accompanied by a hyphen not forming a connection with a different element outside the motif. These 'open' hyphens indicate the position of the physical connection of the motif with another portion of the immunogenic peptide such as a linker sequence or an epitope sequence. For example, a motif of the form "Z<sub>m</sub>-C-X<sub>n</sub>-[CST]-" indicates that the [CST] is the amino acid connected to the other portion of the immunogenic peptide, and Z is a terminal amino acid of the immunogenic peptide. Preferred physical connections are peptide bonds. Repetition of an identical element within a motif can be indicated by placing behind that element a numerical value or a numerical range between parentheses. For example In this respect, "X<sub>n</sub>" refers to n-times "X". X(2) corresponds to X-X or XX; X(2, 5) corresponds to 2, 3, 4 or 5 X amino acids, A(3) corresponds to A-A-A or AAA. To distinguish between the amino acids, those outside the oxidoreductase motif can be called

external amino acids, those within the redox motif are called internal amino acids. Unless stated otherwise X represents any amino acid, particularly an L-amino acid, more particularly one of the 20 naturally occurring L-amino acids.

Any one of the peptides disclosed herein, comprising a T cell epitope of MOG and a  
5 modified peptide motif sequence, having reducing activity is capable of generating a population of antigen-specific cytolytic CD4+ T cells or NKT cells towards antigen-presenting cells.

Accordingly, in its broadest sense, the invention relates to peptides which comprise at least one T-cell epitope of MOG with a potential to trigger an immune reaction, and a  
10 modified oxidoreductase sequence motif with a reducing activity on peptide disulfide bonds. The T cell epitope and the modified oxidoreductase motif sequence may be immediately adjacent to each other in the peptide or optionally separated by one or more amino acids (a so called linker sequence).

Optionally the peptide additionally comprises an endosome targeting sequence and/or  
15 additional "flanking" sequences.

The peptides of the invention comprise an MHC class II T-cell epitope or NKT cell epitope of MOG with a potential to trigger an immune reaction, and a modified oxidoreductase motif. The reducing activity of the motif sequence in the peptide can be assayed for its ability to reduce a sulfhydryl group such as in the insulin solubility assay  
20 wherein the solubility of insulin is altered upon reduction, or with a fluorescence-labelled substrate such as insulin. An example of such assay uses a fluorescent peptide and is described in Tomazzoli et al. (2006) Anal. Biochem. 350, 105–112. Two peptides with a FITC label become self-quenching when they covalently attached to each other via a disulfide bridge. Upon reduction by a peptide in accordance with the  
25 present invention, the reduced individual peptides become fluorescent again.

The modified oxidoreductase motif may be positioned at the amino-terminus side of the T-cell epitope or at the carboxy-terminus of the T-cell epitope.

As explained in detail further on, the peptides of the present invention can be made by chemical synthesis, which allows the incorporation of non-natural amino acids.

Accordingly, "C" in the above recited oxidoreductase motifs represents either cysteine  
30 or another amino acid with a thiol group such as mercaptovaline, homocysteine or other natural or non-natural amino acids with a thiol function. In order to have reducing activity, the cysteines present in a modified oxidoreductase motif should not occur as part of a cystine disulfide bridge. X can be any of the 20 natural amino acids, including

S, C, or T or can be a non-natural amino acid. In particular embodiments X is an amino acid with a small side chain such as Gly, Ala, Ser or Thr. In further particular embodiments, X is not an amino acid with a bulky side chain such as Trp. In further particular embodiments X is not Cysteine. In further particular embodiments at least one X in the modified oxidoreductase motif is His. In other further particular  
5   embodiments at least one X in the modified oxidoreductase is Pro.

Peptides may further comprise modifications to increase stability or solubility, such as modification of the N-terminal NH<sub>2</sub> group or the C terminal COOH group (e.g. modification of the COOH into a CONH<sub>2</sub> group).

10   In the peptides of the present invention comprising a modified oxidoreductase motif, the motif is located such that, when the epitope fits into the MHC or CD1d groove, the motif remains outside of the MHC or CD1d binding groove. The modified oxidoreductase motif is placed either immediately adjacent to the epitope sequence within the peptide [in other words a linker sequence of zero amino acids between motif  
15   and epitope], or is separated from the T cell epitope by a linker comprising an amino acid sequence of 7 amino acids or less. More particularly, the linker comprises 1, 2, 3, 4, 5, 6, or 7 amino acids. Specific embodiments are peptides with a 0, 1, 2 or 3 amino acid linker between epitope sequence and modified oxidoreductase motif sequence. In those peptides where the modified oxidoreductase motif sequence is adjacent to the  
20   epitope sequence this is indicated as position P-4 to P-1 or P+1 to P+4 compared to the epitope sequence. Apart from a peptide linker, other organic compounds can be used as linker to link the parts of the peptide to each other (e.g. the modified oxidoreductase motif sequence to the T cell epitope sequence).

The peptides of the present invention can further comprise additional short amino acid  
25   sequences N or C-terminally of the sequence comprising the T cell epitope and the modified oxidoreductase motif. Such an amino acid sequence is generally referred to herein as a “**flanking sequence**”. A flanking sequence can be positioned between the epitope and an endosomal targeting sequence and/or between the modified oxidoreductase motif and an endosomal targeting sequence. In certain peptides, not  
30   comprising an endosomal targeting sequence, a short amino acid sequence may be present N and/or C terminally of the modified oxidoreductase motif and/or epitope sequence in the peptide. More particularly a flanking sequence is a sequence of between 1 and 7 amino acids, most particularly a sequence of 1, 2, or 3 amino acids.

Preferably Z in the oxidoreductase motif corresponds to the N- or C-terminal end of the immunogenic peptide.

The modified oxidoreductase motif may be located N-terminal from the epitope.

In certain embodiments of the present invention, peptides are provided comprising one  
5 epitope sequence and a modified oxidoreductase motif sequence. In further particular  
embodiments, the modified oxidoreductase motif occurs several times (1, 2, 3, 4 or  
even more times) in the peptide, for example as repeats of the modified oxidoreductase  
motif which can be spaced from each other by one or more amino acids or as repeats  
which are immediately adjacent to each other. Alternatively, one or more modified  
10 oxidoreductase motifs are provided at both the N and the C terminus of the T cell  
epitope sequence.

Other variations envisaged for the peptides of the present invention include peptides  
which contain repeats of a T cell epitope sequence wherein each epitope sequence is  
preceded and/or followed by the modified oxidoreductase motif (e.g. repeats of  
15 "modified oxidoreductase motif-epitope" or repeats of "modified oxidoreductase motif-  
epitope-modified oxidoreductase motif"). Herein the modified oxidoreductase motifs  
can all have the same sequence but this is not obligatory. It is noted that repetitive  
sequences of peptides which comprise an epitope which in itself comprises the  
modified oxidoreductase motif will also result in a sequence comprising both the  
20 'epitope' and a 'modified oxidoreductase motif'. In such peptides, the modified  
oxidoreductase motif within one epitope sequence functions as a modified  
oxidoreductase motif outside a second epitope sequence.

Typically the peptides of the present invention comprise only one T cell epitope. As  
described below a T cell epitope in a protein sequence can be identified by functional  
25 assays and/or one or more in silico prediction assays. The amino acids in a T cell  
epitope sequence are numbered according to their position in the binding groove of  
the MHC proteins. A T-cell epitope present within a peptide consist of between 8 and  
25 amino acids, yet more particularly of between 8 and 16 amino acids, yet most  
particularly consists of 8, 9, 10, 11, 12, 13, 14, 15 or 16 amino acids.

30 In a more particular embodiment, the T cell epitope consists of a sequence of 9 amino  
acids. In a further particular embodiment, the T-cell epitope is an epitope, which is  
presented to T cells by MHC-class II molecules [MHC class II restricted T cell epitopes].  
In an alternative embodiment, the T-cell epitope is an NKT cell epitope, which is  
presented to T cells by CD1d molecules [NKT cell specific epitopes]. Typically T cell

epitope sequence refers to the octapeptide or more specifically nonapeptide sequence which fits into the cleft of an MHC II protein or CD1d protein.

The T cell epitope of the peptides of the present invention can correspond either to a natural epitope sequence of a protein or can be a modified version thereof, provided  
5 the modified T cell epitope retains its ability to bind within the MHC or CD1d cleft, similar to the natural T cell epitope sequence. The modified T cell epitope can have the same binding affinity for the MHC or CD1d protein as the natural epitope, but can also have a lowered affinity. In particular, the binding affinity of the modified peptide is no less than 10-fold less than the original peptide, more particularly no less than 5  
10 times less. Peptides of the present invention have a stabilising effect on protein complexes. Accordingly, the stabilising effect of the peptide-MHC/CD1d complex compensates for the lowered affinity of the modified epitope for the MHC or CD1d molecule.

The sequence comprising the T cell epitope and the reducing compound within the  
15 peptide can be further linked to an amino acid sequence (or another organic compound) that facilitates uptake of the peptide into late endosomes for processing and presentation within MHC class II or CD1d determinants. The late endosome targeting is mediated by signals present in the cytoplasmic tail of proteins and correspond to well-identified peptide motifs. The late endosome targeting is mediated  
20 by signals present in the cytoplasmic tail of proteins and correspond to well-identified peptide motifs such as the dileucine-based [DE]XXXL[LI] (SEQ ID NO: 204) or DXXLL motif (SEQ ID NO: 205) (e.g. DXXXLL, SEQ ID NO 206)), the tyrosine-based YXXO motif or the so-called acidic cluster motif (SEQ ID NO: 207). The symbol O represents amino acid residues with a bulky hydrophobic side chains such as Phe, Tyr and Trp.  
25 The late endosome targeting sequences allow for processing and efficient presentation of the antigen-derived T cell epitope by MHC class II or CD1d molecules. Such endosomal targeting sequences are contained, for example, within the gp75 protein (Vijayasaradhi et al. (1995) J. Cell. Biol. 130, 807-820), the human CD3 gamma protein, the HLA-BM 11 (Copier et al. (1996) J. Immunol. 157, 1017-1027), the  
30 cytoplasmic tail of the DEC205 receptor (Mahnke et al. (2000) J. Cell Biol. 151, 673-683). Other examples of peptides which function as sorting signals to the endosome are disclosed in the review of Bonifacio and Traub (2003) Annu. Rev. Biochem. 72, 395-447. Alternatively, the sequence can be that of a subdominant or minor T cell epitope from a protein, which facilitates uptake in late endosome without overcoming

the T cell response towards the antigen. The late endosome targeting sequence can be located either at the amino-terminal or at the carboxy-terminal end of the antigen derived peptide for efficient uptake and processing and can also be coupled through a flanking sequence, such as a peptide sequence of up to 10 amino acids. When using  
5 a minor T cell epitope for targeting purpose, the latter is typically located at the amino-terminal end of the antigen derived peptide.

Accordingly, the present invention envisages peptides of antigenic proteins and their use in eliciting specific immune reactions. These peptides can either correspond to fragments of proteins which comprise, within their sequence i.e. a reducing compound  
10 and a T cell epitope separated by at most 10, preferably 7 amino acids or less. Alternatively, and for most antigenic proteins, the peptides of the invention are generated by coupling a reducing compound, more particularly a reducing modified oxidoreductase motif as described herein, N-terminally or C-terminally to a T cell epitope of the antigenic protein (either directly adjacent thereto or with a linker of at  
15 most 10, more particularly at most 7 amino acids). Moreover the T cell epitope sequence of the protein and/or the modified oxidoreductase motif can be modified and/or one or more flanking sequences and/or a targeting sequence can be introduced (or modified), compared to the naturally occurring sequence. Thus, depending on whether or not the features of the present invention can be found within the sequence  
20 of the antigenic protein of interest, the peptides of the present invention can comprise a sequence which is 'artificial' or 'naturally occurring'.

The term "**natural**" when referring to a peptide relates to the fact that the sequence is identical to a fragment of a naturally occurring protein (wild type or mutant). In contrast therewith the term "**artificial**" refers to a sequence which as such does not occur in  
25 nature. An artificial sequence is obtained from a natural sequence by limited modifications such as changing/deleting/inserting one or more amino acids within the naturally occurring sequence or by adding/removing amino acids N- or C-terminally of a naturally occurring sequence.

The peptides of the present invention can vary substantially in length. The length of  
30 the peptides can vary from 9 or 11 amino acids, i.e. consisting of an epitope of 7-9 amino acids, adjacent thereto the modified oxidoreductase motif of from 2 to 11 amino acids, up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40 or 50 amino acids. For example, a peptide may comprise an endosomal targeting sequence of 40 amino acids, a flanking sequence of about 2 amino acids, an oxidoreductase motif as

described herein of 2 to 11 amino acids, a linker of 4 to 7 amino acids and a T cell epitope peptide of 7, 8 or 9 amino acids minimal length.

Accordingly, in particular embodiments, the complete peptide consists of between 9 amino acids up to 20, 25, 30, 40, 50, 75 or 100 amino acids. More particularly, where the  
5 reducing compound is a modified oxidoreductase motif as described herein, the length of the (artificial or natural) sequence comprising the epitope and modified oxidoreductase motif optionally connected by a linker (referred to herein as 'epitope-modified oxidoreductase motif' sequence), without the endosomal targeting sequence, is critical. The 'epitope-modified oxidoreductase motif' more particularly has a length of  
10 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 amino acids. Such peptides of 9, 10, 11, 12, 13 or 14 to 19 amino acids can optionally be coupled to an endosomal targeting signal of which the size is less critical.

As detailed above, in particular embodiments, the peptides of the present invention comprise a reducing modified oxidoreductase motif as described herein linked to a T  
15 cell epitope sequence.

In further particular embodiments, the peptides of the invention are peptides comprising T cell epitopes which do not comprise an amino acid sequence with oxidoreductase properties within their natural sequence.

Generally, the peptides of the present invention are not natural (thus no fragments of  
20 proteins as such) but artificial peptides which contain, in addition to a T cell epitope, a modified oxidoreductase motif as described herein, whereby the modified oxidoreductase motif is immediately separated from the T cell epitope by a linker consisting of up to seven, most particularly up to four or up to 2 amino acids.

It has been shown that upon administration (i.e. injection) to a mammal of a peptide  
25 according to the invention (or a composition comprising such a peptide), the peptide elicits the activation of T cells recognising the antigen derived T cell epitope and provides an additional signal to the T cell through reduction of surface receptor. This supra-optimal activation results in T cells acquiring cytolytic properties for the cell presenting the T cell epitope, as well as suppressive properties on bystander T cells.

30 In this way, the peptides or composition comprising the peptides described in the present invention, which contain an antigen-derived T cell epitope and, outside the epitope, a modified oxidoreductase motif can be used for direct immunisation of mammals, including human beings. The invention thus provides peptides of the invention or derivatives thereof, for use as a medicine. Accordingly, the present

invention provides therapeutic methods which comprise administering one or more peptides according to the present invention to a patient in need thereof.

The present invention offers methods by which antigen-specific T cells endowed with cytolytic properties can be elicited by immunisation with small peptides. It has been found that peptides which contain (i) a sequence encoding a T cell epitope from an antigen and (ii) a consensus sequence with redox properties, and further optionally also comprising a sequence to facilitate the uptake of the peptide into late endosomes for efficient MHC-class II or CD1d presentation, elicit suppressor T-cells.

The immunogenic properties of the peptides of the present invention are of particular interest in the treatment and prevention of immune reactions.

Peptides described herein are used as medicament, more particularly used for the manufacture of a medicament for the prevention or treatment of an immune disorder in a mammal, more in particular in a human.

The present invention describes methods of treatment of an immune disorder of a mammal in need for such treatment, by using the peptides of the invention, homologues or derivatives thereof, the methods comprising the step of administering to said mammal suffering or at risk of an immune disorder a therapeutically effective amount of the peptides of the invention, homologues or derivatives thereof such as to reduce the symptoms of the immune disorder. The treatment of both humans and animals, such as, pets and farm animals is envisaged. In an embodiment the mammal to be treated is a human. The immune disorders referred to above are in a particular embodiment selected from allergic diseases and autoimmune diseases. More particularly, such immunogenic peptides are provided for use in treating or alleviating symptoms of MS.

The peptides of the invention or the pharmaceutical composition comprising such as defined herein is preferably administered through sub-cutaneous or intramuscular administration. Preferably, the peptides or pharmaceutical compositions comprising such can be injected sub-cutaneously (SC) in the region of the lateral part of the upper arm, midway between the elbow and the shoulder. When two or more separate injections are needed, they can be administered concomitantly in both arms.

The peptide according to the invention or the pharmaceutical composition comprising such is administered in a therapeutically effective dose. Exemplary but non-limiting dosage regimens are between 50 and 1500 µg, preferably between 100 and 1200 µg. More specific dosage schemes can be between 50 and 250 µg, between 250 and 450

µg or between 850 and 1300 µg, depending on the condition of the patient and severity of disease. Dosage regimen can comprise the administration in a single dose or in 2, 3, 4, 5, or more doses, either simultaneously or consecutively. Exemplary non-limiting administration schemes are the following:

- 5 - A low dose scheme comprising the SC administration of 50 µg of peptide in two separate injections of 25 µg each (100 µL each) followed by three consecutive injections of 25 µg of peptide as two separate injections of 12.5 µg each (50 µL each).
- A medium dose scheme comprising the SC administration of 150 µg of peptide in two separate injections of 75 µg each (300 µL each) followed by three consecutive
- 10 administrations of 75 µg of peptide as two separate injections of 37.5 µg each (150 µL each).
- A high dose scheme comprising the SC administration of 450 µg of peptide in two separate injections of 225 µg each (900 µL each) followed by three consecutive
- 15 administrations of 225 µg of peptide as two separate injections of 112.5 µg each (450 µL each).

Other exemplary non-limiting administration schemes are the following:

- A dose scheme comprising 6 SC administration 2 weeks apart of 450 µg of peptide in two separate injections of 225 µg each.
- 20 - A dose scheme comprising 6 SC administration 2 weeks apart SC of 1350 µg of peptide in two separate injections of 675 µg each.

A particularly but non-limiting dosage regimen of the immunogenic peptide as defined herein is between 50 and 1500 µg, preferably between 450 and 1500 µg. Dosage regimen can comprise the administration in a single dose or in 2, 3, 4, 5, 6 or more

25 doses, either simultaneously or consecutively. Said treatment with the immunogenic peptide can be done 1 to 6 times, such as 1 to 4 times, preferably every 5 to 9 days, such as about every 7 days.

For all the above peptides additional variant are envisaged, wherein between the Histidine flanking residue and the first Cysteine of the oxidoreductase motif, one or two

30 amino acids X are present. Typically these external amino acid(s) X is (are) not His, Cys, Ser or Thr.

The peptides of the present invention can also be used in diagnostic in vitro methods for detecting class II restricted CD4 + T cells or NKT cells in a sample. In this method a sample is contacted with a complex of an MHC class II or CD1d molecule and a peptide according to the present invention. The CD4+ T cells or NKT cells detected by  
5 measuring the binding of the complex with cells in the sample, wherein the binding of the complex to a cell is indicative for the presence of CD4 + T cells or NKT cells in the sample.

The complex can be a fusion protein of the peptide and an MHC class II or CD1d molecule.

10 Alternatively MHC or CD1d molecules in the complex are tetramers. The complex can be provided as a soluble molecule or can be attached to a carrier.

Accordingly, in particular embodiments, the methods of treatment and prevention of the present invention comprise the administration of an immunogenic peptide as described herein, wherein the peptide comprise a T cell epitope of an antigenic protein  
15 which plays a role in the disease to be treated (for instance such as those described above). In further particular embodiments, the epitope used is a dominant epitope.

Peptides in accordance of the present invention will be prepared by synthesising a peptide wherein T cell epitope and modified oxidoreductase motif will be separated by 0 to 7 amino acids. In certain embodiments the modified oxidoreductase motif can be  
20 obtained by introducing 1, 2 or 3 mutations outside the epitope sequence, to preserve the sequence context as occurring in the protein. Typically amino-acids in P-2 and P-1, as well as in P+10 and P+11, with reference to the nonapeptide which are part of the natural sequence are preserved in the peptide sequence. These flanking residues generally stabilize the binding to MHC class II or CD1d. In other embodiments the  
25 sequence N terminal or C terminal of the epitope will be unrelated to the sequence of the antigenic protein containing the T cell epitope sequence.

Thus based upon the above methods for designing a peptide, a peptide is generated by chemical peptide synthesis, recombinant expression methods or in more exceptional cases, proteolytic or chemical fragmentation of proteins.

30 Peptides as produced in the above methods can be tested for the presence of a T cell epitope in in vitro and in vivo methods, and can be tested for their reducing activity in in vitro assays. As a final quality control, the peptides can be tested in in vitro assays to verify whether the peptides can generate CD4+ T cells or NKT cells which are cytolytic via an apoptotic pathway for antigen presenting cells presenting the antigen

which contains the epitope sequence which is also present in the peptide with the modified oxidoreductase motif.

The peptides of the present invention can be generated using recombinant DNA techniques, in bacteria, yeast, insect cells, plant cells or mammalian cells. In view of the limited length of the peptides, they can be prepared by chemical peptide synthesis, wherein peptides are prepared by coupling the different amino acids to each other. Chemical synthesis is particularly suitable for the inclusion of e.g. D-amino acids, amino acids with non-naturally occurring side chains, etc.

Chemical peptide synthesis methods are well described and peptides can be ordered from companies such as Applied Biosystems and other companies.

Peptide synthesis can be performed as either solid phase peptide synthesis (SPPS) or contrary to solution phase peptide synthesis. The best known SPPS methods are t-Boc and Fmoc solid phase chemistry:

During peptide synthesis several protecting groups are used. For example hydroxyl and carboxyl functionalities are protected by t-butyl group, lysine and tryptophan are protected by t-Boc group, and asparagine, glutamine, cysteine and histidine are protected by trityl group, and arginine is protected by the pbf group. If appropriate, such protecting groups can be left on the peptide after synthesis. Peptides can be linked to each other to form longer peptides using a ligation strategy (chemoselective coupling of two unprotected peptide fragments) as originally described by Kent (Schnelzer & Kent (1992) *Int. J. Pept. Protein Res.* 40, 180-193) and reviewed for example in Tam et al. (2001) *Biopolymers* 60, 194-205 provides the tremendous potential to achieve protein synthesis which is beyond the scope of SPPS. Many proteins with the size of 100-300 residues have been synthesised successfully by this method. Synthetic peptides have continued to play an ever increasing crucial role in the research fields of biochemistry, pharmacology, neurobiology, enzymology and molecular biology because of the enormous advances in the SPPS.

Alternatively, the peptides can be synthesised by using nucleic acid molecules which encode the peptides of this invention in an appropriate expression vector which include the encoding nucleotide sequences. Such DNA molecules may be readily prepared using an automated DNA synthesiser and the well-known codon-amino acid relationship of the genetic code. Such a DNA molecule also may be obtained as genomic DNA or as cDNA using oligonucleotide probes and conventional hybridisation methodologies. Such DNA molecules may be incorporated into expression vectors,

including plasmids, which are adapted for the expression of the DNA and production of the polypeptide in a suitable host such as bacterium, e.g. *Escherichia coli*, yeast cell, animal cell or plant cell.

The physical and chemical properties of a peptide of interest (e.g. solubility, stability) are examined to determine whether the peptide is/would be suitable for use in therapeutic compositions. Typically this is optimised by adjusting the sequence of the peptide. Optionally, the peptide can be modified after synthesis (chemical modifications e.g. adding/deleting functional groups) using techniques known in the art.

10 T cell epitopes on their own are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, the recruitment of additional immune cells to the site, and activation of the B cell cascade leading to  
15 production of antibodies. One isotype of these antibodies, IgE, is fundamentally important in the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor where the epitope comprises amino acid residues  
20 essential to receptor recognition, which are contiguous in the amino acid sequence of the protein.

However, upon administration of the peptides with a T-cell epitope and an oxidoreductase motif, the following events are believed to happen:

activation of antigen (i) specific T cells resulting from cognate interaction with the  
25 antigen-derived peptide presented by MHC-class II molecules;  
the reductase sequence reduces T cell surface proteins, such as the CD4 molecule, the second domain of which contains a constrained disulfide bridge. This transduces a signal into T cells. Among a series of consequences related to increased oxidative pathway, important events are increased calcium influx and translocation of the NF- $\kappa$ B  
30 transcription factor to the nucleus. The latter results in increased transcription of IFN- $\gamma$  and granzymes, which allows cells to acquire cytolytic properties via an apoptosis-inducing mechanism; the cytolytic property affects cells presenting the peptide by a mechanism, which involves granzyme B secretion, and Fas-FasL interactions. Since the cell killing effect is obtained via an apoptotic pathway, cytolytic

cells is a more appropriate term for these cells than cytotoxic cells. Destruction of the antigen-presenting target cells prevents activation of other T cells specific for epitopes located on the same antigen, or to an unrelated antigen that would be processed by the same antigen-presenting cell; an additional consequence of T cell activation is to suppress activation of bystander T cells by a cell-cell contact dependent mechanism. In such a case, T cells activated by an antigen presented by a different antigen-presenting cell is also suppressed provided both cytolytic and bystander T cells are in close proximity, namely activated on the surface of the same antigen-presenting cell. The above-postulated mechanism of action is substantiated with experimental data disclosed in the above cited PCT application WO2008/017517 and publications of the present inventors.

Similarly, NKT cell epitopes will reduce the immune response according to the following mechanism, as postulated and shown in WO2012/069568 and publications of the present inventors. When NKT cells are activated by a peptide modified as to contain a thioreductase activity, the latter increases significantly the properties of NKT cells and thereby increases the killing of cells carrying autoantigens by antigen-specific CD4+ NKT cells, which suppresses the immune response against said autoantigens. The participation of NKT cells in the control of immune responses in auto-immune diseases, or against allofactors or allergens has been reported on a number of occasions (Jahng et al Journal of experimental Medicine 199: 947-957, 2004; Van Belle and von Herrath, Molecular Immunology 47: 8-11, 2009) but relatively difficult to describe. In WO2012/069568, it was shown that peptides can be presented by the CD1d molecule. A characteristic of the CD1d molecule is to be made of 2 anti-parallel alpha chains forming a cleft sitting atop of a platform made of two anti-parallel beta chains. The cleft is narrow and deep and accepts only hydrophobic residues, classically deemed to be only lipids. Peptides with hydrophobic residues have the capacity to bind to the CD1d cleft. Besides, as the cleft is open both sides, peptides longer than 7 amino acids can be accommodated. Hydrophobic peptides carrying the CD1d motif are often found in autoantigens, allofactors and allergens, thereby endowing said autoantigen, allofactor or allergen with the capacity to activate CD4+ NKT cells. Direct elimination by killing of cells presenting said autoantigen, allofactor or allergen eliminates the capacity to mount an immune response against these antigens/factors.

The present invention relates to the production of peptides containing hydrophobic residues derived from MOG that confer the capacity to bind to the CD1d molecule. Upon administration, such peptides are taken up by APC, directed to the late endosome where they are loaded onto CD1d and presented at the surface of the APC.

5 Said hydrophobic MOG peptides being characterized by a motif corresponding to the general sequence [FWHY]-XX-[ILMV]-XX-[FWTHY] (SEQ ID NO: 208) or [FW]-XX-[ILMV]-XX-[FW] (SEQ ID NO: 209), in which positions P1 and P7 are occupied by hydrophobic residues such as phenylalanine (F) or tryptophan (W). P7 is however permissive in the sense that it accepts alternative hydrophobic residues to

10 phenylalanine or tryptophan, such as threonine (T) or histidine (H). The P4 position is occupied by an aliphatic residue such as isoleucine (I), leucine (L) or methionine (M). The present invention provides methods for generating antigen-specific cytolytic CD4+ T cells either in vivo or in vitro and, independently thereof, methods to discriminate cytolytic CD4+ T cells from other cell populations such as Foxp3+ Tregs based on

15 characteristic expression data.

The present invention describes in vivo methods for the production of the antigen-specific CD4+ T cells. A particular embodiment relates to the method for producing or isolating the CD4+ T cells by immunising animals (including humans) with the peptides of the invention as described herein and then isolating the CD4+ T cells from the

20 immunised animals. The present invention describes in vitro methods for the production of antigen specific cytolytic CD4+ T cells towards APC. The present invention provides methods for generating antigen specific cytolytic CD4 + T cells towards APC.

In one embodiment, methods are provided which comprise the isolation of peripheral blood cells, the stimulation of the cell population in vitro by an immunogenic peptide according to the invention and the expansion of the stimulated cell population, more particularly in the presence of IL-2. The methods according to the invention have the advantage a high number of CD4+ T cells is produced and that the CD4+ T cells can be generated which are specific for the antigenic protein (by using a peptide comprising

25 an antigen-specific epitope).

30

In an alternative embodiment, the CD4+ T cells can be generated in vivo, i.e. by the injection of the immunogenic peptides described herein to a subject, and collection of the cytolytic CD4+ T cells generated in vivo.

The antigen-specific cytolytic CD4 + T cells towards APC, obtainable by the methods of the present invention are of particular interest for the administration to mammals for immunotherapy, in the prevention of allergic reactions and the treatment of autoimmune diseases. Both the use of allogenic and autogeneic cells are envisaged.

5 Cytolytic CD4+ T cells populations are obtained as described herein below.

Antigen-specific cytolytic CD4+ T cells as described herein can be used as a medicament, more particularly for use in adoptive cell therapy, more particularly in the treatment of acute allergic reactions and relapses of autoimmune diseases such as multiple sclerosis. Isolated cytolytic CD4+ T cells or cell populations, more particularly  
10 antigen-specific cytolytic CD4+ T cell populations generated as described are used for the manufacture of a medicament for the prevention or treatment of immune disorders. Methods of treatment by using the isolated or generated cytolytic CD4+ T cells are disclosed.

As explained in WO2008/017517 cytolytic CD4+ T cells towards APC can be  
15 distinguished from natural Treg cells based on expression characteristics of the cells. More particularly, a cytolytic CD4 + T cell population demonstrates one or more of the following characteristics compared to a natural Treg cell population:

an increased expression of surface markers including CD103, CTLA-4, FasL and ICOS upon activation,

20 intermediate expression of CD25,

expression of CD4, ICOS, CTLA-4, GITR and low or no expression of CD127 (IL7-R), no expression of CD27.

expression of transcription factor T-bet and egr-2 (Krox-20) but not of the transcription repressor Foxp3,

25 a high production of IFN-gamma and no or only trace amounts of IL-10, IL-4, IL-5, IL-13 or TGF-beta.

Further the cytolytic T cells express CD45RO and/or CD45RA, do not express CCR7, CD27 and present high levels of granzyme B and other granzymes as well as Fas ligand.

30 The peptides of the invention will, upon administration to a living animal, typically a human being, elicit specific T cells exerting a suppressive activity on bystander T cells. In specific embodiments the cytolytic cell populations of the present invention are characterised by the expression of FasL and/or Interferon gamma. In specific

embodiments the cytolytic cell populations of the present invention are further characterised by the expression of GranzymeB.

This mechanism also implies and the experimental results show that the peptides of the invention, although comprising a specific T-cell epitope of a certain antigen, can be  
5 used for the prevention or treatment of disorders elicited by an immune reaction against other T-cell epitopes of the same antigen or in certain circumstances even for the treatment of disorders elicited by an immune reaction against other T-cell epitopes of other different antigens if they would be presented through the same mechanism by MHC class II molecules in the vicinity of T cells activated by peptides of the invention.

10 Isolated cell populations of the cell type having the characteristics described above, which, in addition are antigen-specific, i.e. capable of suppressing an antigen-specific immune response are disclosed.

The present invention provides pharmaceutical compositions comprising one or more peptides according to the present invention, further comprising a pharmaceutically  
15 acceptable carrier. As detailed above, the present invention also relates to the compositions for use as a medicine or to methods of treating a mammal of an immune disorder by using the composition and to the use of the compositions for the manufacture of a medicament for the prevention or treatment of immune disorders. The pharmaceutical composition could for example be a vaccine suitable for treating  
20 or preventing immune disorders, especially airborne and foodborne allergy, as well as diseases of allergic origin. As an example described further herein of a pharmaceutical composition, a peptide according to the invention is adsorbed on an adjuvant suitable for administration to mammals, such as aluminium hydroxide (alum). Typically, 50 µg of the peptide adsorbed on alum are injected by the subcutaneous route on 3  
25 occasions at an interval of 2 weeks. It should be obvious for those skilled in the art that other routes of administration are possible, including oral, intranasal or intramuscular. Also, the number of injections and the amount injected can vary depending on the conditions to be treated. Further, other adjuvants than alum can be used, provided they facilitate peptide presentation in MHC-class II or CD1d presentation and T cell  
30 activation. Thus, while it is possible for the active ingredients to be administered alone, they typically are presented as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the present invention comprise at least one active ingredient, as above described, together with one or more pharmaceutically acceptable carriers. The present invention relates to pharmaceutical compositions, comprising, as

an active ingredient, one or more peptides according to the invention, in admixture with a pharmaceutically acceptable carrier. The pharmaceutical composition of the present invention should comprise a therapeutically effective amount of the active ingredient, such as indicated hereinafter in respect to the method of treatment or prevention.

5 Optionally, the composition further comprises other therapeutic ingredients. Suitable other therapeutic ingredients, as well as their usual dosage depending on the class to which they belong, are well known to those skilled in the art and can be selected from other known drugs used to treat immune disorders.

The immunogenic peptide as defined herein may be adsorbed on an adjuvant suitable  
10 for administration to mammals, such as aluminium hydroxide (alum). Typically, 50 µg of the peptide adsorbed on alum are injected by the subcutaneous route on 3 occasions at an interval of 2 weeks. It should be obvious for those skilled in the art that other routes of administration are possible, including, but not limited to, oral, intranasal or intramuscular. Also, the number of injections and the amount injected can vary  
15 depending on the severity of the condition to be treated, and other parameters, such as the age, body weight, general health, sex and diet of the patient. Further, other adjuvants than alum can be used, provided they facilitate peptide presentation in MHC-class II or CD1d and T or NKT cell activation. Thus, while it is possible for the immunogenic peptides to be administered without any adjuvant, they typically are  
20 presented as pharmaceutical formulations. The formulations, both for veterinary and for human use, comprise at least one immunogenic peptide, as above described, together with one or more pharmaceutically acceptable carriers.

The term "**pharmaceutically acceptable carrier**" as used herein with respect to the immunogenic peptide as defined herein means any material or substance with which  
25 the immunogenic peptide is formulated in order to facilitate its application or dissemination to the locus to be treated, for instance by dissolving, dispersing or diffusing the composition, and/or to facilitate its storage, transport or handling without impairing its effectiveness. They include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like.  
30 Additional ingredients may be included in order to control the duration of action of the immunogenic peptide in the pharmaceutical formulation. The pharmaceutically acceptable carrier may be a solid or a liquid or a gas which has been compressed to form a liquid, i.e. the formulations can suitably be used as concentrates, emulsions,

solutions, granulates, dusts, sprays, aerosols, suspensions, ointments, creams, tablets, pellets or powders. Suitable pharmaceutical carriers for use in the pharmaceutical formulations of the peptide are well known to those skilled in the art, and there is no particular restriction to their selection within the present invention. They  
5 may also include additives such as wetting agents, dispersing agents, stickers, adhesives, emulsifying agents, solvents, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to  
10 mammals. The pharmaceutical formulations of the immunogenic peptide may be prepared in any known manner, for instance by homogeneously mixing, coating and/or grinding the active ingredients, in a one- step or multi-steps procedure, with the selected carrier material and, where appropriate, the other additives such as surface-active agents. They may also be prepared by micronisation, for instance in view to  
15 obtain them in the form of microspheres usually having a diameter of about 1 to 10  $\mu\text{m}$ , namely for the manufacture of microcapsules for controlled or sustained release of the immunogenic peptide.

Suitable surface-active agents for use in the pharmaceutical formulations of the immunogenic peptide, also known as emulgent or emulsifier, non-ionic, cationic and/or  
20 anionic materials having good emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water- soluble soaps and water-soluble synthetic surface-active agents. Suitable soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids ( $\text{C}_{10}\text{-C}_{22}$ ), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures  
25 obtainable from coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylarylsulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having  
30 from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives typically contain 8 to 22 carbon atoms.

Examples of alkylarylsulphonates are the sodium, calcium or alcanolamine salts of dodecyl benzene sulphononic acid or dibutyl-naphtalenesulphonic acid or a naphtalene-sulphonic acid/formaldehyde condensation product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardio-lipin, dioctanylphosphatidylcholine, dipalmitoylphosphatidylcholine and their mixtures. Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkyl phenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarene sulphonates and dialkylsulphosuccinates, such as polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, the derivatives typically containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediamino-polypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups. Such compounds usually contain from 1 to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol - polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethyleneglycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants. Suitable cationic surfactants include quaternary ammonium salts, particularly halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C<sub>8</sub>C<sub>22</sub> alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl, oleyl and the like) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

The pharmaceutical dosage forms or pharmaceutical formulations of the immunogenic peptide suitable for injectable use include sterile aqueous solutions or dispersions;

formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the immunogenic peptide in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized immunogenic peptide into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the immunogenic peptide plus any additional desired ingredient from a previously sterile- filtered solution thereof.

Upon formulation, pharmaceutical preparations as defined herein or the peptides as defined herein or the fumarate compound as defined herein can be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

The peptides of the invention or the pharmaceutical composition comprising such as defined herein is preferably administered through sub-cutaneous or intramuscular administration. Preferably, the peptides or pharmaceutical compositions comprising

such can be injected sub-cutaneously (SC) in the region of the lateral part of the upper arm, midway between the elbow and the shoulder. When two or more separate injections are needed, they can be administered concomitantly in both arms.

The peptide according to the invention or the pharmaceutical composition comprising such is administered in a therapeutically effective dose. Exemplary but non-limiting dosage regimens are between 50 and 1500 µg, preferably between 100 and 1200 µg. More specific dosage schemes can be between 50 and 250 µg, between 250 and 450 µg or between 850 and 1300 µg, depending on the condition of the patient and severity of disease. Dosage regimen can comprise the administration in a single dose or in 2, 3, 4, 5, or more doses, either simultaneously or consecutively.

In certain embodiments, the treatment can be repeated several times throughout the disease of the subject. Such consecutive treatments can be done daily, or with an intermission of 1 to 10 days, such as for example every 5 to 9 days such as about every 7 days.

Alternatively, said treatment can be repeated weekly, biweekly, monthly, bimonthly, or every three to four months.

Exemplary non-limiting administration schemes are the following:

- A low dose scheme comprising the SC administration of 50 µg of peptide in two separate injections of 25 µg each (100 µL each) followed by three consecutive injections of 25 µg of peptide as two separate injections of 12.5 µg each (50 µL each).
- A medium dose scheme comprising the SC administration of 150 µg of peptide in two separate injections of 75 µg each (300 µL each) followed by three consecutive administrations of 75 µg of peptide as two separate injections of 37.5 µg each (150 µL each).
- A high dose scheme comprising the SC administration of 450 µg of peptide in two separate injections of 225 µg each (900 µL each) followed by three consecutive administrations of 225 µg of peptide as two separate injections of 112.5 µg each (450 µL each).

Other exemplary non-limiting administration schemes are the following:

- A dose scheme comprising 6 SC administration 2 weeks apart of 450 µg of peptide in two separate injections of 225 µg each.
- A dose scheme comprising 6 SC administration 2 weeks apart SC of 1350 µg of peptide in two separate injections of 675 µg each.

Other exemplary non-limiting administration schemes are the following:

- A dose scheme comprising 6 SC administration 2 weeks apart of 450 µg of peptide in two separate injections of 225 µg each.
- A dose scheme comprising 6 SC administration 2 weeks apart SC of 1350 µg of peptide in two separate injections of 675 µg each.

The immunogenic peptide formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

Other pharmaceutically acceptable forms of the immunogenic peptide can be readily envisaged by the skilled person.

Peptides, homologues or derivatives thereof according to the invention (and their physiologically acceptable salts or pharmaceutical compositions all included in the term "active ingredients") may be administered by any route appropriate to the condition to be treated and appropriate for the compounds, here the proteins and fragments to be administered. Possible routes include regional, systemic, oral (solid form or inhalation), rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intra-arterial, intrathecal and epidural). The preferred route of administration may vary with for example the condition of the recipient or with the diseases to be treated. As described herein, the carrier(s) optimally are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including

subcutaneous, intramuscular, intravenous, intradermal, intraarterial, intrathecal and epidural) administration.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Typical unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents. Peptides, homologues or derivatives thereof according to the invention can be used to provide controlled release pharmaceutical formulations containing as active ingredient one or more compounds of the invention ("controlled release formulations") in which the release of the active ingredient can be controlled and regulated to allow less frequency dosing or to improve the pharmacokinetic or toxicity profile of a given invention compound. Controlled release formulations adapted for oral administration in which discrete units comprising one or more compounds of the invention can be prepared according to conventional methods. Additional ingredients may be included in order to control the duration of action of the active ingredient in the composition. Control release compositions may thus be achieved by selecting appropriate polymer carriers such as for example polyesters, polyamino acids, polyvinyl pyrrolidone, ethylene-vinyl acetate copolymers, methylcellulose, carboxymethylcellulose, protamine sulfate and the like. The rate of drug release and duration of action may also be controlled by incorporating the active ingredient into particles, e.g. microcapsules, microspheres, microemulsions, nanoparticles, nanocapsules and so on. Depending on the route of administration, the pharmaceutical composition may require protective coatings. Pharmaceutical forms

suitable for injection include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation thereof. Typical carriers for this purpose therefore include biocompatible aqueous buffers, ethanol, glycerol, propylene glycol, polyethylene glycol and the like and mixtures thereof. In view of the fact that, when  
5 several active ingredients are used in combination, they do not necessarily bring out their joint therapeutic effect directly at the same time in the mammal to be treated, the corresponding composition may also be in the form of a medical kit or package containing the two ingredients in separate but adjacent repositories or compartments. In the latter context, each active ingredient may therefore be formulated in a way  
10 suitable for an administration route different from that of the other ingredient, e.g. one of them may be in the form of an oral or parenteral formulation whereas the other is in the form of an ampoule for intravenous injection or an aerosol.

Cytolytic CD4 +T cells as obtained in the present invention, induce APC apoptosis after MHC-class II dependent cognate activation, affecting both dendritic and B cells, as  
15 demonstrated in vitro and in vivo, and (2) suppress bystander T cells by a contact-dependent mechanism in the absence of IL-10 and/or TGF-beta. Cytolytic CD4+ T cells can be distinguished from both natural and adaptive Tregs, as discussed in detail in WO2008/017517.

Similarly, NKT cells as obtained in the present invention, i.e. activated by a MOG-derived peptide according to the invention containing a thioreductase activity, the latter  
20 increases significantly the properties of NKT cells and thereby increases the killing of cells carrying MOG autoantigens by antigen-specific CD4+ NKT cells, which suppresses the immune response against said MOG autoantigens. This mechanism is discussed in detail in WO2012/069568.

25 While the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing description. Accordingly, it is intended to embrace all such alternatives, modifications, and variations as follows in the spirit and broad scope of the appended claims. The herein disclosed aspects and  
30 embodiments of the invention are further supported by the following non-limiting examples.

## EXAMPLES

### Example 1: Peptide design

Compared to the P1 peptide HCHGCGGFLRVPCWKI (SEQ ID NO: 65) disclosed in WO2017182528, 4 peptides (P2 to P5) are synthesized comprising an oxidoreductase motif linked to a T cell epitope of the Myelin Oligodendrocyte Glycoprotein (MOG) as shown in the alignment depicted below:

|        |                         |                 |
|--------|-------------------------|-----------------|
| P1:    | HCHGC-GGFLRVPCWKI       | [SEQ ID NO: 65] |
| P2:    | HCPYCVRYFLRVPSWKITLF    | [SEQ ID NO: 25] |
| 10 P3: | HCPYCVRYFLRVPCWKITLF    | [SEQ ID NO: 26] |
| P4:    | KHCPYCVRYFLRVPSWKITLFFK | [SEQ ID NO: 27] |
| P5:    | KHCPYCVRYFLRVPCWKITLFFK | [SEQ ID NO: 28] |

All the 4 peptides comprise the natural human MOG epitope FLRVPCWKI (SEQ ID NO: 1) (P3 and P5) or a variant with a S instead of the C (P2 and P4), the C-terminal TLF flanking sequence naturally occurring in the MOG protein, and an artificial linker VRY. P2 and P3 have an oxidoreductase motif with the sequence HCPYC (SEQ ID NO: 24). P4 and P5 have an oxidoreductase motif with the sequence KHCPYC (SEQ ID NO: 50), and 2 K at their C-termini.

20

### Example 2: Assessment of the oxidoreductase activity of the immunogenic peptides.

The oxidoreductase activity of the immunogenic peptides is determined using a fluorescent assay described in Tomazzolli *et al.* (2006) *Anal. Biochem.* **350**, 105–112.

25 Two peptides with a FITC label become self-quenching when they form a covalent disulfide bond. Upon reduction by a peptide in accordance with the present invention, the reduced individual FITC labelled peptides emit fluorescence again. The activity is expressed as the mean of duplicates. The results are expressed in Relative Fluorescent Units (RFU). All the tested peptides P1 to P5 display an oxidoreductase activity (figure 1).

30

### Example 3: Assessment of the binding activity of the immunogenic peptides to soluble HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 MHC II proteins.

35

To test the binding of the immunogenic peptides to MHCII molecules, a soluble phase competition assay is performed. The increasing concentrations of P1 to P5 peptides compete with biotin-labelled control peptide (high affinity binder of the corresponding MHCII molecule, Eurogentec, Seraing, Belgium) for binding to the soluble HLA-DRB1\*03:01 (also named DR3), HLA-DRB1\*04:01 (also named DR4) or HLA-DRB1\*15:01 (also named DR15) human MHC II proteins (purchased from the Benaroya Research Institute, Seattle, US). As binding approaches its equilibrium (18h), biotin-labelled peptide/MHC II complexes are captured, separated from unbound reagents, and quantitatively detected by time-resolved fluorescence (Eu<sup>3+</sup> streptavidin, Perkin Elmer, Brussels, Belgium). Since the biotinylated control peptide is responsible for the fluorescence signal (Eu<sup>3+</sup> streptavidin/biotin interaction), the decrease in fluorescence intensity reflects the binding of tested peptides. Data are processed and plotted to ascertain dose-dependent binding properties of test peptides. All the tests are performed in triplicates. Figure 2, 3 and 4 show the results of one experiment. It is shown that peptides P2 to P5 bind to HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 with much higher affinity than the control P1 peptide.

**Example 4: Assessment of the role of the linker VRY on binding activity of the immunogenic peptides to soluble HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 MHC II proteins.**

In order to determine whether the improved MHCII binding observed with P2 to P5 as compared to P1 is due to the linker VRY, the following peptides were tested.

P6: -HCHGCVRYFLRVPCWKI [SEQ ID NO: 254]  
P7: -HCPYCGG-FLRVPCWKI [SEQ ID NO: 255]

P6 corresponds to the prior art peptide P1 wherein the linker GG has been replaced by the linker VRY. P7 corresponds to the prior art peptide P1 wherein the oxidoreductase motif HCHGC has been replaced by the HCPYC motif. Both P6 and P7 displayed oxidoreductase activity (not shown). It is shown in Figures 5 to 7 that the peptide P6 binds to HLA-DRB1\*03:01, HLA-DRB1\*04:01 and HLA-DRB1\*15:01 with much higher affinity than the control peptide P1. The P7 peptide exhibits a similar MHCII binding as the P1 peptide.

The same kind of experiments was performed with the following variants of the P4 peptide:

P8: KHCHGCVRYFLRVPSWKITLFFK [SEQ ID NO: 256]  
 P9: KCRC--VRYFLRVPSWKITLFFK [SEQ ID NO: 257]  
 5 P10: KCRPYCVRYFLRVPSWKITLFFK [SEQ ID NO: 257]  
 P11: KHCPYCGG--FLRVPSWKITLFFK [SEQ ID NO: 259]

P8, P9 and P10 peptides corresponds to the peptide P4 wherein the oxidoreductase motif KHCPYC has been replaced by the KHCHGC, KCRC or KCRPYC motifs, respectively. P11 corresponds to the peptide P4 wherein the linker VRY has been replaced by the linker GG. All the peptides displayed oxidoreductase activity (not shown). Replacement of VRY linker by GG induced a strong decrease in HLA-DRB1\*04:01 and HLA-DRB1\*15:01 binding, and to a lesser extent in HLA-DRB1\*03:01 binding (see Figures 8 to 10, compare P4 with P11, logarithmic scale). Modifications of oxidoreductase motifs did not significantly change MHCII binding (see Figures 8 to 10, compare peptides P8, P9 and P10 with P4).

Altogether, these data indicate that the linker VRY enhances MHCII binding of the peptides of the invention independent from the oxidoreductase sequence.

**Example 5: Ability of the immunogenic peptides to induce specific CD4+ T cells with lytic properties.** PBMCs were isolated from blood samples of patients with multiple sclerosis treated by dimethyl fumarate (DMF) on Lymphoprep density gradients. The haplotype of the patients is shown in table 1 below.

25

**Table 1:** Haplotype of the patients included in the present study:

| Tested patients<br>(DMF treated) | Haplotype        |
|----------------------------------|------------------|
| MS017                            | DRB1*03:01/15:01 |
| MS020                            | DRB1*11:01/15:01 |
| MS021                            | DRB1*13:02/15:01 |
| MS024                            | DRB1*03:01/04:01 |
| MS026                            | DRB1*04:02/11:03 |
| MS027                            | DRB1*15:01       |

|       |                  |
|-------|------------------|
| MS028 | DRB1*07:01/15:01 |
| MS029 | DRB1*01:01/13:02 |

- CD14<sup>+</sup> monocytes were isolated from these PBMCs by performing positive immunomagnetic separation with CD14 microbeads (Miltenyi Biotec, 130-050-201) according to the supplier recommendations. CD14<sup>+</sup> monocytes were cultured for six
- 5 days and matured to generate autologous dendritic cells (mDC). CD19<sup>+</sup> B cells were isolated from the CD14<sup>-</sup> PBMCs fraction by performing positive immunomagnetic separation with CD19 microbeads (Miltenyi Biotec, 130-050-301) according to the supplier recommendations. CD19<sup>+</sup> B cells were cultured and immortalized with EBV to generate autologous lymphoblastoid cell lines (LCL).
- 10 Naïve CD4<sup>+</sup> T cells were also purified from the CD14<sup>-</sup> PBMCs fraction by performing negative immunomagnetic separation with naïve CD4<sup>+</sup> T cell isolation kit (Miltenyi Biotec, 130-094-131) according to the supplier recommendations. Naïve CD4<sup>+</sup> T cells were co-cultured with autologous mDC or LCL in the presence of P2 and P4 peptides. The CD4<sup>+</sup> T cells were re-stimulated periodically, about every 10-12 days.
- 15 The ability of the peptides to generate antigen specific CD4<sup>+</sup> T cells was evaluated by flow cytometry analysis of the TCR induced surface activation marker CD154 (CD40L) expression after overnight co-culture at resting state with autologous LCL without (no peptide) or with the peptides (P2 or P4). The surface expression of the lytic marker Fas ligand (CD178) was also evaluated by flow cytometry analysis after overnight co-
- 20 culture at resting state with autologous LCL without (no peptide) or with the peptide (P4).
- The ability of the peptides to induce cytokines secretion in CD4<sup>+</sup> T cells culture supernatants was evaluated by flow cytometry analysis after overnight co-culture at resting state with autologous mDC without (no peptide) or with the peptides (P2 or P4).
- 25 Supernatants were analyzed with the LEGENDplex Human Th Panel (13-plex) (BioLegend, 740721) according to the supplier recommendations.
- The cytolytic activity of the antigen specific CD4<sup>+</sup> T cells was evaluated by quantifying the apoptosis induced on LCL used as antigen presenting cells. Fluorescently labelled autologous LCL, loaded or not with the peptide (P4), were overnight co-cultured at
- 30 resting state with specific CD4<sup>+</sup> T cells, and LCL apoptosis was quantified by flow cytometry through Annexin V staining. Considering the apoptosis percentage of

unloaded LCL, used as control, the percentage of specific apoptosis was calculated as follows:

$$\frac{\% \text{ Annexin V+ of loaded LCL} - \% \text{ Annexin V+ of unloaded LCL}}{100 - \% \text{ Annexin V+ of unloaded LCL}} \times 100$$

## 5 *Results with P2*

We were able to generate P2-specific CD4<sup>+</sup> T cell lines from four different MS patients (MS017, MS022, MS026 and MS027). We showed that stimulation with P2 of three patients' CD4<sup>+</sup> cell lines (MS017 (S9), MS022 (S10) and MS027 (S12)) induced a high frequency of effector cells (CD3<sup>+</sup>CD4<sup>+</sup>CD154<sup>+</sup>) (Figure 11). Moreover, a specific  
10 secretion of cytokines (IL-5 and IL-13) induced by P2 in culture supernatant of MS026 CD4<sup>+</sup> cell line (S11) was observed (Figure 12).

## *Results with P4*

We were able to generate P4-specific CD4<sup>+</sup> T cell lines from eight different MS patients (MS017, MS020, MS021, MS024, MS026, MS027, MS028 and MS029). We showed  
15 that stimulation with P4 of eight patients' CD4<sup>+</sup> cell lines (MS017 (S12), MS020 (S7), MS021 (S9), MS024 (S7), MS026 (S12), MS027 (S12), MS028 (S11) and MS029 (S9)) induced a high frequency of effector cells (CD3<sup>+</sup>CD4<sup>+</sup>CD154<sup>+</sup>) (Figure 13). It was also shown that stimulation with P4 induced a specific increase of effector cells expressing the lytic marker Fas ligand (CD3<sup>+</sup>CD4<sup>+</sup>CD154<sup>+</sup>FasL<sup>+</sup>) for CD4<sup>+</sup> cell lines of patients  
20 MS017 (S9) and MS020 (S10) (Figure 14), thereby demonstrating that P4 is able to induce specific CD4<sup>+</sup> T cells with lytic properties called cytolytic CD4<sup>+</sup> T cells. Moreover, a specific secretion of cytokine (IL-5) induced by P4 in culture supernatant of MS017 (S15), MS024 (S20) and MS026 (S14) CD4<sup>+</sup> cell lines was observed (Figure 15). Furthermore, we showed a specific induction of effector cells  
25 (CD3<sup>+</sup>CD4<sup>+</sup>CD154<sup>+</sup>) after overnight co-culture at resting state of P4-specific CD4<sup>+</sup> cell line with P4 and its corresponding short C-WT T-cell epitope peptide (sequence: DPHFLRVPCWKITLFFK, SEQ ID NO: 29) for CD4<sup>+</sup> cell lines of patients MS017 (S14) and MS026 (S13) (Figure 16). We also showed a specific induction of effector cells (CD3<sup>+</sup>CD4<sup>+</sup>CD154<sup>+</sup>) after overnight co-culture at resting state of P4-specific CD4<sup>+</sup>  
30 cell line with P4 and its corresponding short S-WT T-cell epitope peptide (sequence: KLRHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253) for CD4<sup>+</sup> cell lines of patients MS024 (S20), MS017 (S9), MS026 (S13), MS028 (S11) and MS029 (S9) (Figure 17).

Moreover, a specific secretion of cytokine (IL-5) induced by the P4 peptide and its corresponding short C-WT and long C-WT T-cell epitope peptides (short sequence: DPHFLRVPCWKITLFFK (SEQ ID NO: 29), or long sequence: QYRLRGKLRAEIEIENLHRTFDPHFLRVPCWKITLFVIVPVLGP, SEQ ID NO: 30) in culture supernatant of MS017 (S15) CD4+ cell line was observed (Figure 18). We also observed a specific secretion of cytokine (IL-5) induced by the P4 peptide and its corresponding short S-WT T-cell epitope peptide (sequence: KLHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253) in culture supernatant of MS017 (S12) and MS024 (S20) CD4+ cell lines (Figure 19), thereby indicating that P4-specific CD4+ T cells are able to cross-react with APCs presenting the WT MOG epitope sequence.

Finally, we showed an increase in the percentage of specific LCL apoptosis when labelled autologous LCL, loaded with the P4 peptide or its corresponding short S-WT T-cell epitope peptide (sequence: KLHRTFDPHFLRVPSWKITLFFK, SEQ ID NO: 253), are co-cultured with the P4-specific CD4+ T cell lines from patients MS017 (S7), MS026 (S12), MS028 (S11) and MS029 (S9), further demonstrating the lytic activity of the P4-induced cytolytic CD4+ T cells (Figure 20).

#### **Example 6: Effect of the therapeutic administration of P4 or IMCY-0189 peptides on experimental auto-immune encephalomyelitis (EAE) development in mice.**

##### **Groups of mice and dosing**

The study used a total of 48 female C57BL/6 mice (Taconic Biosciences, 9 weeks old on Day 0). Mice were acclimated for 7 days prior to the first injection. Mice were assigned to groups in a balanced manner to achieve similar average weight across the groups at the start of the study. Table 2 below shows the treatment administered to each group.

**Table 2 - Treatment regimen**

| Group | # animals | Treatment (s.c.) | Dosing days  | Purpose          |
|-------|-----------|------------------|--------------|------------------|
| 1     | 16        | Saline           | 4, 9, 14, 19 | Negative control |
| 2     | 16        | IMCY-0189        | 4, 9, 14, 19 | Test             |
| 3     | 16        | P4               | 4, 9, 14, 19 | Test             |

Dosing of all mice was performed once on each of the days indicated in Table 2, s.c., at a volume of 0.05 mL/site, each mouse receiving injection at two sites, for a total of 5 0.1 mL/mouse/dosing day. IMCY-0189 or P4 peptide total dose was 30 µg per administration.

All dosing was at the same time (+/- 1 hour) each dosing day.

### Compound preparation

10 For Saline treatment, 0.9% NaCl solution was prepared at each dosing day.

#### *IMCY-0189 peptide preparation:*

Lyophilized immunogenic peptide IMCY-0189 with the sequence HCPYCGWYRSPFSRVVHLYR (SEQ ID NO: 260), comprising an oxidoreductase motif HCPYC (SEQ ID NO: 24), a linker GW, a murine Myelin Oligodendrocyte Glycoprotein (MOG<sub>35-55</sub>) MHCII T cell epitope YRSPFSRVV (SEQ ID NO: 261) and a flanking sequence HLYR (SEQ ID NO: 262) (Smart Bioscience) was solubilized immediately before use. Lyophilized IMCY-0189 was thawed at room temperature for 10 minutes, resuspended in Na Acetate buffer 50 mM NaCl 0.9% pH 5.4 and incubated at room temperature for 10 minutes. Reconstituted peptide was then mixed with 20 Imject™ Alum Adjuvant before injection.

#### *P4 peptide preparation:*

Lyophilized immunogenic peptide P4 with the sequence KHCPYCVRYFLRVPSWKITLFKK (SEQ ID NO: 27), comprising an oxidoreductase motif KHCPYC (SEQ ID NO: 50), a linker VRY, a human Myelin Oligodendrocyte Glycoprotein (MOG<sub>201-212</sub>) MHCII T cell epitope FLRVPSWKI (SEQ ID NO: 2) and a flanking sequence TLFFK (SEQ ID NO: 263) (Smart Bioscience) was solubilized immediately before use. Lyophilized P4 was thawed at room temperature for 10

minutes, resuspended in Na Acetate buffer 50 mM NaCl 0.9% pH 5.4 and incubated at room temperature for 10 minutes. Reconstituted peptide was then mixed with Imject™ Alum Adjuvant before injection.

## 5 EAE induction

EAE was induced in all mice as follows:

Day 0, Hour 0 – Immunization with a peptide corresponding to the amino acids 35-55 of MOG (MOG<sub>35-55</sub>)/CFA

Day 0, Hour 2 – Injection of pertussis toxin

10 Day 1, Hour 0 – 2<sup>nd</sup> injection of pertussis toxin (24 hours after initial immunization).

Mice were injected subcutaneously at two sites in the back with the emulsion component (containing MOG<sub>35-55</sub>) of Hooke Kit™ MOG<sub>35-55</sub>/CFA Emulsion PTX, catalog number EK-2110 (lot # 131, Hooke Laboratories, Lawrence MA). One site of  
15 injection was in the area of upper back, approximately 1 cm caudal of the neck line. The second site was in the area of lower back, approximately 2 cm cranial of the base of the tail. The injection volume was 0.1 mL at each site. Each mouse received 200 µg of MOG<sub>35-55</sub>.

Within 2 hours of the injection of emulsion, and then again 24 hours after the injection  
20 of emulsion, the pertussis toxin component of the kit was administered intraperitoneally. The pertussis toxin (lot # 1008, Hooke Laboratories) was administered at 90 ng/dose for both injections and the volume of each injection was 0.1 mL.

## 25 EAE scoring

Animals were scored daily starting from Day 7 to the end of the study. Scoring was performed blind, by a person unaware of both treatment and of previous scores for each mouse. EAE was scored on the scale 0 to 5 as shown in Table 3 below. In-between scores were assigned when the clinical signs fell between two above defined  
30 scores.

**Table 3 – EAE scoring criteria**

| Score | Clinical observations                                                     |
|-------|---------------------------------------------------------------------------|
| 0     | No obvious changes in motor functions                                     |
| 1     | Limp tail                                                                 |
| 2     | Limp tail and weakness of hind legs                                       |
| 3     | Limp tail and complete paralysis of hind legs                             |
| 4     | Limp tail, complete hind leg and partial front leg paralysis              |
| 5     | Complete hind and complete front leg paralysis, or death due to paralysis |

**Serum neurofilaments levels determination**

On Day 28, blood was collected from all mice into gel clot activator tubes and allowed to clot at room temperature for ~30 minutes. Blood is then centrifuged at ~10000 g for 5 minutes. Serum was transferred into Eppendorf tubes and stored at -80°C until shipment to Quanterix™. Serum Neurofilament light (NF-L) protein levels were quantified using Simoa® NF-light Advantage kit, a digital immunoassay for the quantitative determination of NF-L in serum, plasma and CSF. The used antibodies (Uman Diagnostics, Umeå Sweden) also cross react with murine, bovine and macaque NF-L epitopes and as such, this assay can be used for research with these species. All samples were tested in duplicate at a dilution factor of 40x.

**Terminal collection**

At the end of the study, all mice were euthanized, and spines were collected and placed in 10% buffered formalin for histological analysis.

**Histology**

For each spine, one H&E stained slide and one anti-MBP stained slide were prepared and analyzed. Each slide contained a section with samples from lumbar, thoracic and cervical of spinal cord (3 samples). All analysis was performed by a pathologist blinded to the experimental groups and all clinical readouts.

Inflammatory foci of approximately 20 cells were counted in each H&E stained section. When inflammatory infiltrates consisted of more than 20 cells, an estimate was made of how many foci of 20 cells were present.

Demyelination was scored in each anti-MBP (using immunohistochemistry) stained section. In anti-MBP sections, demyelination is observed as conspicuous unstained

areas in white matter tracts and is associated with presence of large vacuoles. The demyelination score represents an estimate of demyelinated area for each section as follows:

- 0 – no demyelination (less than 5% demyelinated area)
- 5      1 – 5 to 20% demyelinated area
- 2 – 20 to 40% demyelinated area
- 3 – 40 to 60% demyelinated area
- 4 – 60 to 80% demyelinated area
- 5 – 80 to 100% demyelinated area

10

### Statistical analysis

AUC, MMS, inflammation and demyelination, and NF-L levels quantification data were analyzed by performing Ordinary one-way ANOVA. Adjustment for multiplicity was performed using Holm-Sidak's method. Significant differences are referred as follows:

15      \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

### Results and interpretation of data

#### *EAE scoring*

EAE development was evaluated by comparing clinical EAE readouts for all groups to the negative control (Saline) group. EAE scoring, AUC (area under the curve) and MMS (mean maximal score) are presented in Figures 21, 22 and 23.

20

Mice of the Saline group (negative control) developed EAE within the expected range for this model. No mice died in this group.

25

Mice treated either with IMCY-0189 or with P4 showed postponed disease onset and reduced end score, and statistically significant reduced AUC and MMS compared to the negative control group. No mice died in these three groups.

#### 30      *Histology*

Histological readouts were evaluated by comparing inflammation and demyelination levels of all groups to the negative control (Saline) group. Inflammation and demyelination data are presented in Figures 24 and 25.

Histological results for the Saline group (negative control) were consistent with the

clinical findings and as expected for this model.

Mice treated with IMCY-0189 showed statistically significantly reduced level of both inflammation and demyelination. Mice treated with P4 showed reduced level of demyelination and reduced level of inflammation. Results of histological analysis were  
5 consistent with the clinical findings.

#### *Serum neurofilaments levels*

Neurofilament light (NF-L) is a 68 kDa cytoskeletal filament protein that is expressed in neurons, as one of the major components of the neuronal cytoskeleton that provide  
10 structural support for the axon. Neurofilaments can be released following axonal damage or neuronal degeneration. NF-L has been shown to associate with neurodegenerative diseases such as multiple sclerosis.

Axonal damage was evaluated by comparing NF-L levels for all groups to the negative control (Saline) group. Data are presented in Figure 26.

15 NF-L levels for the Saline group (negative control) were consistent with the clinical findings and as expected for this model.

Mice treated with IMCY-0189 showed statistically significant reduced NF-L levels compared to the negative control group. Mice treated with P4 also showed statistically significant reduced NF-L levels compared to the negative control group.

20  
**Example 7: Effect of the prophylactic administration of an immunogenic peptide comprising a MOG<sub>35-55</sub> MHCII T cell epitope linked to a HCPYC oxidoreductase motif on experimental auto-immune encephalomyelitis (EAE) development in mice.**

#### **EAE induction**

25 EAE was induced in recipient mice by immunizing donor B6.SJL mice with MOG<sub>35-55</sub>/CFA, and then, 11 days later, by taking their spleens and restimulating them in culture with MOG<sub>35-55</sub> peptide for 3 days. Those cells, now fully encephalitogenic, were  
30 injected on Day 0 into recipient groups of mice, which developed EAE.

#### **Donor mice**

B6.SJL donor mice were acclimated for 13 days before the start of the study and were 9 weeks old at the time of immunization. Donor mice were used to generate encephalitogenic cells as follows:

5 Day -14: Immunization with MOG<sub>35-55</sub>/CFA.

Day -3: Spleen harvest. Mice were euthanized and spleens were harvested, pooled and cell suspension prepared. Cell suspension, at 4 to 5 million cells/mL in T150 flasks, were set up in cultures in the presence of MOG<sub>35-55</sub> peptide (20 µg/mL), IL-12 (20 µg/mL) and anti-IFNγ (7 µg/mL) for 3 days to generate encephalitogenic T cells.

Day 0: Cell collection and transfer. Cells were collected and spun down, resuspended in RPMI1640 (no FCS), counted, and injected into recipient mice at 10 million cells per mouse.

#### 15 Groups of mice (recipient mice) and dosing

Recipient mice were acclimated for 6 days prior to the first injection and were 6 weeks old when treatment started (on Day -21). Mice were assigned to groups in a balanced manner, to achieve similar average weight across the groups at the start of the study. Table 4 below shows the treatment administered to each group.

20 **Table 4 - Recipient mice groups and treatment**

| Group | # mice | Treatment (s.c.) | Dosing days          | Purpose          |
|-------|--------|------------------|----------------------|------------------|
| 1     | 16     | Alum             | -21, -14, -7, +2, +9 | Negative control |
| 2     | 16     | IMCY-0189        | -21, -14, -7, +2, +9 | Test             |

Dosing of all mice was performed once on each of the days indicated in Table 4, s.c., at a volume of 0.05 mL/site, each mouse receiving injection at two sites, for a total of 0.1 mL/mouse/dosing day, corresponding to 100 µg of peptide. All dosing was at the same time (+/- 2 hours) each dosing day.

#### 30 Compound preparation

For Vehicle treatment, Imject™ Alum solution was prepared at each dosing day. IMCY-0189 has the sequence described in example 6. Lyophilized IMCY-0189 was thawed at room temperature for 10 minutes, resuspended in Na Acetate buffer 50 mM pH 5.4 and incubated at room temperature for 5 minutes. Reconstituted peptide was then  
5 mixed with Imject™ Alum Adjuvant before injection.

### **Plasma neurofilaments levels determination**

At termination, blood was collected from all mice into tubes containing K2EDTA and mixed gently. Blood was then centrifuged at ~10000 g for 5 minutes. Plasma was  
10 transferred into Eppendorf tubes and stored at -80°C until shipment to Quanterix™. Plasma Neurofilament light (NF-L) protein levels were quantified using Simoa® NF-light Advantage kit as described in example 6.

EAE scoring, terminal collection, histology analyses and statistical analyses were  
15 performed as described in example 6.

### **Results and interpretation of data**

#### *EAE scoring*

EAE development was evaluated by comparing clinical EAE readouts of the test group  
20 (IMCY-0189) to the negative control group (Alum). EAE scoring, AUC (area under the curve) and MMS (mean maximal score) are presented in Figures 27, 28 and 29.

Mice of the Alum group (negative control) developed typical EAE for this model. No mice died in this group.

All clinical readouts (disease onset, end score, AUC and MMS) of mice treated with  
25 IMCY-0189 were statistically significantly improved, compared to the negative control group. No mice died in this group.

#### *Histology*

Histological readouts were evaluated by comparing inflammation and demyelination  
30 levels of the test group (IMCY-0189) to the negative control group (Alum). Inflammation and demyelination data are presented in Figures 30 and 31.

Histological results for the Alum group (negative control) were consistent with the clinical findings and as expected for this model.

Mice treated with IMCY-0189 showed statistically significantly reduced level of both

inflammation and demyelination. Results of histological analysis were consistent with the clinical findings.

*Plasma neurofilaments levels*

- 5 Neurofilament light (NF-L) is a 68 kDa cytoskeletal filament protein that is expressed in neurons, as one of the major components of the neuronal cytoskeleton that provide structural support for the axon. Neurofilaments can be released following axonal damage or neuronal degeneration. NF-L has been shown to associate with neurodegenerative diseases such as multiple sclerosis.
- 10 Axonal damage was evaluated by comparing NF-L levels of the test group (IMCY-0189) to the negative control group (Alum). Data are presented in Figure 32.
- NF-L levels for the Alum group (negative control) were consistent with the clinical findings and as expected for this model.
- Mice treated with IMCY-0189 showed statistically significantly reduced NF-L levels
- 15 compared to the negative control group.

**CLAIMS**

1. An isolated immunogenic peptide with a length of between 12 and 50 amino acids, said immunogenic peptide comprising:
- a1) an oxidoreductase motif with the sequence  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST],  
5 wherein n is an integer chosen from: 2, 0, 1 or 3, wherein m is an integer selected from 2, 1, 0, or 3, wherein X is any amino acid, wherein Z is any amino acid, wherein X is any amino acid, in which C stands for cysteine, S for serine, T for threonine;
- a2) a T-cell epitope with an amino acid sequence selected from the group consisting of: MHC class II T cell epitopes FLRVPSWKI (SEQ ID NO: 2) and FLRVPCWKI (SEQ  
10 ID NO: 1), or NKT cell epitopes FLRVPCW (SEQ ID NO: 63), and FLRVPSW (SEQ ID NO: 64), and
- wherein said oxidoreductase motif and said epitope are separated by a linker sequence of between 3 to 7 amino acids comprising the sequence VRY.
- 15 2. The peptide according to claim 1, wherein said oxidoreductase motif is selected from the following amino acid motifs:
- (a)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]-,  
wherein n is 0, and wherein m is an integer selected from 0, 1, or 2,  
wherein Z is any amino acid, preferably a basic amino acid preferably selected from:  
20 H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or H, most preferably K;
- (b)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]-,  
wherein n is 1, wherein X is any amino acid, preferably a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably  
25 K or R,  
wherein m is an integer selected from 0, 1, or 2,  
wherein Z is any amino acid, preferably a basic amino acid preferably selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or H, most preferably K;
- 30 (c)  $Z_m$ -[CST]- $X_n$ -C- or  $Z_m$ -C- $X_n$ -[CST]-,  
wherein n is 2, thereby creating an internal  $X^1X^2$  amino acid couple within the oxidoreductase motif, wherein X is any amino acid, preferably wherein at least one X

is a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or R,

wherein m is an integer selected from 0, 1, or 2,

wherein Z is any amino acid, preferably a basic amino acid preferably selected from:

- 5 H, K, R, and a non-natural basic amino acid, such as L-ornithine, more preferably K or H, most preferably K;

(d)  $Z_m\text{-[CST]-X}_n\text{-C-}$  or  $Z_m\text{-C-X}_n\text{-[CST]-}$

wherein n is 3, thereby creating an internal  $X^1X^2X^3$  amino acid stretch within the oxidoreductase motif, wherein X is any amino acid, preferably wherein at least one X

- 10 is a basic amino acid selected from: H, K, R, and a non-natural basic amino acid such as L-ornithine, more preferably K or R,

wherein m is an integer selected from 0, 1, or 2,

wherein Z is any amino acid, preferably a basic amino acid preferably selected from:

- 15 H, K, R, and a non-natural basic amino acid as defined herein, such as L-ornithine, more preferably K or H; or

(h)  $Z_m\text{-[CST]-X}_n\text{-C-}$  or  $Z_m\text{-C-X}_n\text{-[CST]-}$ ,

wherein n is 0 to 3 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  
20 carboxy group (SEQ ID NO: 184 to 203).

3. The peptide according to claim 1 or 2, wherein said T-cell epitope is flanked at its C-terminus by the amino acid sequence TLF leading to the following T-cell epitope-flanker sequence: FLRVPCWKITLF (SEQ ID NO: 3) or FLRVPSWKITLF (SEQ ID NO: 4)

25

4. The peptide according to any one of claims 1 to 3, wherein said immunogenic peptide additionally comprises one or more K amino acid residue(s) flanking the epitope at the C-terminus, leading to the following sequences of linker-T-cell epitope-flanker: FLRVPCWKITLFFK (SEQ ID NO: 5), FLRVPSWKITLFFK (SEQ ID NO: 6),  
30 FLRVPCWKITLFFKK (SEQ ID NO: 7), FLRVPSWKITLFFKK (SEQ ID NO: 8), FLRVPCWKITLFFKKK (SEQ ID NO: 9), or FLRVPSWKITLFFKKK (SEQ ID NO: 10).

5. The peptide according to any one of claims 1 to 4, wherein the oxidoreductase motif has a sequence of  $Z_m\text{-C-XX-C-}$ , with Z being a basic amino acid, preferably

selected from the group consisting of K and H, m being 0, 1, or 2, , preferably wherein the oxidoreductase motif comprises the sequence CPYC (SEQ ID NO: 23), or CHGC (SEQ ID NO: 297).

5

6. The peptide according to claim 5, wherein the oxidoreductase motif has a sequence selected from the group consisting of: HCPYC (SEQ ID NO: 24), KCPYC (SEQ ID NO: 51), KHCPYC (SEQ ID NO: 50), KCRPYC (SEQ ID NO: 216), KHCRPYC (SEQ ID NO: 217), HCHGC (SEQ ID NO: 265), KCHGC (SEQ ID NO: 266), KHCHGC (SEQ ID NO: 267), KCRHGC (SEQ ID NO: 268), and KHCRHGC (SEQ ID NO: 269).

10

7. The peptide according to any one of claims 1 to 4, wherein the oxidoreductase motif has a sequence of  $Z_m$ -C-X-C-, with Z being a basic amino acid, preferably selected from the group consisting of K and H, m being 0, 1, or 2, and X preferably being R.

15

8. The peptide according to claim 7, wherein the oxidoreductase motif has a sequence selected from the group consisting of: CRC, KCRC (SEQ ID NO: 43), HCRC (SEQ ID NO: 270) and KHCRC (SEQ ID NO: 271).

20

9. The peptide according to any one of claims 1 to 4, wherein said peptide comprises or consists of any one of the amino sequences selected from the group consisting of:

KCRCVRYFLRVPSWKITLFFK (SEQ ID NO: 272),  
25 KCRCVRYFLRVPCWKITLFFK (SEQ ID NO: 273),  
KCRCVRYFLRVPSWKITLFFK (SEQ ID NO: 274),  
KCRCVRYFLRVPCWKITLFFK (SEQ ID NO: 275),  
KCRCVRYFLRVPSWKITLFF (SEQ ID NO: 276),  
KCRCVRYFLRVPCWKITLFF (SEQ ID NO: 277),  
30 KCRPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 257),  
KCRPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 278),  
KCRPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 279),  
KCRPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 280),  
KCRPYCVRYFLRVPSWKITLFF (SEQ ID NO: 281),

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KCRPYCVRYFLRVPCWKITLF (SEQ ID NO: 282),  
KHCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 27),  
KHCPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 28),  
KHCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 283),  
5 KHCPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 284),  
KHCPYCVRYFLRVPSWKITLF (SEQ ID NO: 285),  
KHCPYCVRYFLRVPCWKITLF (SEQ ID NO: 286),  
HCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 287),  
HCPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 288),  
10 HCPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 289),  
HCPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 290),  
HCPYCVRYFLRVPSWKITLF (SEQ ID NO: 25),  
HCPYCVRYFLRVPCWKITLF (SEQ ID NO: 26),  
CPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 291),  
15 CPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 292),  
CPYCVRYFLRVPSWKITLFFK (SEQ ID NO: 293),  
CPYCVRYFLRVPCWKITLFFK (SEQ ID NO: 294),  
CPYCVRYFLRVPSWKITLF (SEQ ID NO: 295), and  
CPYCVRYFLRVPCWKITLF (SEQ ID NO: 296).

20

10. A polynucleotide encoding the peptide according to any one of claims 1 to 9, wherein said polynucleotide is selected from the group comprising DNA, pDNA, cDNA, RNA, and mRNA or modified versions thereof.

25 11. A pharmaceutical composition comprising the peptide according to any one of claims 1 to 9, or the polynucleotide according to claim 10.

12. The peptide according to any one of claims 1 to 9, the polynucleotide according to claim 10, or the pharmaceutical composition according to claim 11, for use as a  
30 medicament.

13. The peptide, polynucleotide, or pharmaceutical composition according to claim 12, for use in treating of, preventing and/or for reducing the symptoms of a

demyelinating disorder, preferably wherein said demyelinating disorder is a disease or disorder caused by MOG auto-antigens or anti-MOG antibodies.

14. The peptide, polynucleotide, or pharmaceutical composition for use according to claim 12, wherein said disorder is selected from: Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO).

15. The peptide, polynucleotide, or pharmaceutical composition according to any one of claims 12 to 14, for use in treating of, preventing and/or for reducing the symptoms of MS, wherein the subject has an HLA-DRB1\* type selected from the group consisting of: HLA-DRB1\*15:01, HLA-DRB1\*03:01, HLA-DRB1\*04:01, and HLA-DRB1\*07:01, preferably wherein the subject has HLA-DRB1\*04:01 or HLA-DRB1\*15:01.

16. The peptide, polynucleotide, or pharmaceutical composition according to any one of claims 12 to 14, for use in treating of, preventing and/or for reducing the symptoms of NMO or reducing the symptoms of NMO, wherein the subject has an HLA type selected from the group consisting of: HLA-DRB1\*03:01 and HLA-DPB1\*05:0114.

17. The peptide, polynucleotide, or pharmaceutical composition for use according to claim 12 or 14, wherein said MS is selected from: Clinically Isolated Syndrome (CIS), relapse-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), Acute Fulminant Multiple Sclerosis and MS-suspected radiology isolated syndrome (RIS).

25

18. The peptide, polynucleotide, or pharmaceutical composition for use according to any one of claims 12 to 17, wherein said subject is being, has been, or is going to be treated with a fumarate compound.

19. An in vitro method for the generation of a population of cytolytic CD4+ T cells, against APC presenting MOG epitopes, comprising the steps of:

- providing peripheral blood cells;
- contacting said cells in vitro with the peptide of any one of claims 1 to 9, or the polynucleotide according to claim 10; and

- expanding said cells in the presence of IL-2.

20. A method for the generation of a population of cytolytic CD4+ T cells, against APC presenting MOG epitopes, comprising the steps of:

- 5 - administering to a subject an effective amount of the peptide of any one of claims 1 to 9, or the polynucleotide according to claim 10;  
- obtaining said cytolytic CD4+ T cells from a peripheral blood cell population of said subject.

10 21. A method for the generation of a population of NKT cells, against APC presenting MOG epitopes, comprising the steps of:

- administering to a subject an effective amount of the peptide of any one of claims 1 to 9, or the polynucleotide according to claim 10;  
- obtaining said NKT cells from a peripheral blood cell population of said subject.

15

22. A population of cytolytic CD4+ T cells or NKT cells, against APC presenting MOG epitopes, obtainable by the method of claims 19, 20, or 21.

23. A population of cytolytic CD4+ T cells or NKT cells, against APC presenting  
20 MOG epitopes, obtainable by the method of claims 19, 20, or 21, for use as a medicament.

24. A population of cytolytic CD4+ T cells or NKT cells for use according to claim 23, for use in the treatment of, ameliorating the symptoms of, and/or preventing of a  
25 demyelinating disorder or reducing the symptoms of a demyelinating disorder.

25. A pharmaceutical composition comprising the peptide of any one of claims 1 to 9, the polynucleotide according to claim 10, or the CD4+ T cells or NKT cells according to claim 24, or any mixture thereof, and optionally further comprising a  
30 pharmaceutically acceptable carrier.

26. The pharmaceutical composition of claim 25, optionally further comprising an additional active ingredient suitable for treatment of a demyelinating disorder, or

reducing the symptoms of a demyelinating disorder or preventing a demyelinating disorder.

27. The pharmaceutical composition of claim 25 or 26, for use as a medicament.

28. The pharmaceutical composition for use according to claim 27, for use in treating of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder, preferably caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies, most preferably Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO).

29. Use of an immunogenic peptide according to any one of claims 1 to 9, the polynucleotide according to claim 10, or the CD4+ T cells or NKT cells according to claim 22, or any mixture thereof, for the manufacture of a medicament for treating of, ameliorating the symptoms of, and/or preventing of a demyelinating disorder, preferably caused or aggravated by MOG auto-antigens and/or anti-MOG antibodies, most preferably Multiple Sclerosis (MS) or Neuromyelitis Optica (NMO).

30. A method for treating of, ameliorating the symptoms of, and/or preventing a demyelinating disorder in a subject, comprising the step of providing the peptide according to claims 1 to 9, the polynucleotide according to claim 10, or the CD4+ T cells or NKT cells of claim 22, or any mixture thereof, to a subject.

31. The method according to claim 29, wherein said demyelinating disorder is selected from: Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Optic Neuritis, Acute Disseminated Encephalomyelitis, Balo's Disease, HTLV-I Associated Myelopathy, Schilder's Disease, Transverse Myelitis, Idiopathic inflammatory demyelinating diseases, vitamin B12-induced central nervous system neuropathies, Central pontine myelinolysis, Myelopathies including tabes dorsalis, Leukodystrophies such as Adrenoleukodystrophy, Leukoencephalopathies such as Progressive multifocal leukoencephalopathy (PML), Vanishing White Matter Disease, and Rubella induced mental retardation.

32. The method according to claim 30 or 31, further comprising a step of administering a fumarate compound to said subject.

33. The method according to claim 32, wherein said fumarate compound is selected from the group consisting of: monomethyl fumarate (MMF), dimethyl fumarate (DMF), compounds that can be metabolized into MMF *in vivo*, monomethyl fumarate  
5 prodrugs such as diroximel fumarate or tepilamide fumarate, or a combination of any one or more thereof, or a deuterated form, a clathrate, a solvate, a tautomer, a stereoisomer, or a pharmaceutically acceptable salt of any one or more thereof, or a combination of any one of the foregoing.
- 10 34. An *in vitro* method for detecting MHC class II restricted CD4+ T cells specific for a MOG antigen in a sample comprising the steps of;
- contacting a subject sample with a complex of an isolated MHC class II molecules and a peptide according to claims 1 to 9, or the polynucleotide according to claim 10;
  - detecting CD4+ T cells by measuring the binding of said complex with cells in said  
15 sample, wherein the binding of the complex to a cell is indicative for the presence of CD4+ T cells in said sample.