

PEPTIDES FOR VACCINE AGAINST BIRCH ALLERGY

Field of the Invention

The present invention relates to compositions for preventing or treating allergy
5 to birch.

Background of the Invention

T-cell antigen recognition requires antigen presenting cells (APCs) to present
antigen fragments (peptides) on their cell surface in association with molecules of the
10 major histocompatibility complex (MHC). T cells use their antigen specific T-cell
receptors (TCRs) to recognise the antigen fragments presented by the APC. Such
recognition acts as a trigger to the immune system to generate a range of responses to
eradicate the antigen which has been recognised.

Recognition of external antigens by the immune system of an organism,
15 such as man, can in some cases result in diseases, known as atopic
conditions. Examples of the latter are the allergic diseases including asthma, atopic
dermatitis and allergic rhinitis. In this group of diseases, B lymphocytes generate
antibodies of the IgE class (in humans) which bind externally derived antigens, which
are referred to in this context as allergens since these molecules elicit an allergic
20 response. Production of allergen-specific IgE is dependent upon T lymphocytes which
are also activated by (are specific for) the allergen. Allergen-specific IgE antibodies
bind to the surface of cells such as basophils and mast cells by virtue of the expression
by these cells of surface receptors for IgE.

Crosslinking of surface bound IgE molecules by allergen results in
25 degranulation of these effector cells causing release of inflammatory mediators such as
histamine, 5-hydroxytryptamine and lipid mediators such as the sulphidoleukotrienes. In
addition to IgE-dependent events, certain allergic diseases such as asthma are
characterised by IgE-independent events.

Allergic IgE-mediated diseases are currently treated with agents which provide
30 symptomatic relief or prevention. Examples of such agents are anti-histamines, β 2
agonists, and glucocorticosteroids. In addition, some IgE-mediated diseases are treated
by desensitisation procedures that involve the periodic injection of allergen

components or extracts. Desensitisation treatments may induce an IgG response that competes with IgE for allergen, or they may induce specific suppressor T cells that block the synthesis of IgE directed against allergen. This form of treatment is not always effective and poses the risk of provoking serious side effects, particularly
5 general anaphylactic shock. This can be fatal unless recognised immediately and treated with adrenaline. A therapeutic treatment that would decrease or eliminate the unwanted allergic-immune response to a particular allergen, without altering the immune reactivity to other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

10 Pollen allergens are recognised as a major cause of allergic diseases in humans and animals, including asthma, allergic rhinitis and allergic dermatitis. At least 10% of the population of the USA suffers from pollen allergies at various times and to varying extents. Proteins present in tree pollen, in particular from trees of the order Fagales, for example birch, alder, hazel, hornbeam and oak, are particularly important. Of these
15 species, birch pollen allergens are the most frequent initiators of allergic responses to tree pollen (Jarolim *et al*: Allergy 1989; 44(6):385-95). For example, approximately 25% of hayfever sufferers are responsive to birch pollen. Hayfever is the common term for a form of seasonal allergy characterised by sneezing, runny nose and itching eyes. Allergy to tree pollen is most problematic during the spring months, with the birch
20 pollen season typically occurring around April (in the northern hemisphere). However, some related types of tree such as alder and hazel can release airborne pollen as early as January (northern hemisphere). These are followed by elm, willow and ash in March, with oak in late April and early May.

It has been calculated that for adults in the United States, hayfever is the 5th
25 leading chronic disease and a major cause of work absenteeism, resulting in nearly 4 million missed or lost workdays each year, resulting in a total cost of more than \$700 million in total lost productivity. Allergies are also the most frequently reported chronic condition in children, limiting activities for more than 40% of them. Each year, allergies account for more than 17 million outpatient office visits in the United
30 States; seasonal allergies such as hayfever account for more than half of these allergy visits.

A therapeutic or preventative treatment would therefore be of great benefit to

humans that suffer or are at risk of suffering from tree allergy.

Summary of the Invention

The present inventors have discovered that certain polypeptide fragments derived
5 from the major allergens in the pollens of birch species are useful in desensitising
individuals to these allergens. Polypeptide fragments derived from Bet v2, Bet v1, Bet v3,
Bet v4, Bet v6 and Bet v7 of birch (family name: *Betulaceae*) are particularly useful.

The polypeptides of the invention were selected as MHC class II-binding T cell
epitopes through use of *in silico* analysis to predict peptide-MHC interactions and MHC
10 class II binding assays. Additional epitopes were identified by homology.

A difficulty associated with approaches to desensitisation based on peptide
immunisation lies in how to select an appropriate size and region of the allergen as the
basis for the polypeptide to be used for immunisation. The size of the polypeptide of
choice is crucial. If the polypeptide is too small, the vaccine would not be effective in
15 inducing an immunological response. If the polypeptides are too large, or if the whole
antigen is introduced into an individual, there is the risk of inducing adverse reactions,
such as anaphylaxis, which may be fatal.

The polypeptides of the invention have been selected to retain T cell specificity
whilst being small enough in size to not possess significant tertiary structure that would
20 enable them to retain the conformation of an IgE-binding epitope of the whole molecule.
The polypeptides of the invention therefore do not induce significant crosslinking of
adjacent specific IgE molecules on cells such as mast cells and basophils and consequently
do not cause significant histamine release.

An advantage of the invention is the ability of the polypeptides to broadly target
25 Major Histocompatibility Complex (MHC) molecules. T cell receptors (TCRs) are highly
variable in their specificity. Variability is generated, as with antibody molecules, through
gene recombination events within the cell. TCRs recognise antigen in the form of short
peptides bound to molecules encoded by the genes of the Major Histocompatibility
Complex (MHC). These gene products are the same molecules that give rise to "tissue
30 types" used in transplantation and are also referred to as Human Leukocyte Antigen
molecules (HLAs) which terms may be used interchangeably. Individual MHC molecules
possess peptide binding grooves which, due to their shape

and charge are only capable of binding a limited group of polypeptides. The polypeptides bound by one MHC molecule may not necessarily be bound by other MHC molecules.

When a protein molecule such as an antigen or allergen is taken up by antigen presenting cells such as B lymphocytes, dendritic cells, monocytes and macrophages, the molecule is enzymatically degraded within the cell. The process of degradation gives rise to polypeptide fragments of the molecule which, if they are of the appropriate size, charge and shape, may then bind within the peptide binding groove of certain MHC molecules and be subsequently displayed upon the surface of antigen presenting cells. If the polypeptide/MHC complexes are present upon the antigen presenting cell surface in sufficient numbers they may then activate T cells which bear the appropriate polypeptide/MHC-specific T cell receptors.

Due to the polymorphic nature of the MHC, individuals in an outbred population such as man will express different combinations of MHC molecules on their cell surfaces. Since different MHC molecules can bind different polypeptides from the same molecule based on the size, charge and shape of the polypeptide, different individuals will display a different repertoire of polypeptides bound to their MHC molecules. Identification of universal MHC-binding polypeptide epitopes in an outbred population such as man is more difficult than in inbred animals (such as certain strains of laboratory mice). On the basis of differential MHC expression between individuals and the inherent differences in polypeptide binding and presentation which this brings, it is unlikely that a single polypeptide can be identified which will be of use for desensitisation therapy in man.

The polypeptides of the invention, however, provide a broad coverage of efficacy over the human population by targeting multiple different MHC molecules. A vaccine formulated with a polypeptide of the invention would therefore have broad utility. Accordingly, the present invention provides a composition suitable for use in preventing or treating allergy to birch pollen by tolerisation comprising:

- i) the polypeptide of SEQ ID NO: 74 (BIR12B; AKYMVIQGEPRVIRGK), or a variant thereof, and
- ii) the polypeptide of SEQ ID NO: 53 (Bir02J; PAARMFKAFILEGDKLVPK), or a variant thereof,

wherein a variant of a polypeptide is:

- I) up to 30 amino acids in length and comprises the sequence of said polypeptide, or
- II) 9 to 30 amino acids in length and comprises a sequence that has at least 65% homology to the sequence of said polypeptide and that is capable of tolerising to said polypeptide, or
- III) 9 to 30 amino acids in length and comprises a sequence of at least 9 contiguous amino acids of the sequence of said polypeptide, or a sequence that has at least 65% homology to said at least 9 contiguous amino acids, which sequence of at least 9 contiguous amino acids or homologous sequence is capable of tolerising to said polypeptide.

Also provided is

- a composition suitable for use in preventing or treating allergy to birch pollen comprising at least one polynucleotide sequence which encodes each of:
 - i) the polypeptide of SEQ ID NO: 74 (BIR12B; AKYMVIQGEPRVIRGK), or a variant thereof as defined above, and
 - ii) the polypeptide of SEQ ID NO: 53 (Bir02J; PAARMFKAFILEGDKLVPK), or a variant thereof as defined above;
- a composition as defined above for use in a method of preventing or treating allergy to birch pollen; and
- an *in vitro* method of determining whether T cells recognize a composition as defined above comprising contacting said T cells with said composition and detecting whether said T cells are stimulated by said composition.

Description of the sequences mentioned herein

SEQ ID NOS: 1 to 80 provide the polypeptide sequences of the invention as set out in Tables 1 to 8. SEQ ID NOS: 1 to 34 and 45 to 70 correspond to peptides derived from Bet v1.

- 5 SEQ ID NOS: 71 to 76 correspond to peptides derived from Bet v2. SEQ ID NOS: 35, 36 and 77 correspond to peptides derived from Bet v3. SEQ ID NOS: 37 to 39, 78 and 79 correspond to peptides derived from Bet v4. SEQ ID NOS: 40 to 43 and 80 correspond to peptides derived from Bet v6. SEQ ID NO: 44 corresponds to a peptide derived from Bet v7.

10 **Detailed description of the invention**

- The invention concerns polypeptides which can be used in tolerisation. Such polypeptides may comprise, consist of, or consist essentially of the sequences shown in any of SEQ ID NOS: 1 to 80. Variants of these specific polypeptides may also be used. The variants
- 15 may comprise, consist of, or consist essentially of sequences which are fragments of either any of SEQ ID NOS: 1 to 80 or homologues of any of SEQ ID NOS: 1 to 80.

The invention also concerns comprising

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polypeptides or polynucleotides of the invention. Such compositions are suitable for use in preventing or treating birch allergy by tolerisation. Such tolerisation will typically be to an epitope (for example a MHC class II-binding T cell epitope) present in any of SEQ ID NOS: 1 to 80.

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Tree species

Species of tree from the family *Betulaceae*, commonly known as birch, are responsible for a high proportion of tree allergy worldwide, particularly allergies associated with tree pollen, such as hayfever. Other important tree species include
10 alder, hazel, hornbeam and oak.

Birch trees, for example Silver Birch (*Betula pendula*), tolerate a wide range of habitats, with soil pH from approximately 3.5 to approximately 7. They are native to most of Europe and parts of Asia, but are common throughout the world, being found in the temperate, boreal, and arctic zones of the Northern Hemisphere, especially in
15 Canada and other parts of North America. Birch trees typically flower between April and May (Northern Hemisphere).

Peptide fragments of birch pollen allergens

The present inventors have identified the regions in certain birch pollen
20 allergen proteins which comprise MHC Class II-binding T cell epitopes. The present inventors have also shown that regions corresponding to MHC Class II-binding T cell epitopes within the major birch pollen allergens are highly conserved between different isoforms of said allergens. Based on this information, polypeptides derived from the relevant regions of each protein are suitable for preventing or treating birch allergy by
25 tolerisation to all isoforms of that protein.

The polypeptides are derived directly or by homology from the protein allergens Bet v2 (SEQ ID NOS: 71 to 76), Bet v1 (SEQ ID NOS: 1 to 34 and 45 to 70), Bet v3 (SEQ ID NOS: 35, 36 and 77), Bet v4 (SEQ ID NOS: 37 to 39, 78 and 79), Bet v6 (SEQ ID NOS: 40 to 43 and 80) and Bet v7 (SEQ ID NO: 44). The terms “peptide”
30 and “polypeptide” are used interchangeably herein. The above proteins are also referred to herein as “the allergens”. Tables 1 to 7 set out the sequences of the peptides of the invention (SEQ ID NOS: 1 to 80), indicating the

parent protein from which each peptide derives. The composition of the invention comprises at least one polypeptide selected from SEQ ID NOS: 1 to 80 or a variant of any thereof.

Disclosed herein is a composition for use in the prevention or treatment of birch
 5 allergy by tolerisation which may comprise at least two, at least three, preferably at least four different polypeptides selected from any of SEQ ID NOS: 1 to 80, or a variant of any thereof. It is preferred that none of the selected polypeptides are variants of the same original sequence defined by any one of SEQ ID NOS: 1 to 80. In other words, it is preferred that each of the three or four polypeptides are different original baseline
 10 sequences defined by any one of SEQ ID NOS: 1 to 80, or are variants of different original baseline sequences defined by any one of SEQ ID NOS: 1 to 80.

Preferably, the composition will comprise polypeptides which derive from more than one allergen. For example, the composition may comprise one or more polypeptides or variants thereof derived from Bet v 2 and one or more polypeptides or variants thereof
 15 derived from Bet v 1. Additional polypeptides may optionally be included which derive from Bet v 3, Bet v 4, Bet v 6 and/or Bet v 7. Accordingly, one disclosed composition comprises

- i) at least one of the polypeptides of SEQ ID NO: 74, 72, 71, 73, 75 and 76 (which are derived from Bet v 2), or a variant of any thereof as defined herein; and
- 20 ii) at least one of the polypeptides of SEQ ID NOS: 1 to 34 and 45 to 70 (which are derived from Bet v 1), or a variant of any thereof; and optionally
- iii) at least one of the polypeptides of:
 - (a) SEQ ID NOS: 35, 36 and 77 (which are derived from Bet v 3), or a variant of any thereof as defined herein; and/or
 - 25 (b) SEQ ID NOS: 37 to 39, 78 and 79 (which derived from Bet v 4), or a variant of any thereof as defined herein; and/or
 - (c) SEQ ID NO:s 40 to 43 and 80 (which are derived from Bet v 6), or a variant of any thereof as defined herein; and/or
 - (d) SEQ ID NO: 44 (which is derived from Bet v 7), or a variant thereof as defined
 - 30 herein.

The composition may thus comprise any combination of one or more polypeptides selected from group (i), one or more polypeptides selected from group (ii)

and optionally one or more polypeptides from group (iii) (a) to (d) as defined above. Groups (i), (ii) and (iii) (a) to (d) correspond to peptides derived from different Bet allergens, as described above. Combining polypeptides derived from different Bet allergens may allow for broad coverage of birch pollen allergy observed in the general
 5 population by providing tolerising epitopes from more than one birch pollen allergen.

Non-limiting examples of compositions selected as defined above include:

One, two or more polypeptides selected from SEQ ID NO: 74, 72, 71, 73, 75 and 76 or variants of any thereof, at least one polypeptide selected from group (ii) or variant of any thereof, and optionally at least one polypeptide or variant thereof selected from groups (iii)
 10 (a) and/or (b); or

One, two or more polypeptides selected from SEQ ID NO: 74, 72, 71, 73, 75 and 76 or variants of any thereof and two, three, four or five polypeptides selected from group (ii) or variants of any thereof, and optionally at least one polypeptide or variant thereof selected from groups (iii) (a) and/or (b); or

15 One, two or more polypeptides selected from SEQ ID NO: 74, 72, 71, 73, 75 and 76 or variants of any thereof and two, three, four or five polypeptides selected from group (ii) or variants of any thereof, and at least one polypeptide from group (iii) b).

One such composition comprises:

(i) at least one of the polypeptides of SEQ ID NO: 74 (BIR12B; AKYMVIQGEPGRVIRGK), SEQ ID NO: 72 (BIR11; FPQFKPQEITGIMK), SEQ ID NO: 71 (BIR10; GSVWAQSSSFPQFK), SEQ ID NO: 73 (BIR12A; PTGMFVAGAKYMVIQGR), SEQ ID NO: 75 (BIR13; IKYMVIQGEAGAVIRGK and SEQ ID NO: 76 (BIR14; EAGAVIRGKKGSGGIT), or a variant of any thereof, and
 20 ii) at least one of the polypeptides of SEQ ID NO: 53 (Bir02J; PAARMFKAFILEGDKLVPK), SEQ ID NO: 48 (Bir01I; FNYETETTSVIPAAARK), SEQ ID NO: 54 (Bir04; PGTIKKISFPEGFPFKYV), SEQ ID NO: 67 (Bir09; ETLLRAVESYLLAHSDAY), SEQ ID NO: 60 (BIR07; SNEIKIVATPDGGSILK), and SEQ ID NO: 63 (Bir07C; SNEIKIVATPEGGSILK), or a variant of any thereof.

Another composition further comprise at least one additional polypeptide of (i) or (ii) or
 30 variant thereof not selected above. Another composition further comprises at least one additional polypeptide of SEQ ID NO:

77 (BIR15; SLNTRLRLRRIFDLFDK) or SEQ ID NO: 78 (BIR16A; AERERIFKRFDANGEGK), or a variant of any thereof. A preferred composition comprises:

- (a) the polypeptide Bir12B (AKYMVIQGEPRVIRGK), or a variant thereof;
 - 5 (b) the polypeptide Bir02J (PAARMFKAFILEGDKLVPK), or a variant thereof;
- and

- (c) the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof;

A particularly preferred composition comprises the polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir09 (ETLLRAVESYLLAHS DAY) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, and the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof, and optionally no further polypeptides.

A further particularly preferred composition comprises the polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof, and the polypeptide Bir09B (KEMGETLLRAVESYLLAHS) or a variant thereof, and optionally no further polypeptides.

A further particularly preferred composition comprises the polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, and the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof, and optionally no further

polypeptides.

Variants of the polypeptides of SEQ ID NOS: 1 to 80 are mentioned herein. A variant of any of SEQ ID NOS: 1 to 80 will typically be functional. By functional it is meant that the variant is one which:

- (a) comprises or consists of a sequence which binds to the same MHC class II molecule as the corresponding polypeptide of SEQ ID NOS: 1 to 80; and/or
- (b) comprises or consists of a sequence which is recognised by a T cell which recognises the corresponding polypeptide of SEQ ID NOS: 1 to 80; and/or
- (c) is capable of inducing a late phase response in an individual with birch allergy; and/or
- (d) is capable of tolerising an individual to the corresponding polypeptide.

Recognition by a T cell may be tested by measuring the ability of a peptide or variant to induce T cell proliferation in a sample of T cells. The induction of a late phase response may also be tested in this way when the sample of T cells is taken from an individual with birch allergy. Methods of testing the induction of T cell proliferation are well known in the art and one such method is exemplified in Example 8.

Variants of SEQ ID NOS: 1 to 80 may be fragments derived by truncation, e.g.

by removal of one or more amino acids from the N and/or C-terminal ends of a polypeptide. Fragments may also be generated by one or more internal deletions, provided that the core 9 amino acids that makes up the T cell epitope is not substantially disrupted.

5 For example, a variant of SEQ ID NO: 1 may comprise a fragment of SEQ ID NO: 1, i.e. a shorter sequence. This may include a deletion of one, two, three or four amino acids from the N-terminal end of SEQ ID NO: 1 or from the C-terminal end of SEQ ID NO: 1. Such deletions may be made from both ends of SEQ ID NO: 1.

 A variant of SEQ ID NO: 1 may include additional amino acids (for example
10 from the sequence of the parent protein from which the peptide derives) extending beyond the end(s) of SEQ ID NO: 1. A variant of a polypeptide may typically be a longer polypeptide of up to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acids in length which comprises the sequence of the corresponding polypeptide of SEQ ID NOS: 1 to 80.

15 A variant may include a combination of the deletions and additions discussed above. For example, amino acids may be deleted from one end of SEQ ID NO: 1, but additional amino acids from the full length parent protein sequence may be added at the other end of SEQ ID NO: 1. The same discussion of variants above also applies to SEQ ID NOS: 2 to 80.

20 A variant may alternatively be a polypeptide of 9 to 30, 11 to 20 or 13 to 17 amino acids in length which comprises a sequence that has at least 65% sequence identity to the sequence of the corresponding polypeptide of SEQ ID NOS: 1 to 80. More preferably a suitable variant may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% amino acid identity to the
25 corresponding polypeptide of SEQ ID NOS: 1 to 80.

 A variant may be a polypeptide of length 9 to 30, 11 to 20 or 13 to 17 amino acids which comprises a sequence of, or a sequence that has, at least 65% sequence identity to at least 9 (for example at least 10, 11, 12 or 13) or more contiguous amino acids of the sequence of the corresponding polypeptide of SEQ ID NOS: 1 to 80. These
30 contiguous amino acids may typically comprise a MHC class II epitope, for example which binds to any of the MHC molecules mentioned herein.

 A variant may include one or more amino acid substitutions from the

amino acid sequence of any of SEQ ID NOS: 1 to 80 or a fragment thereof. A variant may comprise sequence having at least 65% sequence identity to at least 9 or more contiguous amino acids in any of SEQ ID NOS: 1 to 80. More preferably a suitable variant may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 5 90%, at least 95%, or at least 98% amino acid identity to at least 9 contiguous amino acids of any of SEQ ID NOS: 1 to 80. This level of amino acid identity may be seen at any section of the peptide, although it is preferably the core region. The level of amino acid identity is over at least 9 contiguous amino acids but it may be at least 10, 11, 12, 13, 14, 15 or at least 16 or 17 amino acids, depending on the size of the peptides of 10 comparison. Accordingly, any of the above-specified levels of identity may be across the entire length of sequence.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson et al., 1994, supra) with the following parameters:

- 15 Pairwise alignment parameters -Method: accurate, Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10; Multiple alignment parameters -Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: GPSNDQEKR.
- 20 Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

A variant may comprise 1, 2, 3, 4, 5 or more, or up to 10 amino acid substitutions from any of SEQ ID NOS: 1 to 80. Substitution variants preferably involve the replacement of one or more amino acids with the same number of amino 25 acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic 30 amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

Ala	aliphatic, hydrophobic, neutral	Met	hydrophobic, neutral
Cys	polar, hydrophobic, neutral	Asn	polar, hydrophilic, neutral
Asp	polar, hydrophilic, charged (-)	Pro	hydrophobic, neutral
Glu	polar, hydrophilic, charged (-)	Gln	polar, hydrophilic, neutral
Phe	aromatic, hydrophobic, neutral	Arg	polar, hydrophilic, charged (+)
Gly	aliphatic, neutral	Ser	polar, hydrophilic, neutral
His	aromatic, polar, hydrophilic, charged (+)	Thr	polar, hydrophilic, neutral
Ile	aliphatic, hydrophobic, neutral	Val	aliphatic, hydrophobic, neutral
Lys	polar, hydrophilic, charged(+)	Trp	aromatic, hydrophobic, neutral
Leu	aliphatic, hydrophobic, neutral	Tyr	aromatic, polar, hydrophobic

Further variants include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof.

Amino acids used in the sequences may also be modified, e.g. labelled, providing the function of the peptide is not significantly adversely affected.

Where the variant has a sequence that varies from the sequence of any of SEQ ID NOS: 1 to 80 or a fragment thereof, the substitutions may occur across the full length of the sequence, within the sequence of any of SEQ ID NOS: 1 to 80 or outside the sequence of any of SEQ ID NOS: 1 to 80. For example, the variations described herein, such as additions, deletions, substitutions and modifications, may occur within the sequence of any of SEQ ID NOS: 1 to 80. A variant may comprise or consist essentially of the amino acid sequence of any of SEQ ID NOS: 1 to 80 in which one, two, three, four or more amino acid substitutions have been made. A variant may comprise a fragment of the parent protein that is larger than any of SEQ ID NOS: 1 to 80. In this embodiment, the variations described herein, such as substitutions and modifications, may occur within and/or outside the sequence of any of SEQ ID NOS: 1 to 80. For example, one or more positively charged residues may be added at the N and/or C terminus of the native sequence of the peptide of any of SEQ ID NOS: 1 to 80.

The variants of the invention are 9 to 30 amino acids in length

inclusive. Preferably, they may be from 9 to 20 or more preferably 13 to 17 amino acids in length. The variants may be the same length as the polypeptide sequences in any one of SEQ ID NOS: 1 to 80.

Polypeptides may be chemically derived from a polypeptide allergen, for example
 5 by proteolytic cleavage or can be derived in an intellectual sense from the polypeptide allergen, for example by making use of the amino acid sequence of the polypeptide allergen and synthesising peptides based on the sequence. Peptides may be synthesised using methods well known in the art.

The term "peptide" includes not only molecules in which amino acid residues are
 10 joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al* (1997) J. Immunol.159, 3230-3237. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Meziere *et al* (1997) show that, at least
 15 for MHC class II and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

Similarly, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the carbon atoms of the
 20 amino acid residues is used; it is particularly preferred if the linker moiety has substantially the same charge distribution and substantially the same planarity as a peptide bond. It will also be appreciated that the peptide may conveniently be blocked at its N-or C-terminus so as to help reduce susceptibility to exoproteolytic digestion. For example, the N-terminal amino group of the peptides may be protected by reacting with a carboxylic
 25 acid and the C-terminal carboxyl group of the peptide may be protected by reacting with an amine. Other examples of modifications include glycosylation and phosphorylation. Another potential modification is that hydrogens on the side chain amines of R or K may be replaced with methylene groups (-NH₂ → -NH(Me) or -N(Me)₂).

Analogues of polypeptides may also include polypeptide variants that increase or
 30 decrease the polypeptide's half-life *in vivo*. Examples of analogues capable of increasing the half-life of polypeptides include

peptoid analogues of the peptides, D-amino acid derivatives of the peptides, and peptide-peptoid hybrids. A further embodiment of the variants disclosed herein comprises D-amino acid forms of the polypeptide. The preparation of polypeptides using D-amino acids rather than L-amino acids greatly decreases any unwanted
 5 breakdown of such an agent by normal metabolic processes, decreasing the amounts of agent which needs to be administered, along with the frequency of its administration.

Polypeptides may be derived from splice variants of the parent proteins encoded by mRNA generated by alternative splicing of the primary transcripts encoding the parent protein chains. Polypeptides may also be derived from amino acid
 10 mutants, glycosylation variants and other covalent derivatives of the parent proteins which retain at least an MHC-binding property of the allergens. Exemplary derivatives include molecules wherein the polypeptides are covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid. Further included are naturally occurring variants of the parent
 15 proteins found in different mites. Such a variant may be encoded by an allelic variant or represent an alternative splicing variant.

Variants as described above may be prepared during synthesis of the peptide or by post- production modification, or when the peptide is in recombinant form using the known techniques of site- directed mutagenesis, random mutagenesis, or enzymatic
 20 cleavage and/or ligation of nucleic acids.

Typical examples of variants as described herein may be as follows:

- a variant of Bir01I is Bir01F (FNYETEATSVIPAARK), Bir01G (FNYEIEATSVIPAARK) or Bir01H (FNYEIETTSVIPAAARK); and/or
- a variant of Bir02J is Bir02E (PAARLFKAFILEGDTLIPK), Bir02G (PAARLFKAFILEGDNLIPK), Bir02I (PAARMFKAFILD) or Bir02D (PAARMFKAFILDGDKLVPK); and/or
- a variant of Bir09 is selected from Bir09A (GETLLRAVESYLLAHS), Bir09B (KEMGETLLRAVESYLLAHS) or Bir09C (KEKGETLLRAVESYLLAHS); and/or
- a variant of Bir16B is Bir16A (AERERIFKRFDANGEGK).

It will be understood that SEQ ID NOS: 1 to 80 are polypeptide sequences which comprise a T cell epitope that consists of a core of typically 9 amino acids, which are the minimal essential sequence required for MHC class II binding. However, the polypeptides of SEQ ID NOS: 1 to 80 may also comprise additional
 5 residues flanking the core. The peptides may therefore comprise a region containing a T cell epitope, in which some residues may be modified without affecting the function of the epitope. Thus, for example, the sequences of any of SEQ ID NOS: 1 to 80 may be altered to improve their solubility, and accordingly a variant of any of SEQ ID NOS: 1 to 80 will preferably be more soluble than the corresponding polypeptide of SEQ ID
 10 NOS: 1 to 80 under equivalent conditions. Methods for evaluating the solubility of peptides are well known in the art and one such method is exemplified in Example 9.

Improved solubility is advantageous for the tolerisation of subjects to allergens from which the peptides derive, since administration of poorly soluble agents to subjects causes undesirable, non-tolerising inflammatory responses. The solubility of
 15 the peptides may be improved by altering the residues which flank the region containing a T cell epitope. A polypeptide may be engineered to be more soluble such that it comprises:

- i) N terminal to the residues of the polypeptide which flank a T cell epitope: one to six contiguous amino acids corresponding to the one to six contiguous amino acids
 20 immediately N terminal to said residues in the sequence of the protein from which the polypeptide derives; and/or
- ii) C terminal to the residues of the polypeptide which flank a T cell epitope: one to six contiguous amino acids corresponding to the one to six contiguous amino acids immediately C terminal to the said residues in the sequence of the protein from
 25 which the polypeptide derives; or
- iii) both N and C terminal to the residues of the polypeptide which flank a T cell epitope, at least one amino acid selected from arginine, lysine, histidine, glutamate and aspartate.

Optionally, the polypeptides may additionally be engineered to be more soluble
 30 such that:

- i) any cysteine residues in the native sequence of the polypeptide are replaced with serine

or 2-aminobutyric acid; and /or

ii) any hydrophobic residues in the upto three amino acids at the N or C terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

5 iii) any two consecutive amino acids comprising the sequence Asp-Gly in the upto four amino acids at the N or C terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

iv) one or more positively charged residues are added at the N and/or C terminus of the native sequence of the polypeptide.

10 Preferably the polypeptides and variants of the invention are capable of causing T cell proliferation in at least 20 % of samples of T cells, wherein each sample is obtained from different birch allergic individuals in the population. The compositions of the invention are preferably capable of inducing T cell proliferation in 30 % or more samples of T cells obtained from of a panel of birch allergic individuals. More preferably, the
15 compositions are capable of inducing T cell proliferation in 35% or more, 40 % or more, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, or 90 % or more of samples obtained from sensitized individuals in a panel. The number of individuals in a panel of birch allergic individuals may be any number greater than one, for example at least 2, 3, 5, 10, 15, 20, 30, 50, 80, or at least 100 individuals.

20 It is preferred if the polypeptides, variants and compositions of the invention cause T cell proliferation, but do not lead to the release of histamine from enriched basophils or mast cell preparations from a sensitised individual. There may be some histamine release, but preferably the peptides, variants and compositions do not cause significant amounts of histamine to be released. Significant histamine release may be considered to be the
25 release of 20% or more of the total available leukocyte histamine when a sample of leukocytes from an individual is stimulated with a composition *in vitro*. A peptide, variant or composition of the invention preferably causes the release of less than 5%, less than 4%, less than 3%, less than 2% or less than 1% of the total available leukocyte histamine when a sample of leukocytes from an individual is stimulated with a composition *in vitro*.
30 A normal individual typically has an approximate leukocyte histamine content of 150ng/10⁷ cells.

Suitable peptides or variants capable of binding to TCRs may be derived

empirically or selected according to known criteria. Within a single polypeptide there are certain residues which contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable regions of the T cell receptor (Allen et al (1987) Nature 327: 713-5).

5 Within the residues contributing to T cell receptor interaction, a hierarchy has been demonstrated which pertains to dependency of T cell activation upon substitution of a given peptide residue. Using polypeptides which have had one or more T cell receptor contact residues substituted with a different amino acid, several groups have demonstrated profound effects upon the process of T cell activation. Evavold & Allen
10 (1991) Nature 252: 1308-10) demonstrated the dissociation of T cell proliferation and cytokine production. In this *in vitro* model, a T cell clone specific for residues 64-76 of haemoglobin (in the context of I-E^k), was challenged with a peptide analogue in which a conservative substitution of aspartic acid for glutamic acid had been made. This substitution did not significantly interfere with the capacity of the analogue to bind to
15 I-E^k.

Following *in vitro* challenge of a T cell clone with this analogue, no proliferation was detected although IL-4 secretion was maintained, as was the capacity of the clone to help B cell responses. In a subsequent study the same group demonstrated the separation of T cell-mediated cytotoxicity from cytokine production. In
20 this instance, the former remained unaltered while the latter was impaired. The efficacy of altered peptide ligands *in vivo* was initially demonstrated in a murine model of EAE (experimental allergic encephalomyelitis) by McDevitt and colleagues (Smilek *et al* (1991) Proc Natl Acad Sci USA 88 : 9633-9637). In this model EAE is induced by immunisation with the encephalitogenic peptide Ac1-11 of MBP (myelin basic
25 protein). Substitution at position four (lysine) with an alanine residue generated a peptide which bound well to its restricting element (A α^u A β^u), but which was non-immunogenic in the susceptible PL/JxSJLF1 strain and which, furthermore prevented the onset of EAE when administered either before or after immunisation with the encephalitogenic peptide. Thus, residues can be identified in polypeptides which affect
30 the ability of the polypeptides to induce various functions of T-cells.

Advantageously, polypeptides may be designed to favour T-cell proliferation and induction of desensitisation. Metzler and Wraith have demonstrated improved

tolerogenic capacity of polypeptides in which substitutions increasing peptide-MHC affinity have been made (Metzler & Wraith(1993) *Int Immunol* ~ : 1159-65). That an altered polypeptide ligand can cause long-term and profound anergy in cloned T cells was demonstrated by Sloan-Lancaster *et al* (1993) *Nature* 363: 156-9.

5 The compositions disclosed herein are capable of inducing a late phase response in an individual that is sensitised to the allergens. The term "late phase response" includes the meaning as set forth in *Allergy and Allergic Diseases* (1997) A. B. Kay (Ed.), Blackwell Science, pp 1113-1130. The late phase response may be any late phase response (LPR). Preferably, the polypeptides are capable of inducing a late asthmatic
10 response (LAR) or a late rhinitic response, or a late phase skin response or a late phase ocular response. Whether or not a particular peptide can give rise to a LPR can be determined using methods well known in the art; a particularly preferred method is that described in Cromwell O, Durham SR, Shaw RJ, Mackay J and Kay AB. Provocation tests and measurements of mediators from mast cells and basophils in asthma and allergic
15 rhinitis. In: *Handbook of Experimental Immunology* (4) Chapter 127, Editor: Weir DM, Blackwell Scientific Publications, 1986.

 Thus, preferably, the individual polypeptides and variants disclosed herein are able to induce a LPR in an individual who has been sensitised to the allergens. Whether or not an individual has been sensitised to the allergens may be determined by well known
20 procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radioallergosorbent test (RAST) for measurement of allergen specific IgE. Whether or not a particular individual is expected to benefit from treatment may be determined by the physician based, for example, on such tests.

25 Desensitising or tolerising an individual to the allergens means inhibition or dampening of allergic tissue reactions induced by the allergens in appropriately sensitised individuals. It has been shown that T cells can be selectively activated, and then rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to desensitisation of the patient for a particular allergen. The desensitisation manifests itself
30 as a reduction in response to an allergen or allergen-derived polypeptide, or preferably an elimination of such a response, on second and further administrations of the allergen or allergen-derived polypeptide. The second administration may be made after

a suitable period of time has elapsed to allow desensitisation to occur; this is preferably any period between one day and several weeks. An interval of around two weeks is preferred.

Although the compositions disclosed herein are able to induce a LPR in a birch
 5 allergic individual, it should be appreciated that when a composition is used to treat a patient it is preferable that a sufficiently low concentration of the composition is used such that no observable LPR will occur but the response will be sufficient to partially desensitise the T cells such that the next (preferably higher) dose may be given, and so on. In this way the dose is built up to give full desensitisation but often without ever inducing
 10 a LPR in the patient. Although, the composition or polypeptide is able to do so at a higher concentration than is administered.

The compositions disclosed herein preferably are capable of inducing a late phase response in 50 % or more of a panel of birch allergic individuals from the population. More preferably, the compositions are capable of inducing a LPR in 55% or more, 60 % or
 15 more, 65 % or more, 70% or more, 75% or more, 80% or more, 85% or more, or 90 % or more of sensitized individuals in a panel. Whether or not the compositions are able to induce a LPR in a certain percentage of a panel of subjects can be determined by methods which are well known in the art.

20 *Nucleic acids and vectors*

The individual polypeptides that make up the compositions and products of the invention may be administered directly, or may be administered indirectly by expression from an encoding sequence. For example, a polynucleotide may be provided that encodes a polypeptide of the invention, such as any of the polypeptides described above. A
 25 polypeptide of the invention may thus be produced from or delivered in the form of a polynucleotide which encodes, and is capable of expressing, it. Any reference herein to the use, delivery or administration of a polypeptide of the invention is intended to include the indirect use, delivery or administration of such a polypeptide via expression from a polynucleotide that encodes it.

30 Accordingly, disclosed herein is a composition for use in preventing or treating allergy to birch by tolerisation comprising at least one polynucleotide sequence which when expressed causes the production of a composition according to the

invention for use in preventing or treating allergy to birch by tolerisation.

The terms “nucleic acid molecule” and “polynucleotide” are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting
5 examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide of the invention may be provided in isolated or purified form. A nucleic acid sequence which “encodes” a selected polypeptide is a nucleic acid
10 molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. For the purposes of the invention, such nucleic acid sequences can include,
15 but are not limited to, cDNA from viral, prokaryotic or eukaryotic mRNA, genomic sequences from viral or prokaryotic DNA or RNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Polynucleotides can be synthesised according to methods well known in the art, as described by way of example in Sambrook et al (1989, Molecular Cloning - a
20 laboratory manual; Cold Spring Harbor Press).

Polynucleotide molecules may be provided in the form of an expression cassette which includes control sequences operably linked to the inserted sequence, thus allowing for expression of the polypeptide *in vivo* in a targeted subject. These expression cassettes, in turn, are typically provided within vectors (e.g., plasmids or
25 recombinant viral vectors) which are suitable for use as reagents for nucleic acid immunization. Such an expression cassette may be administered directly to a host subject. Alternatively, a vector comprising a polynucleotide may be administered to a host subject. Preferably the polynucleotide is prepared and/or administered using a genetic vector. A suitable vector may be any vector which is capable of carrying a
30 sufficient amount of genetic information, and allowing expression of a polypeptide.

Expression vectors are routinely constructed in the art of molecular biology and

may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for expression of a polypeptide. Other suitable vectors would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook *et al.*

Thus, a polypeptide may be provided by delivering such a vector to a cell and allowing transcription from the vector to occur. Thus, also disclosed herein is a vector for use in preventing or treating allergy to birch pollen by tolerisation comprising four or more polynucleotide sequences which encode a different polypeptide as disclosed herein. Preferably, a polynucleotide as disclosed herein or used in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector.

“Operably linked” refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given regulatory sequence, such as a promoter, operably linked to a nucleic acid sequence is capable of effecting the expression of that sequence when the proper enzymes are present. The promoter need not be contiguous with the sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the nucleic acid sequence and the promoter sequence can still be considered “operably linked” to the coding sequence.

A number of expression systems have been described in the art, each of which typically consists of a vector containing a gene or nucleotide sequence of interest operably linked to expression control sequences. These control sequences include transcriptional promoter sequences and transcriptional start and termination sequences. The vectors of the invention may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. A “plasmid” is a vector in the form of an extrachromosomal genetic element. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of

a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell. The vectors may also be adapted to be used *in vivo*, for example to allow *in vivo* expression of the polypeptide.

5 A “promoter” is a nucleotide sequence which initiates and regulates transcription of a polypeptide-encoding polynucleotide. Promoters can include inducible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), repressible promoters (where expression of a polynucleotide sequence operably linked to the
10 promoter is repressed by an analyte, cofactor, regulatory protein, etc.), and constitutive promoters. It is intended that the term “promoter” or “control element” includes full-length promoter regions and functional (e.g., controls transcription or translation) segments of these regions.

A polynucleotide, expression cassette or vector as disclosed herein may
15 additionally comprise a signal peptide sequence. The signal peptide sequence is generally inserted in operable linkage with the promoter such that the signal peptide is expressed and facilitates secretion of a polypeptide encoded by coding sequence also in operable linkage with the promoter.

Typically a signal peptide sequence encodes a peptide of 10 to 30 amino acids
20 for example 15 to 20 amino acids. Often the amino acids are predominantly hydrophobic. In a typical situation, a signal peptide targets a growing polypeptide chain bearing the signal peptide to the endoplasmic reticulum of the expressing cell. The signal peptide is cleaved off in the endoplasmic reticulum, allowing for secretion of the polypeptide via the Golgi apparatus. Thus, a polypeptide may be provided to an
25 individual by expression from cells within the individual, and secretion from those cells.

Alternatively, polynucleotides may be expressed in a suitable manner to allow presentation of a polypeptide by an MHC class II molecule at the surface of an antigen presenting cell. For example, a polynucleotide, expression cassette or vector may be
30 targeted to antigen presenting cells, or the expression of encoded peptide may be preferentially stimulated or induced in such cells.

The polynucleotide, expression cassette or vector may encode an adjuvant, or an adjuvant may otherwise be provided. As used herein, the term "adjuvant" refers to any material or composition capable of specifically or non-specifically altering, enhancing, directing, redirecting, potentiating or initiating an antigen-specific immune response.

5 Polynucleotides of interest may be used *in vitro*, *ex vivo* or *in vivo* in the production of a peptide of the invention. Such polynucleotides may be administered or used in the prevention or treatment of allergy by tolerisation.

Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859 and 5,589,466. The nucleic acid molecule can be introduced
10 directly into the recipient subject, such as by standard intramuscular or intradermal injection; transdermal particle delivery; inhalation; topically, or by oral, intranasal or mucosal modes of administration. The molecule alternatively can be introduced *ex vivo* into cells that have been removed from a subject. For example, a polynucleotide, expression cassette or vector of the invention may be introduced into APCs of an
15 individual *ex vivo*. Cells containing the nucleic acid molecule of interest are re-introduced into the subject such that an immune response can be mounted against the peptide encoded by the nucleic acid molecule. The nucleic acid molecules used in such immunization are generally referred to herein as "nucleic acid vaccines."

The polypeptides, polynucleotides, vectors or cells disclosed herein may be
20 present in a substantially isolated form. They may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated. They may also be in a substantially purified form, in which case they will generally comprise at least 90%, e.g. at least 95%, 98% or 99%, of the proteins, polynucleotides, cells or dry mass of the preparation.

25

Antigen presenting cells (APCs)

Also disclosed herein is the use *in vitro* of a method of producing a population of APCs that present polypeptides on their surface, that may be subsequently used in therapy. Such a method may be carried out *ex vivo* on a sample of cells that have been obtained
30 from a patient. The APCs produced in this way therefore form a pharmaceutical agent that can be used in the treatment or

prevention of birch allergy by tolerisation. The cells should be accepted by the immune system of the individual because they derive from that individual. Delivery of cells that have been produced in this way to the individual from whom they were originally obtained, thus forms therapy.

5

Formulations and compositions

Polypeptides, polynucleotides, vectors and cells may be provided to an individual either singly or in combination. Each molecule or cell may be provided to an individual in an isolated, substantially isolated, purified or substantially purified
 10 form. For example, a polypeptide may be provided to an individual substantially free from the other polypeptides. Alternatively, four or more polypeptides in the composition may be coupled chemically together, using standard peptide coupling reagents, to provide a single polypeptide containing the preferred epitopes. Such polypeptides would be screened for basophil histamine release to confirm lack of
 15 histamine release as per the individual peptides. In a further embodiment, four or more polypeptides in the composition may be provided as part of a single polypeptide chain i.e by recombinant means from an encoding polynucleotide. The four or more polypeptides may be fused contiguously, or may alternatively be separated by appropriate linkers.

20 Whilst it may be possible for the polypeptides, polynucleotides or compositions to be presented in raw form, it is preferable to present them as a pharmaceutical formulation. Thus, the present invention also provides a pharmaceutical composition for use in preventing or treating allergy to birch by tolerisation comprising a composition according to the invention together with one or more pharmaceutically
 25 acceptable carriers or diluents and optionally one or more other therapeutic ingredients. The carrier (s) must be 'acceptable' in the sense of being compatible with the other ingredients and not deleterious to the recipient thereof. Typically, carriers for injection, and the final formulation, are sterile and pyrogen free. Preferably, the carrier or diluent is thioglycerol or thioanisole.

30 Formulation of a composition can be carried out using standard pharmaceutical formulation

chemistries and methodologies all of which are readily available to the reasonably skilled artisan.

For example, compositions containing one or more molecules or cells can be combined with one or more pharmaceutically acceptable excipients or vehicles.

- 5 Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances and the like, may be present in the excipient or vehicle. These excipients, vehicles and auxiliary substances are generally pharmaceutical agents that do not induce an immune response in the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not
- 10 limited to, liquids such as water, saline, polyethyleneglycol, hyaluronic acid, glycerol, thioglycerol and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of
- 15 pharmaceutically acceptable excipients, vehicles and auxiliary substances is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

- Such compositions may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable compositions may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose
- 20 containers containing a preservative. Compositions include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such compositions may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a composition for parenteral
- 25 administration, the active ingredient is provided in dry (for e.g., a powder or granules) form for reconstitution with a suitable vehicle (e. g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated
- 30 according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a

non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono-or di-glycerides.

- 5 Other parentally-administrable compositions which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble
10 polymer, or a sparingly soluble salt.

Alternatively, the polypeptides or polynucleotides may be encapsulated, adsorbed to, or associated with, particulate carriers. Suitable particulate carriers include those derived from polymethyl methacrylate polymers, as well as PLG microparticles derived from poly(lactides) and poly(lactide-co-glycolides). See, e.g.,
15 Jeffery et al. (1993) Pharm. Res. 10:362-368. Other particulate systems and polymers can also be used, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules.

The formulation of any of the polypeptides, polynucleotides or cells mentioned herein will depend upon factors such as the nature of the substance and the method of
20 delivery. Any such substance may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), topically, parenterally, subcutaneously, by inhalation, intravenously, intramuscularly, intrasternally, transdermally, intradermally, sublingually, intranasally, buccally or by infusion techniques. The substance may also
25 be administered as suppositories. A physician will be able to determine the required route of administration for each particular individual.

A composition may comprise a suitable concentration of each polypeptide/polynucleotide/cell to be effective without causing adverse reaction. Typically, the concentration of each polypeptide in the composition will be in the range
30 of 0.03 to 200 nmol/ml. More preferably in the range of 0.3 to 200 nmol/ml, 3 to 180 nmol/ml, 10 to 150 nmol/ml, 50 to 200nmol/ml or 30 to 120

nmol/ml. The composition or formulations should have a purity of greater than 95% or 98% or a purity of at least 99%.

An adjuvant may be used in combination with the polypeptide/polynucleotides/cells. The adjuvant is preferably administered in an amount which is sufficient to augment the effect of the polypeptide/polynucleotides/cells or vice versa. The adjuvant or other therapeutic agent may be an agent that potentiates the effects of the molecule or cell. For example, the other agent may be an immunomodulatory molecule or an adjuvant which enhances the response to the peptide or cell.

The polypeptides, polynucleotides, cells or compositions disclosed herein may be used for therapy in combination with one or more other therapeutic agents. The agents may be administered separately, simultaneously or sequentially. They may be administered in the same or different compositions. Accordingly, in a method disclosed herein, the subject may also be treated with a further therapeutic agent.

A composition may therefore be formulated which comprises a molecule and/or cell and also one or more other therapeutic molecules. A composition of the invention may alternatively be used simultaneously, sequentially or separately with one or more other therapeutic compositions as part of a combined treatment.

Non-limiting examples of adjuvants include vitamin D, rapamycin and glucocorticoid steroids such as dexamethasone, fluticasone, budesonide, mometasone, beclomethasone, hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone, betamethasone and triamcinolone. A preferred glucocorticoid is dexamethasone.

Therapeutic methods and individual to be treated

The present invention relates to polypeptides, polynucleotides, vectors and cells that are capable of desensitising or tolerising human individuals to the allergens described above and are therefore useful in the prevention or treatment of birch allergy. The invention provides compositions for use in preventing or treating allergy to birch by tolerisation. The invention also provides a method of tolerising or desensitizing a birch allergic individual comprising

administering, either singly or in combination the polypeptides/polynucleotides/cells as described above.

The individual to be treated or provided with the composition of the invention is preferably human. It will be appreciated that the individual to be treated may be known to be sensitised to the allergens, at risk of being sensitised or suspected of being sensitised. The individual can be tested for sensitisation using techniques well known in the art and as described herein. Alternatively, the individual may have a family history of allergy to birch. It may not be necessary to test an individual for sensitisation to birch because the individual may display symptoms of allergy when exposed to birch. By exposure is meant proximity to, for example, a birch plant, or a substance or product derived from a birch plant, or a substance or product containing or comprising either of the above. The substance or product derived from a birch plant is typically birch pollen. By proximity is meant 10 metres or less, 5 metres or less, 2 metres or less, 1 metre or less, or 0 metres from the items described above. Symptoms of allergy can include itchy eyes, runny nose, breathing difficulties, red itchy skin or rash.

The individual to be treated may be of any age. However, preferably, the individual may be in the age group of 1 to 90, 5 to 60, 10 to 40, or more preferably 18 to 35.

Preferably, the individual to be treated is from a population that has MHC allele frequencies within the range of frequencies that are representative of the Caucasian population. Reference population allele frequencies for 11 common DRB1 allele families are shown in Table 1 (Data from HLA Facts Book, Parham and Barber).

Table 1

DRB1	1	3	4	7	8	11	12	13	14	15	16
%	6.4	14.7	15.7	8.8	3.4	8.3	3.9	14.7	2.9	17.6	2.5
Reference population %	9.4	11.1	12.8	13.2	3.7	13.4	2.3	10.2	3.2	10.7	3.6

Reference frequencies were obtained by analysis of multiple studies reporting frequencies and the figures shown are mean values. Preferably therefore, the individual to be treated is from a population that has equivalent MHC allele frequencies as the reference population for the alleles referred to Table 1 (such as for at least 1, 2, 3, 4, 5 or all of the alleles), for example within the ranges of those figures plus or minus 1, 2, 3, 5, 10, 15 or 20%.

Preferably the individual is from a population where the allele frequencies of the following DRB1 alleles is:

- 4 – at least 9%
- 10 7 – at least 10%
- 11 – at least 8%.

The individual may have had allergy to birch for at least 2 weeks, 1 month, 6 months, 1 year or 5 years. The individual may suffer from a rash, nasal congestion, nasal discharge and/or coughing caused by the allergy. The individual may or may not have been administered with other compositions/compounds which treat birch allergy. The individual may live in a geographical region which has:

- a temperate, boreal or arctic climate, and/or:
- a typical soil pH in the range of about 3.5, to about 7.5.

The individual typically suffers from allergy to birch pollen in a particular season. The season typically corresponds to the flowering season of birch, which is typically spring, preferably early spring (for example from April to May in the Northern hemisphere). The allergic individual is typically allergic to birch pollen from any tree in the subgenus *Betula*, for example *Betula pendula* or *Betula pubescens*.

25 *Combination immunotherapy*

Since many individuals are allergic, or may require desensitizing to several polypeptide antigens, also disclosed is a means of desensitizing individuals that are allergic to multiple antigens. “Tolerance” induced in an individual to a first polypeptide antigen or allergen can create in the individual a “tolergeneic environment” wherein inappropriate immune responses to other antigens can be downregulated in order to provide tolerance to other antigens.

This finding means that individuals allergic to multiple allergens can be treated

in a greatly reduced time period, and that individuals seriously allergic to some allergens (e.g., peanuts) but more mildly allergic to other allergens (e.g., cat dander) can benefit from a therapy wherein tolerance to the milder allergen is established and then this tolerogenic environment is used to provide tolerance to the other, more
5 extreme allergen. In addition, individuals suffering from an autoimmune disorder who are additionally sensitised (or otherwise immune) to an unrelated antigen or allergen can benefit from a treatment regime wherein tolerance to the unrelated antigen or allergen is first established and then this tolerogenic environment is used to provide tolerance to the autoantigen associated with the autoimmune disorder.

10 A method is therefore provided for desensitising a birch allergic individual to birch allergen as described above and one or more further different polypeptide antigens. The method entails, in a first step, administering to the individual a composition (primary composition) as described herein and wherein the administration is carried out in a manner sufficient to generate a hyporesponsive state against birch
15 allergen. Once a hyporesponsive state has been established toward birch allergen, or at least a shift toward desensitisation has occurred, the method entails administration of a secondary composition comprising a second, different polypeptide antigen to which the individual is to be sensitised. Administration of the secondary composition is carried out in such a way as to take advantage of the tolerogenic environment established by
20 use of the primary composition, where it is now possible to establish tolerance to the second, different polypeptide antigen. The secondary composition is coadministered with either the first primary composition or a larger fragment of the birch allergen. By “coadministered” it is meant either the simultaneous or concurrent administration, e.g., when the two are present in the same composition or administered in separate
25 compositions at nearly the same time but at different sites, as well as the delivery of polypeptide antigens in separate compositions at different times. For example, the secondary composition may be delivered prior to or subsequent to delivery of the first composition at the same or a different site. The timing between deliveries can range from about several seconds apart to about several minutes apart, several hours apart, or
30 even several days apart. Furthermore, different delivery methods can be employed.

The second polypeptide antigen is preferably an allergen different to the birch

allergen. Suitable allergens for use in the method can of course be obtained and/or produced using known methods. Classes of suitable allergens include, but are not limited to, dust mite allergens, pollens, animal dander (especially cat dander), grass allergens, molds, dusts, antibiotics, stinging insect venoms, and a variety of

5 environmental (including chemicals and metals), drug and food allergens. Common tree allergens include pollens from cottonwood, poplar, ash, birch, maple, oak, elm, hickory, and pecan trees; common plant allergens include those from mugwort, ragweed, English plantain, sorrel-dock and pigweed; plant contact allergens include those from poison oak, poison ivy and nettles; common grass allergens include rye

10 grass, Timothy, Johnson, Bermuda, fescue and bluegrass allergens; common allergens can also be obtained from molds or fungi such as *Alternaria*, *Fusarium*, *Hormodendrum*, *Aspergillus*, *Micropolyspora*, *Mucor* and thermophilic actinomycetes; epidermal allergens can be obtained from house or organic dusts (typically fungal in origin), or from animal sources such as feathers, and dog dander; common food

15 allergens include milk and cheese (dairy), egg, wheat, nut (e.g., peanut), seafood (e.g., shellfish), pea, bean and gluten allergens; common environmental allergens include metals (nickel and gold), chemicals (formaldehyde, trinitrophenol and turpentine), Latex, rubber, fiber (cotton or wool), burlap, hair dye, cosmetic, detergent and perfume allergens; common drug allergens include local anesthetic and salicylate allergens;

20 antibiotic allergens include penicillin, tetracycline and sulfonamide allergens; and common insect allergens include bee, wasp and ant venom, and cockroach calyx allergens. Particularly well characterized allergens include, but are not limited to, the major cat allergen Fel d1, bee venom phospholipase A2 (PLA) (Akdis et al. (1996) *J. Clin. Invest.* 98:1676-1683) and the multi-epitopic recombinant grass allergen

25 rKBG8.3 (Cao et al. (1997) *Immunology* 90:46-51). These and other suitable allergens are commercially available and/or can be readily prepared as extracts following known techniques.

Preferably, the second polypeptide allergen is a whole tree pollen allergen or allergen fragment selected from the list of allergen sequences and database accession

30 numbers (NCBI Entrez accession numbers) below. NCBI is the National Center for Biotechnology information and is a division of the US National Institutes of Health. The NCBI web site, from which access to the database may be sought, is

www.ncbi.nlm.nih.gov/. Allergen sequences and database accession numbers (NCBI Entrez accession numbers):

Olive tree

5 Olive sequences

416610 Ole e 1

EDIPQPPVSQFHQQVYCDTCRAGFITELSEFIPGASLRLQCKDKENGDVTFTE
VGYTRAEGLYSMLVERDHLKNEFCEITLISSGRKDCNEIPTEGWAKPSLKFKLNT
10 VNGTTRTVNPLGFFKKEALPKCAQVYNKLGMYPPNM

Tree allergen sequences (mainly birch) sequences:

15 130975 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIM
KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI
YEEPVTPGQCNMVVERLGDYLIDQGL

20 1942360 Bet v 2

MSWQTYVDEHLMCDIDGQGEELAASAIVGHDGSVWAQSSSFPQFKPQEITGIM
KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI
YEEPVTPGQCNMVVERLGDYLIDQGL

25 166953 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIM
KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI
YEEPVTPGQCNMVVERLGDYLIDQGL

30 541814 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIM
KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI

YEEPVTPGQCNMVVERLGDYLIDQGL

2488678 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIM

5 KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI
YEEPVTPGQCNMVVERLGDYLIDQGL

1829894 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIM

10 KDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGI
YEEPVTPGQCNMVVERLGDYLIDQGL

1168696 Bet v 3

15 MPCSTEAMEKAGHGHASTPRKRSLSNSSFRLRSESLNTRLRLRRIFDLFDKNSDG
IITVDELSRALNLLGLETDLSELESTVKSFTREGNIGLQFEDFISLHQSLNDSYFA
YGGEDDDNEEDMRKSILSQEEADSFSGGFKVFDEDDGDGYISARELQMVLGKL
GFSEGSEIDRVEKMIVSVDSNRDGRVDFFEFKDMMRSVLVRSS

20 809536 Bet v 4

MADDHPQDKAERERIFKRFDANGDGKISAAELGEALKTLGSITPDEVKHMMA
EIDTDGDGFISFQEFTDFGRANRGLLKDVAKIF

543675 Que a I - Quercus alba=oak trees (fragment)

25 GVFTXESQETSVIAPAXLFLKALFL

543509 Car b I - Carpinus betulus=hornbeam trees (fragment)

GVFNIEAETPSVIPAAARLFKSYVLDGDKLIPKVAPQAIXK

30 543491 Aln g I - Alnus glutinosa=alder trees (fragment)

GVFNIEAETPSVIPAAARLFKAFILDGDKLLPKVAPEAVSSVENI

1204056 Rubisco

VQCMQVWPPLGLKKFETLSYLPPLSSEQLAKEVDYLLRKNLIPCLEFELEHG
FV
YREHNRSPGYDGRYWTMWKLPMFGCNDSSQVLKELEECKKAYPSAFIRIIGF
DDK

5

Additional tree allergen sequences (NCBI entrez accession number):

131919; 128193; 585564; 1942360; 2554672; 2392209; 2414158; 1321728; 1321726;
1321724; 1321722; 1321720; 1321718; 1321716; 1321714; 1321712; 3015520;
10 2935416; 464576; 1705843; 1168701; 1168710; 1168709; 1168708; 1168707;
1168706; 1168705; 1168704; 1168703; 1168702; 1842188; 2564228; 2564226;
2564224; 2564222; 2564220; 2051993; 1813891; 1536889; 534910; 534900; 534898;
1340000; 1339998; 2149808; 66207; 2129477; 1076249; 1076247; 629480; 481805;
81443; 1361968; 1361967; 1361966; 1361965; 1361964; 1361963; 1361962;
15 1361961; 1361960; 1361959; 320546; 629483 ; 629482; 629481; 541804; 320545;
81444; 541814.; 629484; 474911; 452742; 1834387; 298737; 298736; 1584322;
1584321; 584320; 1542873; 1542871; 1542869; 1542867; 1542865; 1542863;
1542861; 1542859; 1542857; 1483232; 1483230; 1483228; 558561; 551640; 488605;
452746; 452744; 452740; 452738; 452736; 452734; 452732; 452730; 452728;
20 450885; 17938; 17927; 17925; 17921; 297538; 510951; 289331; 289329; 166953 .

Cedar sequences

493634 Cry j IB precursor

25 MDSPCLVALLVFSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADCAVGFGSST
MGGKGGDLYTVTNSDDDPVNPPGTLRYGATRDRPLWIIFSGNMNIKLKMPMY
IAGYKTFDGRGAQVYIGNGGPCVFIKRVSNVHHGLYLYGCSTSVLGNVLINESF
GVEPVHPQDGDALTLRTATNIWIDHNSFSNSSDGLVDVTLTSTGVTISNNLFFN
HHKVMSLGHDDA YSDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANN
30 YDPWTIYAIGGSSNPITLSEGNSTAPNESYKKQVTIRIGCKTSSSCSNVWVWQST
QDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENGNA TPHTQNAGVLTCSLSK
RC

493632 Cry j IA precursor

MDSPCLVALLVLSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADCAVGFSS
TMGGKGGDLYTVTNSDDDPVNPAPGTLRYGATRDRPLWIFSGNMNIKLKMP
5 MYIAGYKTFDGRGAQVYIGNGGPCVFIKRVSNVHHGLHLYGCSTSVLGNVLIN
ESFGVEPVHPQDGDALTLRTATNIWIDHNSFSNSSDGLVDVTLSSGTISNNLF
FNHHKVMLLGHDDAYSDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVN
NNYDPWTIYAIGGSSNPTILSEGNSTAPNESYKKQVTIRIGCKTSSSSCSNWW
QSTQDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENGNA TPQLTKNAGVLTCS
10 LSKRC

1076242 Cry j II precursor - Japanese cedar

MAMKLIAPMAFLAMQLIIMAAAEDQSAQIMLDSVVEKYLRNRSRLRKVEHSR
HDAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKNPSAMLLVPGSKKFVVN
15 NLFFNGPCQPHFTFKVDGILAA YQNPASWKNNRIWLQFAKLTGFTLMGKGVID
GQ GKQWWAGQCKWVNGREICNDRDRPTAIKFDSTGLIIQGLKLMNSPEFHL
VFGNCEGVKIIGISITAPRDS PNTDGIDIFASKNFHLQKNTIGTGDDCVAIGTGSS
NIVIEDLICPGHGISIGSLGRENSRAEVS YVHVNGAKFIDTQNGLRIKTWQGS
GMASHIYENVEMINSENPIINQFYCTASACQNQRSAVQIQDVTYKNIRGTSA
20 TAAAIQLKCSDSMPCKDIKLS DISLKLTS GKIASC LNDNANGYFSGHVIPACKNL
SPSAKRKESKSHKHPKTMVENMRA YDKGNRTRILLGSRPPNCTNKCHGCSP
CKAKLVIVHRIMPQEYYPQRWICSCHGKIYHP

1076241 Cry j II protein - Japanese cedar

MAMKFIAPMAFVAMQLIIMAAAEDQSAQIMLDS DIEQYLRNRSRLRKVEHSRH
DAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKKPSAMLLVPGNKKFVVN
25 NLFFNGPCQPHFTFKVDGILAA YQNPASWKNNRIWLQFAKLTGFTLMGKGVID
GQ GKQWWAGQCKWVNGREICNDRDRPTAIKFDSTGLIIQGLKLMNSPEFHL
VFGNCEGVKIIGISITAPRDS PNTDGIDIFASKNFHLQKNTIGTGDDCVAIGTGSS
30 NIVIEDLICPGHGISIGSLGRENSRAEVS YVHVNGAKFIDTQNGLRIKTWQGS
GMASHIYENVEMINSENPIINQFYCTASACQNQRSAVQIQDVTYKNIRGTSA
TAAAIQLKCSDSMPCKDIKLS DISLKLTS GKIASC LNDNANGYFSGHVIPACKNL

SPSAKRKESKSHKHPKTVMVKNMGAYDKGNRTRILLGSRPPNCTNKCHGCSP
CKAKLVIVHRIMPQEYYPQRWMCSRHGKIYHP

541803 Cry j I precursor - Japanese cedar

5 MDSPCLVALLVLSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADCAVGFGSS
TMGGKGGDLTYVTNSDDDPVNPPGTLRYGATRDRPLWIFSGNMNIKLKMPM
YIAGYKTFDGRGAQVYIGNGGPCVFIKRVSNVHHGLHLYGCSTSVLGNVNLINES
FGVEPVHPQDGDALTLRTATNIWIDHNSFSNSSDGLVDVTLSSSTGVTISNNLFF
NHHKVMMLGHDDAYSDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANN
10 NYDPWTIYAIGGSSNPTILSEGENSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQS
TQDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENGNA TPQLTKNAGVLTCSLS
KRC

541802 Cry j I precursor - Japanese cedar

15 MDSPCLVALLVFSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADCAVGFGSST
MGGKGGDLTYVTNSDDDPVNPAPGTLRYGATRDRPLWIFSGNMNIKLKMPM
YIAGYKTFDGRGAQVYIGNGGPCVFIKRVSNVHHGLYLYGCSTSVLGNVNLINES
FGVEPVHPQDGDALTLRTATNIWIDHNSFSNSSDGLVDVTLTSTGVTISNNLFF
NHHKVMSLGHDDAYSDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANN
20 NYDPWTIYAIGGSSNPTILSEGENSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQS
TQDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENGNA TPHLTQNAGVLTCSLS
KRC

Delivery methods

25 Once formulated the compositions of the invention can be delivered to a subject
in vivo using a variety of known routes and techniques. For example, a composition
can be provided as an injectable solution, suspension or emulsion and administered via
parenteral, subcutaneous, epidermal, intradermal, intramuscular, intraarterial,
intraperitoneal, intravenous injection using a conventional needle and syringe, or using
30 a liquid jet injection system, or using a patch. Compositions can also be administered
topically to skin or mucosal tissue, such as nasally, intratracheally, intestinal, rectally
or vaginally, or provided as a finely divided spray suitable for respiratory or pulmonary

administration. Other modes of administration include oral administration, suppositories, sublingual administration, and active or passive transdermal delivery techniques.

Where a polypeptide of the invention is to be administered, it is preferred to administer the polypeptide to a site in the body where it will have the ability to contact suitable antigen presenting cells, and where it, or they, will have the opportunity to contact T cells of the individual. Where an APC is to be administered, it is preferred to administer the APC to a site in the body where it will have the ability to contact, and activate, suitable T cells of the individual.

Delivery regimes

Administration of the polypeptides/polynucleotides/cells (such as the composition containing a plurality of peptides) may be by any suitable method as described above. Suitable amounts of the polypeptide may be determined empirically, but typically are in the range given below. A single administration of each polypeptide may be sufficient to have a beneficial effect for the patient, but it will be appreciated that it may be beneficial if the polypeptide is administered more than once, in which case typical administration regimes may be, for example, once or twice a week for 2-4 weeks every 6 months, or once a day for a week every four to six months. As will be appreciated, each polypeptide or polynucleotide, or combination of polypeptides and/or polynucleotides may be administered to a patient singly or in combination.

Dosages for administration will depend upon a number of factors including the nature of the composition, the route of administration and the schedule and timing of the administration regime. Suitable doses of a molecule of the invention may be in the order of up to 15µg, up to 20µg, up to 25µg, up to 30µg, up to 50µg, up to 100µg, up to 500 µg or more per administration. Suitable doses may be less than 15µg, but at least 1ng, or at least 2ng, or at least 5ng, or at least 50ng, or least 100ng, or at least 500ng, or at least 1µg, or at least 10µg. For some molecules of the invention, the dose used may be higher, for example, up to 1 mg, up to 2 mg, up to 3 mg, up to 4 mg, up to 5 mg or higher. Such doses may be provided in a liquid formulation, at a concentration suitable to allow an appropriate volume for administration by the selected route.

Kits

Also disclosed is a combination of components described herein which are packaged in the form of a kit in a container. Such kits may comprise a series of components to allow for a treatment of the invention. For example, a kit may comprise one or more different polypeptides, polynucleotides and/or cells of the invention, or one or more polypeptides, polynucleotides or cells of the invention and one or more additional therapeutic agents suitable for simultaneous administration, or for sequential or separate administration. The kit may optionally contain other suitable reagent(s) or instructions and the like.

The invention is illustrated by the following Examples:

Example 1

MHC Class II binding search

The aim of this study is to identify a distinct panel of peptides with strong affinities for the eight most common human MHC Class II HLA-DRB1* allotypes. In order to identify binding peptides in the major birch allergens Bet v1, Bet v2, Bet v3, Bet v4, Bet v6, an *in silico* approach was carried out using the commercially available EpiMatrix algorithm (EpiVax Inc.) This is a bioinformatic analysis of peptides from a sequence for the potential to be accommodated within the binding groove of MHC class II HLA-DR molecules.

EpiMatrix is a matrix-based algorithm that ranks 9 amino acid residue sequences, overlapping by 8 amino acids, from any polypeptide sequence by estimated probability of binding to each of the selected MHC molecules. (De Groot et al., AIDS Research and Human Retroviruses 13:539-41 (1997). The procedure for developing matrix motifs was published by Schafer et al, Vaccine 16:1880-4 (1998). In this Example, binding potential for HLA DR1, DR3, DR4, DR7, DR8, DR11, DR13 and DR15 is assessed. Putative MHC ligands are selected by scoring each 9-mer frame in a protein sequence. This score is derived by comparing the sequence of the 9-mer to the matrix of amino acid sequences known to bind to each MHC allele. Retrospective studies have demonstrated that EpiMatrix accurately predicts published MHC ligands (Jesdale et al., in Vaccines '97 (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.,

1997)). Successful Prediction of peptides which bind to multiple MHC molecules has also been confirmed.

The EpiMatrix data for each allergen is shown either:

As overlapping 9 mer peptide data with the binding (Z) score for each allele
 5 and the number of 'hits' for the eight alleles (Z scores of equal to or greater than the top 5% of predicted binders); or

As a cluster report where the data from analysing multiple sequences from the database is 'clustered' to give an overview of binding for all variants of the protein. The "EpiMatrix hits" refers to the number of high predicted Z binding scores for the
 10 eight alleles within that sequence whilst the "EpiMatrix Cluster Score" is derived from the number of hits normalized for the length of the cluster. Cluster Score is thus the excess or shortfall in predicted aggregate MHC binding properties relative to a random peptide standard. A cluster score above 10 is considered to indicate broad MHC binding properties.

15 EpiMatrix analyses were performed on the entire sequences of known isoforms of Bet v1, listed below with their corresponding NCBI accession numbers:

Bet v1 L	P43185;	Bet v1 E	P43178;
Bet v1 M/N	P43186;	Bet v1 D/H	P43177;
Betv1 K	P43184;	Bet v1 C	P43176;
Bet v1 J	P43183;	Bet v1 B	P45431;
Bet v1 G	P43180;	Bet v1 A	P15494;
Bet v1 F/I	P43179;		

EpiMatrix analyses were also performed on additional known Bet v1 sequences indexed by accession number in Table 2.

20 These analyses identified core peptides (and their flanking sequences) derived from the above sequences which are predicted to have good MHC class-II binding.

These sequences are shown below in Tables 1 and 2. As shown, many of the peptides identified are highly conserved between different Bet v1 isoforms.

In Tables 1 and 2:

25 "Residues in main sequence" gives the location of the peptide within the sequences that were analysed. The core peptide (underscored middle amino acids in bold) defines the actual binding sequence that was identified during the analysis. The stabilizing flanks (N-terminal and C-terminal, not bold) were included for use with the core sequence and are typically required to aid manufacture of the peptides. "Number of

hits” refers to the number of high predicted binding affinities for all MHC types tested within the sequence. The “EpiMatrix Cluster Score” is derived from the number of hits normalized for the length of the cluster. Cluster Score is thus the excess or shortfall in predicted aggregate MHC binding properties relative to a random peptide standard. A score above 10 is considered to indicate broad MHC binding properties.

Table 1 - Bet v1

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HITS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO.	SEQ ID NO.
P43185	13 - 28	VIPAARMFKAFILDGD	0.78	6	11.3	P1	1
P15494	100 - 114	SNEIKIVATPDGGS	0.03	7	15.57	P2	2
P43176	100 - 114	CNEIKIVATPDGGS	0.25	7	15.57	P3	3
P43177	100 - 114	SNEIKIVATPDGGCV	0.23	7	15.57	P4	4
P43178	100 - 116	SNEIKIVATPNGGSILK	0.02	10	20.13	P5	5
P43179	100 - 116	SNEIKIVATPNGGSILK	0.02	10	20.13	P6	
P43180	100 - 114	SNEIKIVATPDGGCV	0.23	7	15.57	P7	
P43183	100 - 116	SNEIKIVATPNGGSILK	0.02	10	20.13	P8	
P43184	100 - 114	CNEIKIVATPDGGS	0.25	7	15.57	P9	
P43185	100 - 114	SNEIKIVATPDGGCV	0.23	7	15.57	P10	
P43186	100 - 114	CNEIKIVATPDGGS	0.25	7	15.57	P11	
P45431	100 - 114	CNEIKIVATPDGGS	0.25	7	15.57	P12	
P43178	112 - 126	GSILKINNKYHTKGD	-1.08	6	12.34	P13	
P43179	112 - 126	GSILKINNKYHTKGD	-1.08	6	12.34	P14	
P43183	112 - 126	GSILKINNKYHTKGD	-1.08	6	12.34	P15	
P15494	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P16	6
P43176	142 - 160	EALLRAVESYLLAHS DAYN	0.04	8	12.06	P17	7
P43177	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P18	
P43178	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P19	
P43179	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P20	
P43180	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P21	
P43183	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P22	
P43184	142 - 160	EALLRAVESYLLAHS DAYN	0.04	8	12.06	P23	
P43185	142 - 160	ETLLRAVESYLLAHS DAYN	-0.09	8	12.06	P24	
P43186	142 - 160	EALLRAVESYLLAHS DAYN	0.04	8	12.06	P25	
P45431	142 - 160	EALLRAVESYLLAHS DAYN	0.04	8	12.06	P26	

Table 1A

EpiMatrix analysis of Bet v1 Sequence: P15494: predicted multiple HLA DR allele binding region: FNYETETTSVIPAAARLFKAFILDGDNLF (4-31).

Frame Start	AA Sequence	Frame Stop	DRB1*01:01 Z-Score	DRB1*03:01 Z-Score	DRB1*04:01 Z-Score	DRB1*07:01 Z-Score	DRB1*08:01 Z-Score	DRB1*11:01 Z-Score	DRB1*13:01 Z-Score	DRB1*15:01 Z-Score	Hits
4	FNYETETTS	12	.96	1.29	1.58	-.42	.10	.93	-.21	.27	0

5	NYETETTSV	13	.32	-.86	.59	.81	-1.32	-.80	-.75	.22	0
6	YETETTSVI	14	2.15	1.37	2.25	1.72	1.09	1.15	-.03	.70	3
7	ETETTSVIP	15	-.11	-1.34	.48	.58	-.89	-.60	-.84	-.03	0
8	TETTSVIPA	16	.16	-1.10	.97	.51	-.86	-.69	-.48	.04	0
9	ETTSVIPAA	17	-.04	.16	.64	-.18	-.89	.43	-.39	-.17	0
10	TTSVIPAAR	18	.64	.66	-.12	-.56	.14	.47	.50	-.43	0
11	TSVIPAARL	19	1.52	-.44	.23	.47	-.63	.28	-.96	-.33	0
12	SVIPAARLF	20	.69	-.06	-.07	1.07	-.44	.25	1.05	-.20	0
13	VIPAARLFK	21	.67	2.00	.65	.50	1.21	1.32	1.98	1.21	2
14	IPAARLFKA	22	.55	1.17	.00	-.28	.32	.16	1.27	1.60	0
15	PAARLFKAF	23	-1.29	-1.65	-1.57	.28	-.29	-.88	-.32	-.58	0
16	AARLFKAFI	24	1.13	1.39	-.25	-.68	1.25	1.50	1.32	-.06	0
17	ARLFKAFIL	25	2.12	.89	.25	1.84	1.11	.18	1.54		3
18	RLFKAFLD	26	-1.54	-.90	-1.35	-.86	1.38	-1.29	-.38	-.67	0
19	LFKAFLDGL	27	.86	1.13	1.48	.68	.94	.39	1.14	1.37	0
20	FKAFILDGD	28	-.17	.58	.09	-.43	1.90	.24	.30	.96	1
21	KAFILDGDN	29	-.49	-1.35	-.67	-.69	-.59	-.02	-2.05	.29	0
22	AFILDGDNL	30	1.32	-.28	-.21	.67	-.85	-.69	-.80	.30	0
23	FILDGDNLF	31	-.02	2.27	1.73	1.07	.47	-.09	-.22	.74	2

Table 2 – Bet v 1

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HTS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO	SEQ ID NO
CAA04829	1 - 16	GVFNYEIGATSVIPAA	0.84	7	11.23	P27	8
2122374C	14 - 34	IAPARLFKSFVLADNLIIPKV	0.62	9	13.67	P28	9
ABC41588	97 - 111	CNEIKLVATPDGGST	-0.15	7	15.45	P29	10
ABC41605	97 - 111	SKEIKLAAAPDGGSI	0.01	6	13.05	P30	11
ABC41615	97 - 111	SNEIKIVATPDGGCI	0.25	7	15.57	P31	12
ABC41617	97 - 111	CNEIKLVATPDGGSI	0.20	7	15.45	P32	13
ABC41596	97 - 113	CNEIKIVAAPGGGSILK	0.54	9	17.42	P33	14
ABC41602	97 - 113	CNEIKIVPAPGGGSILK	0.34	9	16.98	P34	15
ABC41609	97 - 113	SYEIKIVAAPGGGSILK	0.48	9	17.42	P35	16
IQMR_A	99 - 113	SNEIKIVATGDGGSI	0.11	5	10.32	P36	17
CAA96546	100 - 114	CNEIKIVAAPDGGSI	0.41	7	14.59	P37	18
CAA96547	100 - 114	SNEIKIVATPDGRSI	-0.25	7	15.57	P38	19
CAA07324	100 - 114	SNEIKLVATPDGGSI	-0.02	7	15.45	P39	20
CAA07327	100 - 114	CNEIKIVATPDGGCV	0.45	7	15.57	P40	21
CAA07318	100 - 114	SNEIKIVTTPDGGCV	0.06	7	15.57	P41	22
AAD26561	100 - 114	SNEIKIVATPDGGPI	-0.03	7	15.57	P42	23
ABC41589	109 - 125	GSILKIRNKYHTKGDHE	-1.41	9	15.34	P43	24
ABC41609	139 - 150	AGLFKAIVENYLV	0.82	5	10.95	P44	25
ABC41583	139 - 150	ETLLRAVESYLL	0.58	6	13.44	P45	26
ABC41589	139 - 150	EALLRAVESYLL	0.78	6	13.44	P46	27
ABC41602	139 - 150	EALFRAVESYLL	0.70	7	16.84	P47	28
CAA96544	139 - 156	EKAVGLLKAVESYLLAHS	0.43	7	12.67	P48	29
CAA07319	141 - 160	GETLLRAVEGYLLAHS DAYN	-0.09	8	11.13	P49	30
AAD26561	142 - 156	ETLLRAVESYPLAHS	-0.05	6	13.44	P50	31
2122374C	142 - 160	AGLFKAIVENYLV AHPNAYN	0.02	10	15.5	P51	32

CAA96539	142 - 160	ETLLRAVERYLLAHS DAYN	-0.29	10	18.69	P52	33
2122374A	142 - 160	EALFRAVESYLLAHS DAYN	-0.02	9	15.46	P53	34

Example 2

EpiMatrix analyses as above were performed on the entire sequence of a known isoform of Bet v 3 (NCBI accession no: P43187). These analyses identified a core peptide (and its flanking sequence) derived from the above sequence which is predicted to have good MHC class-II binding. The sequence is shown below in Table 3.

Headings and notes for Table 3 are as with Table 1 above.

Table 3 - Bet v 3

INPUT SEQUENCE	RESIDUES IN SEQUENCE (incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HITS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO	SEQ ID NO
P43187	188 - 205	VDEFEFKDMRSVLVRSS	0.16	10	14.02	P54	35

- 10 A sequence at residues 80 to 94 of P43187, TVKSFTREGNIGLQF (Peptide ID NO. P55, SEQ ID NO: 36), was also predicted to have good MHC-Class II binding.

Additional *in silico* analysis of other birch allergen sequences from Bet v 3 is shown here:

Table 3A

- 15 EpiMatrix analysis of Bet v3 Sequence: GI1168696 SPP43187: predicted multiple HLA DR allele binding region SLNTRLRLRRIFDLFDK (35-50).

Frame start	sequence	end	DRB1*01 01 Z-Score	DRB1*03 301 Z-Score	DRB1*04 401 Z-Score	DRB1*07 01 Z-Score	DRB1*08 01 Z-Score	DRB1*11 101 Z-Score	DRB1*13 01 Z-Score	DRB1*15 01 Z-Score	Hits
35	SLNTRLRLRR	43	.28	.97	-.38	.38	.64	.53	1.73	.68	1
36	LNTLRLRRI	44	1.30	.87	-.15	.46	.82	1.35	1.41	.17	0
37	NTLRLRRIF	45	-.24	.12	-1.52	.43	1.21	1.13	1.42	-.47	0
38	TLRLRRIFD	46	.47	.40	-.46	.42		.66	.49	-.34	1
39	LRLRRIFDL	47	1.09	.83	.08		1.68	.31	1.94		4
40	RLRRIFDLF	48	-1.83	-.86	-1.40	-.38	-.94	-1.34	-.63	-.87	0
41	LRRIFDLFD	49	.84	.60	1.39	.46	1.41	.88	.39	1.55	0
42	RRIFDLFDK	50	-.31	.38	-.31	-.58	.85	.33	1.05	1.61	0

Example 3

- 20 EpiMatrix analyses as above were performed on the entire sequence of known isoforms of Bet v 4 (NCBI accession nos: Q39419, CAA73147). These analyses identified core peptides (and their flanking sequences) derived from the above sequences which are predicted to have good MHC class-II binding. These sequences

are shown below in Table 4. Headings and notes for Table 4 are as with Table 1 above.

Table 4 - Bet v 4

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HITS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO.	SEQ ID NO.
Q39419	10 - 27	AERERIFKRFDANGDGKI	-1.19	8	13.83	P56	37
Q39419	67 - 81	FTDFGRANRGLLKDV	-0.38	7	13.49	P57	38
CAA73147	67 - 81	FTDFGRANRGLLKDV	-0.23	7	13.53	P58	39

5 Example 4

EpiMatrix analyses as above were performed on the entire sequence of a known isoform of Bet v 6 (NCBI accession no: O65002). These analyses identified core peptides (and their flanking sequences) derived from the above sequences which are predicted to have good MHC class-II binding. These sequences are shown below in

10 Table 5. Headings and notes for Table 5 are as with Table 1 above.

Table 5 - Bet v 6

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HITS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO.	SEQ ID NO.
O65002	43 - 63	PVKGKLVKFRGLGVTLHGD	0.04	9	14.09	P59	40
O65002	67 - 90	HESLVKAFKQVDVVISVGHQLA	0.52	11	15.54	P60	41
O65002	149 - 170	YVSSNFFAGYFLPTLAQPLTS	0.46	10	12.92	P61	42
O65002	258 - 274	PINVILAINHSVFVKGD	0.83	7	10.54	P62	43

Example 5

EpiMatrix analyses as above were performed on the entire sequence of a known isoform of Bet v 7 (NCBI accession no: CAC84116). These analyses identified a core peptide (and its flanking sequence) derived from the above sequence which is predicted to have good MHC class-II binding. This sequence is shown below in Table 6.

Headings and notes for Table 6 are as with Table 1 above.

20 Table 6 - Bet v 7

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydrophobicity	EpiMatrix HITS (Excl. FLANKS)	EpiMatrix CLUSTER SCORE (Excl. FLANKS)	Peptide ID NO	SEQ ID NO
CAC84116	34 - 48	AENFRALCTGEKNG	-0.77	5	10.46	P63	44

Example 5A

Additional *in silico* analysis of other birch allergen sequences from Bet v 2 is shown here:

5 Table 6A

EpiMatrix analysis of Bet v2 Sequence: GI1942360_PDB1CQA: predicted multiple HLA DR allele binding region: SVWAQSSSFQFKPQEITGIMK (33-54).

Frame start	sequence	end	DRB1*01 01 Z-Score	DRB1*03 301 Z-Score	DRB1*04 401 Z-Score	DRB1*07 01 Z-Score	DRB1*08 01 Z-Score	DRB1*11 101 Z-Score	DRB1*13 01 Z-Score	DRB1*15 01 Z-Score	Hits
33	SVWAQSSSF	41	.75	.40	1.04	1.19	-.29	-.22	-.17	.71	0
34	VWAQSSSF	42	.90	.82	1.31	.46	-.02	.21	.64	1.01	0
35	WAQSSSFQ	43	1.70	.36	2.24		1.23	1.44	.12	1.53	3
36	AQSSSFQF	44	-.65	.38	-.28	-.01	-1.26	-.18	-.18	-.56	0
37	QSSSFQFK	45	-.31	.01	.24	.04	-1.01	.00	-1.52	-.51	0
38	SSSFQFKP	46	-.45	-.73	-1.50	-1.02	-.04	-.76	-.02	.21	0
39	SSFQFKPQ	47	-1.10	-2.37	-.57	.24	-.52	-.64	-1.22	-.98	0
40	SFQFKPQE	48	-.23	1.11	-.75	-1.69	1.34	.47	1.32	-.32	0
41	FQFKPQEI	49	1.55	.73	.67	.99	.88	.49	.09	2.02	1
42	PQFKPQEI	50	-.26	.27	-.85	.12	1.00	.28	.59	-.60	0
43	QFKPQEIT	51	-1.28	-.42	-.84	-.60	-.27	-1.13	.23	.00	0
44	FKPQEITGI	52	1.94	1.68	1.69	1.76	.07	.45	1.37	1.43	4
45	KPQEITGIM	53	.15	.15	.32	.12	-.39	.10	-1.15	-.26	0
46	PQEITGIMK	54	-.06	-1.11	-.06	-.24	-1.55	-.85	-1.48	-.15	0

Table 6B

- 10 EpiMatrix analysis of Bet v2 Sequence: GI1942360_PDB1CQA: predicted multiple HLA DR allele binding region: IKYMVIQGEAGAVIRGKKGSGG (72-93).

Frame start	sequence	end	DRB1*01 01 Z-Score	DRB1*03 301 Z-Score	DRB1*04 401 Z-Score	DRB1*07 01 Z-Score	DRB1*08 01 Z-Score	DRB1*11 101 Z-Score	DRB1*13 01 Z-Score	DRB1*15 01 Z-Score	Hits
72	IKYMVIQGE	80	1.21	1.60	1.25	.49	1.87	1.30	-.52	.97	1
73	KYMVIQGEA	81	.45	.63	-.35	.37	.56	1.73	.28	.53	1
74	YMVIQGEAG	82	1.68	.22	1.47	.98	.61	1.06	-.37	.26	1
75	MVIQGEAGA	83	1.49	2.23	1.51	-.42	1.07	1.87	2.27	.85	3
76	VIQGEAGAV	84	1.12	1.46	-.18	1.12	-.11	.16	-.07	2.02	1
77	IQGEAGAVI	85		1.64	1.28	.54	.46	.67	.50	.86	1
78	QGEAGAVIR	86	.56	.61	-.43	-.01	-.68	-1.08	.29	.12	0
79	GEAGAVIRG	87	.02	.36	.41	-.45	-.52	-1.38	-.79	.28	0
80	EAGAVIRGK	88	-.12	-.55	.17	-.08	-.44	.08	-.23	-1.21	0
81	AGAVIRGKK	89	-.65	.95	-1.14	.00	.75	1.71	.58	.53	1
82	GAVIRGKKG	90	.82	-1.39	-.20	-.20	-.70	-.26	-1.31	.08	0
83	AVIRGKKGS	91	-.08	.16	-.55	-.21	1.97	1.49	1.43	1.07	1
84	VIRGKKGSG	92	.10		-.64	-.90	1.35	1.18	.92	1.79	2
85	IRGKKGSGG	93	1.18	1.05	.64	.02	2.09	.95	1.11	1.83	2

Example 6

Based on the analyses performed in Examples 1 to 5A, the following peptides shown in Table 7 were designed for screening in subsequent assays. The design process involved modification of native sequences to enhance solubility and other physicochemical characteristics. For example, for Bir12A, residues in parent 62-77R indicates that the peptide sequence of Bir12A corresponds to residues 62 to 77 of the parent sequence, with an additional R residue added to the C terminus to improve solubility. Similarly, for Bir01F, G, H and I, residues in parent 4-18K indicates that these peptide sequences correspond to residues 4 to 18 of the parent sequence, with an additional K residue added to the C terminus to improve solubility.

Table 7

Peptide	Sequence	Residues	SEQ. ID. NO
BIR01F	FNYETEATSVIPAARK	4-18K (P43185) Bet v1	45
BIR01G	FNYEIEATSVIPAARK	4-18K (P43179) Bet v1	46
BIR01H	FNYEIETTSVIPAAARK	4-18K (P43177) Bet v1	47
BIR01I	FNYETETTSVIPAAARK	4-18K (P15494) Bet v1	48
BIR02D	PAARMFKAFILDGDKLVPK	15-33(P43185) Bet v1	49
BIR02E	PAARLFKAFILEGDTLIPK	15-33 (P43184) Bet v1	50
BIR02G	PAARLFKAFILEGDNLI PK	15-33 (P41380) Bet v1	51
BIR02I	PAARMFKAFILD	15-26 (P41385) Bet v1	52
BIR02J	PAARMFKAFILEGDKLVPK	D to E variant of BIR02D	53
BIR04	PGTIKKISFPEGFPFKYV	51-68 (P43185) Bet v1	54
BIR05	SPFKYVKERVDEVDHA	63-78 (P43186) Bet v1	55
BIR05A	FPFKYVKDRVDEVDHT	63-78 (P43185) Bet v1	56
BIR06	ANFKYSYSMIEGGALGD	78-94(P43186) Bet v1	57
BIR06B	TNFKYSYSVIEGGPVG D	78-94 (P43183) Bet v1	58
BIR06D	TNFKYNYSVIEGGPIG	78-93 (P) Bet v1	59
BIR07	SNEIKIVATPDGGSILK	100-116 Bet v1	60
BIR07A	SNEIKIVATPNGGSILK	100-116 Bet v1	61
BIR07B	SNEIKIVATPQGG SILK	100-116 Bet v1	62
BIR07C	SNEIKIVATPEGGSILK	100-116 Bet v1	63
BIR07D	SNEIKIVATPGGGSILK	100-116 Bet v1	64
BIR08	GSILKINNKYHTKGD	112-126 Bet v1	65
BIR08A	SILKISNKYHTKGD	113-125 (P43186) Bet v1	66
BIR09	ETLLRAVESYLLAHSDAY	142-159 Bet v1	67
BIR09A	GETLLRAVESYLLAHS	141-156 Bet v1	68
BIR09B	KEMGETLLRAVESYLLAHS	138-156 Bet v1	69
BIR09C	KEKGETLLRAVESYLLAHS	M to K variant of above	70
BIR10	GSVWAQSSSFPOFK	33-45 (P25816) Bet v 2	71
BIR11	FPQFKPQEITGIMK	41-54 (AAB44348) Bet v2	72
BIR12A	PTGMFVAGAKYMVIOGR	62-77R (P35079) Phl p12	73
BIR12B	AKYMVIOGEPGRVIRGK	70-86 (P35079) Phl p12	74

BIR13	GIKYMVIQGEAGAVIRGK	71-88 (AAB44348) Bet v2	75
BIR14	EAGAVIRGKKGGGGIT	80-95 (P25816) Bet v2	76
BIR15	SLNTLRLRRIFDLFDK	35-50 Bet v3	77
BIR16A	AERERIFKRFDANGEGK	10-26 D to E variant Bet v4	78
BIR16B	AERERIFKRFDAGGEGK	N to G variant of above	79
BIR17	VKGKLVKFKGLGVTLHLG	44-62 Bet v6	80

Example 7

In vitro binding analysis

The peptides identified as being potential MHC Class II-binding are pre-screened for solubility in an aqueous, acidic milieu and the peptides are tested in an in vitro MHC Class II binding assay.

Methods

The assay employed is a competitive MHC class II binding assay, wherein each peptide is analysed for its ability to displace a known control binder from each of the human MHC class II allotypes investigated. The allotypes and control peptides used in this study are typically those shown below:

Control peptides used in the in vitro binding assays

Allotype	Control Peptide	Sequence
DRB1*0301	Myco. tuberculosis/leprae hsp 65 2-16	AKTIA YDEEARRGLE
DRB1*1101	Influenza haemagglutinin 307-319	PKYVKQNTLKLAT
DRB1*1501	Human myelin basic protein 85-99	ENPVVHFFKNIVTPR

Each of the peptides from Tables 1 to 7 are analysed in the competition assay and screened for relative binding compared to the control peptides. Due to the nature of the competitive assay the data for each peptide is determined as a ratio of its own IC₅₀ to that of the control peptide. Thus, a peptide that has an IC₅₀ value that is parity to the control peptide has an identical binding affinity, while peptides with a ratio less than one have a higher affinity and those with a ratio greater than one have a lower affinity.

Solubility in aqueous solution is an essential criterion for a peptide to be an effective therapeutic agent. Therefore, as a consequence of the solubility screen very hydrophobic peptides with a high frequency of large hydrophobic amino acid residues in multiple binding registers will be eliminated. This is a characteristic of promiscuous HLA-DRB1* binders. Peptides which bind to one or more of the MHC Class II

allotypes are identified. It would be expected that such peptides would have the ability to bind similar allotypes that have not been tested through the homology of MHC structures.

5 **Example 8**

The following methods are applied to the same peptides as in Example 7.

Cell proliferation assay

The cell proliferation assay is performed on PBMC's (140×10^6 cells required for all parameters to be tested). Proliferation is measured by the incorporation of the radiolabelled compound 3H-thymidine. In more detail, 100µl of the appropriate antigen or peptide concentration is distributed into the appropriate wells of 96 well plates. The plates are then placed into a humidified 5% CO₂ incubator set at 37°C for a maximum of 4 hours. PBMC's isolated as described above are prepared to a concentration of 2×10^6 cells/ml in complete medium at room temperature. 100µl of cell solution is then distributed into each of the wells of the 96 well plates containing antigen/peptide. The plates are then incubated for 6 to 8 days. The cultures are pulsed with tritiated thymidine solution by adding 10µl of tritiated thymidine stock solution (1.85MBq/ml in serum-free RPMI medium) to each well. The plates are then returned to the incubator for between 8 and 16 hours. Cultures are then harvested using a Canberra Packard FilterMate™ 196 cell harvester. Dried filter mats are counted using an appropriate beta scintillation counter.

Counts from wells containing peptide are compared statistically to wells containing media alone (12 wells per group). The non-parametric Mann-Whitney test is used. The same statistical test is used for all subjects. A statistically significant difference between media only wells and peptide-stimulated wells is considered a positive stimulation of PBMC's by the peptide.

Cytokine release assay

The 36 peptides were manufactured at small scale (approximately 10mg batch size, non-GMP). The purity of each peptide was at least 95% by HPLC. 96 well culture plates containing peptides and controls (the negative control was culture medium and the positive controls were staphylococcal enterotoxin B (SEB) 25ng/ml and whole birch pollen allergen extract 100 µg/ml) were prepared in advance and stored at -20°C

prior to the day of assay. Peptides were added to wells in a volume of 100 μ l containing peptides at a concentration of 200 μ g/ml, such that subsequent addition of 100 μ l of cells would create a final assay concentration of 100 μ g/ml.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised blood by Ficoll density gradient centrifugation. A 100 μ l aliquot of a 5×10^6 cell/ml PBMC suspension was then added to each well and the plates placed in a humidified 5% CO₂ incubator at 37°C for 5 days. Following stimulation, culture supernatants (100 μ l) were harvested for testing by multiplex bead assay.

Multiplex cytokine bead assays (IL-10, IL-13, Interferon gamma (IFN-g)) were performed on thawed culture supernatants according to the manufacturer's instructions. Single measurements were performed for each culture supernatant sample. After completion of the multiplex assay, individual cytokine levels were determined by interpolation from the standard curve generated in the assay. A positive result was taken as being greater than 100 pg/ml for the IL-13 and IFN-g assays or >4 times the background for the IL-10 assays. The number of responders out of 47 birch allergic subjects tested was calculated for each peptide for the three cytokines. Results for IL-13 or IFN-g are summarized in Table 9.

Table 8

% responders indicates the proportion of subjects in which each peptide induced IL-13 or IFN-g above a threshold level of 100pg/ml

Peptide	% responders	Peptide	% responders
Bir02J	48	Bir15	27
Bir01I	42	Bir16B	27
Bir01F	38	Bir01H	25
Bir12B	38	Bir06D	25
Bir01G	33	Bir07B	25
Bir04	33	Bir07D	25
Bir09	33	Bir10	25
Bir02E	31	Bir14	25
Bir02G	31	Bir17	25
Bir02I	31	Bir05A	23
Bir07	31	Bir06	23
Bir07C	31	Bir07A	23
Bir09A	31	Bir13	23
Bir09B	31	Bir06B	19
Bir11	31	Bir08A	19
Bir16A	31	Bir05	17
Bir02D	29	Bir08	17
Bir09C	27	Bir12A	17

Peptides which induce positive response in a high proportion of subjects are desirable for inclusion in a vaccine. As shown, the top performing peptides were

Bir02J (top of the 02 series), Bir 01I (top of the 01 series) and Bir12B. The core of any vaccine should ideally contain these peptides. The second best performing peptides were Bir04 and Bir09 (top of the 09 series) which may be added to the core mixture of Bir02J, Bir01I and Bir12B. The third best performing peptides were Bir07, Bir07C, Bir11 and Bir16A. Additional peptides from this group may be added to the vaccine mixture to further increase coverage. Bir15 was the fourth best performing peptide and may also be added to the vaccine mixture. In terms of other peptides in the various series, Bir01F, 01G or 01H, in that order of preference, are useful variants of Bir01I; Bir02E, 02G, 02I or lastly 02D are useful variants of Bir02J; Bir09A, 09B or lastly 09C are useful variants of Bir09; and Bir16B is a useful variant of Bir16A. A possible preferred mixture would therefore include Bir02J, Bir01I, Bir12B, Bir04, Bir09, Bir07C and Bir16A. Bir11 and/or Bir15 may also be included, or alternatively substituted for Bir07C and/or Bir16A.

In terms of IL-10 release, Bir01I, listed above as one of the top 3 peptides for IL-13 or IFN-g production, induced IL-10 responses in 49% of individuals. Bir02I also induced IL-10 production in a high proportion of individuals (43%). Inclusion of a strong IL-10 inducing peptide may assist in the induction of tolerance following vaccination.

20 Example 9 – Solubility screening

A) Introduction

Table 9-1: Birch peptides to be included in solubility testing

Peptide	Sequence	Mw (Da)	Length (a.a.)	Theoretical Isoelectric point (pI)
BIR01I	FNYETETTSVIPAAARK	1825.92	16	6.14
BIR02I	PAARMFKAFILD	1378.74	12	9.18
BIR02J	PAARMFKAFILEGDKLVPK	2130.26	19	9.72
BIR04	PGTIKKISFPEGFPFKYV	2054.12	18	9.56
BIR07C	SNEIKIVATPEGGSILK	1754.98	17	5.86
BIR09	ETLLRAVESYLLAHS DAY	2050.04	18	4.65
BIR09B	KEMGETLLRAVESYLLAHS	2146.11	19	5.50
BIR12B	AKYMVIIQGEPRVIRGK	1901.07	17	10.28
BIR16A	AERERIFKRFDANGEGK	2022.04	17	8.63

B)

Solubility Testing

45 A series of matrices containing 260mM trehalose and spanning a pH range of

3.0 to 7.0 plus a solution modified with 2mM HCl were prepared as indicated in Appendix 2. The solubility of each of the nine peptides was evaluated in each of the matrices in accordance with Appendix 1. Where solubility was achieved initially, but the peptide precipitated out of solution subsequently then an additional quantity of the relevant matrix was added to try and achieve solubility of the peptide at ca. 200µM.

Details of the Birch reference peptides used are indicated in Table 9-2. All peptides were manufactured by Bachem AG, Bubendorf, Switzerland.

Table 9-2. Details of Birch peptides

Peptide	Batch no.	MW (Da)	Peptide purity (%)	Peptide content (%)
BIR11	1028882	1827.03	99.2	87.1
BIR02I	1028883	1379.69	99.1	86.2
BIR02J	1028884	2131.61	97.4	84.1
BIR04	1028885	2055.45	98.1	88.3
BIR07C	1029310	1756.03	97.9	89.8
BIR09	1028886	2051.28	97.5	86.9
BIR09B	1029311	2147.48	97.3	89.4
BIR12B	1028887	1902.30	97.3	85.9
BIR16A	1028888	2023.24	98.4	88.1

C) Results

The results of the solubility screening are displayed in tables 9-3 to 9-8 below:

Table 3: 2mM HCl and 260mM trehalose dihydrate, pH 2.65

2mM hydrochloric Acid			Weight of peptide 'as is' (mg)	Volume required (µL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (µmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	0.975	50	19.50	16.849	9.222
BIR02I	Completely dissolved	Completely dissolved	1.175	50	23.50	20.075	14.550
BIR02J	Completely dissolved	Completely dissolved	1.180	50	23.60	19.332	9.069
BIR04	Completely dissolved	Completely dissolved	1.025	50	20.50	17.758	8.639
BIR07C	Completely dissolved	Completely dissolved	1.148	100	11.48	10.092	5.747
BIR09	Completely dissolved - filter debris	Completely dissolved - filter debris	1.147	1250	0.92	0.777	0.379
BIR09B	Completely dissolved	Completely dissolved	1.220	100	12.20	10.612	4.942
BIR12B	Completely dissolved	Completely dissolved	1.022	100	10.22	8.542	4.490
BIR16A	Completely dissolved	Completely dissolved	1.243	50	24.86	21.551	10.652

Table 4^a 10mM sodium citrate and 260mM trehalose dihydrate, pH 3.01

Citrate buffer pH 3.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.071	650	1.65	1.424	0.779
BIR021	Completely dissolved	Completely dissolved	1.112	1100	1.01	0.864	0.626
BIR021	Completely dissolved in 100μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.150	3000	0.38	0.314	0.147
BIR04	Completely dissolved	Completely dissolved	1.129	50	22.58	19.559	9.516
BIR07C	Completely dissolved	Completely dissolved	1.108	850	1.30	1.146	0.653
BIR09	Undissolved in 1.5mL, diluted to 3mL still undissolved	Undissolved	1.111	3000	0.37	0.314	0.153
BIR09B	Completely dissolved	Completely dissolved	1.162	100	11.62	10.108	4.707
BIR12B	Completely dissolved	Completely dissolved	1.094	200	5.47	4.572	2.403
BIR16A	Completely dissolved	Completely dissolved	1.076	150	7.17	6.219	3.074

Table 4^b 10mM sodium citrate and 260mM trehalose dihydrate, pH 3.99

Citrate buffer pH 4.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.026	450	2.28	1.970	1.078
BIR021	Cloudy solution with suspended material. Frothing on vortex	Undissolved	1.051	3000	0.35	0.299	0.217
BIR021	Completely dissolved in 50μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.155	3000	0.39	0.315	0.148
BIR04	Completely dissolved	Completely dissolved	1.018	50	20.36	17.636	8.580
BIR07C	Clear solution with suspended material. Frothing on vortex	Clear solution with suspended material	1.126	3000	0.38	0.330	0.188
BIR09	Cloudy solution with undissolved material	Cloudy solution with undissolved material	1.074	3000	0.36	0.303	0.148
BIR09B	Completely dissolved	Completely dissolved	1.140	100	11.40	9.916	4.618
BIR12B	Completely dissolved	Completely dissolved	1.032	100	10.32	8.626	4.534
BIR16A	Completely dissolved	Completely dissolved	1.123	150	7.49	6.490	3.208

Table 5: 10mM sodium citrate and 260mM trehalose dihydrate, pH 5.02

Citrate buffer pH 5.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.036	450	2.30	1.989	1.089
BIR021	Cloudy solution with suspended material	Completely dissolved	1.085	3000	0.36	0.309	0.224
BIR021	Completely dissolved in 150μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.029	3000	0.34	0.281	0.132
BIR04	Completely dissolved	Completely dissolved	1.076	50	21.52	18.641	9.069
BIR07C	Completely dissolved	Completely dissolved	1.167	1350	0.86	0.760	0.433
BIR09	Clear solution with suspended material	Clear solution with suspended material	1.115	3000	0.37	0.315	0.154
BIR09B	Completely dissolved	Completely dissolved	1.275	100	12.75	11.091	5.165
BIR12B	Completely dissolved	Completely dissolved	1.167	200	5.84	4.877	2.564
BIR16A	Completely dissolved	Completely dissolved	1.158	50	23.16	20.077	9.923

Table 6: 10mM sodium citrate and 260mM trehalose dihydrate, pH 6.01

Citrate buffer pH 6.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.117	150	7.45	6.434	3.522
BIR021	Cloudy solution with suspended material	Cloudy solution with suspended material	1.143	3000	0.38	0.325	0.236
BIR021	Completely dissolved in 100μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.172	3000	0.39	0.320	0.150
BIR04	Completely dissolved. Evidence of filter debris	Completely dissolved. Filter debris	1.011	100	10.11	8.758	4.261
BIR07C	Completely dissolved in 350μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.045	3000	0.35	0.306	0.174
BIR09	Completely dissolved	completely dissolved	1.084	1050	1.03	0.875	0.426
BIR09B	Completely dissolved	Completely dissolved	1.230	250	4.92	4.280	1.993
BIR12B	Completely dissolved. Evidence of filter debris	Completely dissolved. Filter debris	1.026	450	2.28	1.906	1.002
BIR16A	Completely dissolved	Completely dissolved	1.293	50	25.86	22.418	11.080

Table 7: 10mM potassium dihydrogen phosphate and 260mM trehalose dihydrate, pH 6.03

Phosphate buffer pH 6.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.107	50	22.14	19.130	10.470
BIR021	Completely dissolved	Completely dissolved	1.145	100	11.45	9.781	7.089
BIR021	Completely dissolved	Completely dissolved	1.112	100	11.12	9.109	4.273
BIR04	Completely dissolved	Completely dissolved	0.986	100	9.86	8.541	4.155
BIR07C	Completely dissolved in 250μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.245	3000	0.42	0.365	0.208
BIR09	Clear solution with suspended material	Clear solution with suspended material	1.037	3000	0.35	0.293	0.143
BIR09B	Completely dissolved in 100μL, redissolved in 3mL after 24hrs	Precipitated out, redissolved	1.086	3000	0.36	0.315	0.147
BIR12B	Completely dissolved	Completely dissolved	1.192	50	23.84	19.926	10.474
BIR16A	Completely dissolved	Completely dissolved	1.077	50	21.54	18.673	9.229

Table 8: 10mM potassium dihydrogen phosphate and 260mM trehalose dihydrate, pH 7.03

Phosphate Buffer pH 7.0			Weight of peptide 'as is' (mg)	Volume required (μL)	Solubility (mg per ml 'as is')	Solubility (mg per ml)	Solubility (μmol per ml)
Peptide	Comments	Solubility after 24 Hours					
BIR11	Completely dissolved	Completely dissolved	1.039	50	20.78	17.955	9.827
BIR021	Completely dissolved	Completely dissolved	1.190	100	11.90	10.163	7.368
BIR021	Completely dissolved	Completely dissolved	1.231	100	12.31	10.084	4.730
BIR04	Completely dissolved	Completely dissolved	1.075	50	21.50	18.624	9.061
BIR07C	Completely dissolved	Completely dissolved	1.005	150	6.70	5.890	3.354
BIR09	Completely dissolved	Completely dissolved	1.138	750	1.52	1.286	0.627
BIR09B	Completely dissolved	Completely dissolved	1.085	50	21.70	18.876	8.790
BIR12B	Completely dissolved	Completely dissolved	1.031	50	20.62	17.234	9.060
BIR16A	Completely dissolved	Completely dissolved	1.099	50	21.98	19.055	9.418

Example 9 - Annex 1
PEPTIDE SOLUBILITY STUDIES

Solubility Methodology

- 5 The formulation vehicles were prepared and measurement of pH taken.
 - Weighing of peptides.
 - Approximately 1mg was required for each evaluation.
 - Materials were dispensed into containers suitable for subsequent solubility evaluation, i.e. clear glass HPLC vials (with screw cap).
- 10 • Evaluation of solubility (for each matrix).
 - Aliquots of matrix (50 to 100~L) were added as required.
 - The peptide solubility was interpreted by visual inspection.
 - The description of the sample characteristics following addition of each aliquot of the solvent was recorded.
- 15 - Repeat visual assessment of solubility after 24 hours.
 - Where a peptide precipitated out of solution after 24 hours, additional buffer was added to produce a final concentration of ca. 0.2mM (200nmol per mL should equate to roughly 0.35mg/mL).
 - Calculation of peptide solubilities (initial evaluation).
 - 20 - Based on absolute amount of powder weighed.
 - Determination of molar concentration at which solubility was achieved using peptide molecular masses and peptide content and purity values.

Calculations

$$25 \quad \text{Solubility mg/ml 'as is'} = \frac{\text{weight(mg)} \times 1000}{\text{dilution}(\mu\text{l})}$$

$$\text{Solubility mg/ml} = \frac{\text{weight(mg)} \times 1000 \times \% \text{Content} \times \% \text{Purity}}{\text{dilution}(\mu\text{l})}$$

$$30 \quad \text{Solubility } \mu\text{mol/ml} = \frac{\text{weight(mg)} \times 1000 \times \% \text{Content} \times \% \text{Purity} \times \frac{1}{\text{MolWt}} \times 1000}{\text{dilution}(\mu\text{l})}$$

Example 9 - Annex 2BUFFERS FOR INITIAL SOLUBILITY AND STABILITY SCREENING

Each matrix was prepared at a concentration of 10mM of the buffering agent. Each
5 buffer contained 260mM Trehalose dihydrate (FW 378.3).

Preparation of matrix

The procedure indicated is for the preparation of 100mL of each buffer, but
alternative volumes can be prepared by adjusting the quantities.

- 10 • 0.1M stock solutions of sodium citrate and potassium dihydrogen phosphate were prepared.
- Weight of trehalose dihydrate equivalent to 260mM was transferred to an appropriate mixing vessel containing 70-80mL of distilled deionised water and allowed to dissolve.
- 10mL of the appropriate stock 0.1M buffer solution was added to the mixing vessel
- 15 and stirred .
- The pH of the matrix was adjusted to the desired value by adding 2mM hydrochloric acid or 0.1M sodium hydroxide as required.
- The solutions were finally diluted to 100g weight and the pH re-assessed.

Buffers for initial solubility and stability screening, shown as Buffer salt or pH
20 modifier / pH:

2mM HCl and 260mM trehalose dihydrate/ pH 2.65

10mM sodium citrate and 260mM trehalose dihydrate/ pH 3.01

10mM sodium citrate and 260mM trehalose dihydrate/ pH 3.99

10mM sodium citrate and 260mM trehalose dihydrate/ pH 5.02

25 10mM sodium citrate and 260mM trehalose dihydrate/ pH 6.01

10mM potassium dihydrogen phosphate and 260mM trehalose dihydrate/ pH 6.03

10mM potassium dihydrogen phosphate and 260mM trehalose dihydrate/ pH 7.03

Example 10 - Histamine release assay

30 The purpose of this assay was to identify compositions that are capable of activating blood basophils (as a surrogate for tissue mast cells) resulting in histamine release that may result in allergic reactions during therapy. A composition comprising

a mixture of peptides that induce histamine release frequently may be considered unsuitable for use as a vaccine.

Histamine release requires the crosslinking of adjacent specific IgE molecules on the surface of the basophil. The peptides being evaluated were small (11 to 18 amino acids in length) and should not, therefore, possess significant tertiary structure that would enable them to retain the conformation of an IgE-binding epitope of the whole molecule. Furthermore, peptide monomers in solution, even if they are bound by IgE, should not be able to crosslink adjacent IgE molecules.

Histamine release from fresh peripheral whole blood from birch allergic subjects was evaluated. Peripheral blood basophils were used as a surrogate for tissue mast cells which were not practical to assay. Blood was incubated in vitro with mixtures of peptides identified as suitable based on the results of Examples 1 to 9 above. Specifically, the following mixtures were assayed:

Mix 1 – BIR01I, BIR02J, BIR04, BIR12B, BIR16A, BIR07C

Mix 2 – BIR01I, BIR02J, BIR04, BIR12B, BIR16A, BIR07C, BIR09

Mix 3 – BIR01I, BIR02J, BIR04, BIR12B, BIR16A, BIR07C, BIR09B

Mix 4 – BIR01I, BIR02I, BIR04, BIR12B, BIR16A, BIR07C

Mix 5 – BIR01I, BIR02I, BIR04, BIR12B, BIR16A, BIR07C, BIR09

Mix 6 – BIR01I, BIR02I, BIR04, BIR12B, BIR16A, BIR07C, BIR09B

Histamine release in response to whole birch allergen extract was measured in each subject to confirm basophil sensitisation. A positive control, representing total histamine release, generated by freeze/thawing the cells twice, was included in each assay. A negative control for spontaneous histamine release was generated by incubating cells in buffer only.

The assay was performed using the Immunotech Histamine Release Immunoassay kit according to the manufacturer's instructions. Following the *in vitro* challenge of blood basophils with peptide mixtures, whole allergen or buffer in microtitre plate wells, supernatants were removed and the histamine in the samples converted to acyl histamine. Acylated samples were tested by a competitive acyl histamine ELISA.

Peptide mixtures were assayed for their ability to induce histamine release over a 5 log₁₀ range (1 to 10,000 ng/ml). The concentration range assayed was selected

based on theoretical *in vivo* doses of peptide that may be achieved during therapy. For example, a 31µg dose (approximately 3 nmol/peptide equivalent) of each peptide entering a blood volume of 5 litres, would result in a blood concentration of 6ng/ml, at the lower end of the histamine release assay dose range. The whole birch allergen
5 extract was used over the same concentration range.

Single measurements were performed for each dilution. After completion of the ELISA, individual histamine levels were determined by interpolation from the standard curve generated in the ELISA assay. Results from samples were adjusted to allow for dilution. Where two or more consecutive dilutions of a peptide/allergen preparation
10 elicited >15% of the total histamine release seen in the freeze thawed positive control (>15% of positive control), or where a single value of >15% of positive control was achieved at the highest concentration tested (10 µg/mL for peptides), this was considered a "*positive histamine release*".

A total of 40 histamine release assays were completed during the study. Of
15 these 5 assays were rejected because of failure to meet appropriate QC controls, e.g. due to unacceptably high levels (>15% of positive control) of spontaneous release in the medium plus buffer negative control wells.

The mixtures tested all showed good histamine release properties. The study findings are summarised as follows: (WA = whole allergen)

Mix	Peptide conc:µg/ml	Average % histamine release	Mix	Peptide conc:µg/ml	Average % histamine release
1	10	1%	5	10	2%
1	1	2%	5	1	2%
1	0.1	3%	5	0.1	2%
1	0.01	3%	5	0.01	2%
1	0.001	2%	5	0.001	2%
2	10	2%	6	10	3%
2	1	2%	6	1	2%
2	0.1	3%	6	0.1	1%
2	0.01	3%	6	0.01	1%
2	0.001	2%	6	0.001	2%
3	10	4%	WA	10	65%
3	1	2%	WA	1	38%
3	0.1	3%	WA	0.1	38%
3	0.01	2%	WA	0.01	42%
3	0.001	2%	WA	0.001	43%
4	10	2%			
4	1	2%			
4	0.1	2%			
4	0.01	1%			
4	0.001	1%			

CLAIMS

1. A composition suitable for use in preventing or treating allergy to birch pollen by tolerisation comprising:

- i) the polypeptide of SEQ ID NO: 74 (BIR12B; AKYMVIQGEPGRVIRGK), or a variant thereof, and
- ii) the polypeptide of SEQ ID NO: 53 (Bir02J; PAARMFKAFILEGDKLVPK), or a variant thereof,

wherein a variant of a polypeptide is:

- I) up to 30 amino acids in length and comprises the sequence of said polypeptide, or
- II) 9 to 30 amino acids in length and comprises a sequence that has at least 65% homology to the sequence of said polypeptide and that is capable of tolerising to said polypeptide, or
- III) 9 to 30 amino acids in length and comprises a sequence of at least 9 contiguous amino acids of the sequence of said polypeptide, or a sequence that has at least 65% homology to said at least 9 contiguous amino acids, which sequence of at least 9 contiguous amino acids or homologous sequence is capable of tolerising to said polypeptide.

2. A composition according to claim 1, further comprising at least one additional polypeptide of SEQ ID NO: 72 (BIR11; FPQFKPQEITGIMK), SEQ ID NO: 71 (BIR10; GSVWAQSSSFPQFK), SEQ ID NO: 73 (BIR12A; PTGMFVAGAKYMVIQGR), SEQ ID NO: 75 (BIR13; IKYMVIQGEAGAVIRGK, SEQ ID NO: 76 (BIR14; EAGAVIRGKKKGSGGIT), SEQ ID NO: 48 (Bir01I; FNYETETTSVIPAAARK), SEQ ID NO: 54 (Bir04; PGTIKKISFPEGFPFKYV), SEQ ID NO: 67 (Bir09; ETLRAVESYLLAHSDAY), SEQ ID NO: 60 (BIR07; SNEIKIVATPDGGSILK), SEQ ID NO: 63 (Bir07C; SNEIKIVATPEGGSILK), SEQ ID NO: 77 (BIR15; SLNTLRLRRIFDLFDK) or SEQ ID NO: 78 (BIR16A; AERERIFKRFDANGEGK), or a variant of any thereof; wherein a variant of a

polypeptide is as defined in claim 1(I), (II) or (III).

3. A composition according to claim 1 or 2 comprising:

- (a) the polypeptide Bir12B (AKYMVIQGEPRVIRGK), or a variant thereof;
 - (b) the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof;
- and

- (c) the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof;

wherein a variant of a polypeptide is as defined in claim 1(I), (II) or (III).

4. A composition according to any one of the preceding claims, comprising the polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir09 (ETLLRAVESYLLAHS DAY) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, and the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof; wherein a variant of a polypeptide is as defined in claim 1(I), (II) or (III).

5. A composition according to any one of the preceding claims, comprising the polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof, and Bir09B (KEMGETLLRAVESYLLAHS); wherein a variant of a polypeptide is as defined in claim 1(I), (II) or (III).

6. A composition according to any one of the preceding claims, comprising the

polypeptide Bir12B (AKYMVIQGEPRVIRGK) or a variant thereof, the polypeptide Bir02J (PAARMFKAFILEGDKLVPK) or a variant thereof, the polypeptide Bir01I (FNYETETTSVIPAAARK) or a variant thereof, the polypeptide Bir04 (PGTIKKISFPEGFPFKYV) or a variant thereof, the polypeptide Bir07C (SNEIKIVATPEGGSILK) or a variant thereof, and the polypeptide Bir16A (AERERIFKRFDANGEGK) or a variant thereof; wherein a variant of a polypeptide is as defined in claim 1(I), (II) or (III).

7. A composition according to any one of the preceding claims, wherein:

- said variant of Bir01I is Bir01F (FNYETEATSVIPAARK), Bir01G (FNYEIEATSVIPAARK) or Bir01H (FNYEIETTSVIPAAARK); and/or
- said variant of Bir02J is Bir02E (PAARLFKAFILEGDTLIPK), Bir02G (PAARLFKAFILEGDNLIPK), Bir02I (PAARMFKAFILD) or Bir02D (PAARMFKAFILDGDKLVPK); and/or
- said variant of Bir09 is Bir09A (GETLLRAVESYLLAHS), Bir09B (KEMGETLLRAVESYLLAHS) or Bir09C (KEKGETLLRAVESYLLAHS); and/or
- said variant of Bir16A is Bir16B (AERERIFKRFDAGGEGK).

8. The composition according to any one of the preceding claims, wherein one or more of the polypeptides have one or more modifications selected from the following:

- (i) N terminal acetylation;
- (ii) C terminal amidation;
- (iii) one or more hydrogens on the side chain amines of Arginine and/or Lysine replaced with a methylene group;
- (iv) glycosylation; and
- (v) phosphorylation.

9. The composition according to any one of the preceding claims wherein at least one of the polypeptides has been engineered to be soluble such that it comprises:

- i) N terminal to the residues of the polypeptide which flank a T cell epitope: one to

six contiguous amino acids corresponding to the two to six contiguous amino acids immediately N terminal to said residues in the sequence of the protein from which the polypeptide derives; and/or

ii) C terminal to the residues of the polypeptide which flank a T cell epitope: one to six contiguous amino acids corresponding to the one to six contiguous amino acids immediately C terminal to the said residues in the sequence of the protein from which the polypeptide derives; or

iii) both N and C terminal to the residues of the polypeptide which flank a T cell epitope, at least one amino acid selected from arginine, lysine, histidine, glutamate and aspartate,

wherein the polypeptide has a solubility of at least 3.5mg/ml and the T cell epitope has a solubility of less than 3.5mg/ml.

10. The composition according to any one of the preceding claims wherein at least one of the polypeptides has been engineered to be soluble such that additionally:

i) any cysteine residues in the native sequence of the polypeptide are replaced with serine or 2-aminobutyric acid; and /or

ii) any hydrophobic residues in the upto three amino acids at the N or C terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

iii) any two consecutive amino acids comprising the sequence Asp-Gly in the upto four amino acids at the N or C terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

iv) one or more positively charged residues are added at the N and/or C terminus of the native sequence of the polypeptide.

11. The composition according to any one of the preceding claims which is a solution comprising each polypeptide at a concentration in the range of 0.03 to 200 nmol/ml.

12. The composition according to claim 11, wherein said concentration is in the range of 0.3 to 200 nmol/ml.
13. The composition according to claim 11, wherein said concentration is in the range of 50 to 200nmol/ml.
14. The composition according to claim 11, wherein said concentration is in the range of 30 to 120 nmol/ml.
15. A composition suitable for use in preventing or treating allergy to birch pollen comprising at least one polynucleotide sequence which encodes each of:
 - i) the polypeptide of SEQ ID NO: 74 (BIR12B; AKYMVIQGEPGRVIRGK), or a variant thereof as defined in claim 1 (I), (II) or (III), and
 - ii) the polypeptide of SEQ ID NO: 53 (Bir02J; PAARMFKAFILEGDKLVPK), or a variant thereof as defined in claim 1 (I), (II) or (III).
16. The composition according to any one of the preceding claims which is a pharmaceutically acceptable composition further comprising a pharmaceutically acceptable carrier or diluent.
17. The composition according to claim 16, which is formulated for oral administration, nasal administration, topical administration, subcutaneous administration, sublingual administration, intradermal administration, buccal administration, epidermal administration, or for administration by inhalation, by injection, or by a patch.
18. The composition according to any one of the preceding claims for use in a method of preventing or treating allergy to birch pollen.
19. The composition according to any one of claims 1 to 17 additionally

comprising a further polypeptide allergen for use in tolerising an individual to the further polypeptide allergen.

20. An *in vitro* method of determining whether T cells recognize a composition as defined in claim 1 comprising contacting said T cells with said composition and detecting whether said T cells are stimulated by said composition.

21. A method according to claim 20 which is carried out to determine whether an individual has, or is at risk of having, an allergy to birch pollen.

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N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Cys Asn Glu Ile Lys Ile Val Ala Thr Pro Asp Gly Gly Cys Val
1 5 10 15

<210> 22
<211> 15
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<213> Betulaceae

<400> 22

Ser Asn Glu Ile Lys Ile Val Thr Thr Pro Asp Gly Gly Cys Val
1 5 10 15

<210> 23
<211> 15
<212> PRT
<213> Betulaceae

<400> 23

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Asp Gly Gly Pro Ile
1 5 10 15

<210> 24
<211> 17
<212> PRT
<213> Betulaceae

<400> 24

Gly Ser Ile Leu Lys Ile Arg Asn Lys Tyr His Thr Lys Gly Asp His
1 5 10 15

Glu

<210> 25
<211> 12
<212> PRT
<213> Betulaceae

<400> 25

Ala Gly Leu Phe Lys Ala Val Glu Asn Tyr Leu Val
1 5 10

<210> 26
<211> 12
<212> PRT
<213> Betulaceae

<400> 26

Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu
1 5 10

<210> 27
<211> 12
<212> PRT
<213> Betulaceae

<400> 27

Glu Ala Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu
1 5 10

<210> 28

<211> 12

<212> PRT

<213> Betulaceae

<400> 28

Glu Ala Leu Phe Arg Ala Val Glu Ser Tyr Leu Leu
1 5 10

<210> 29

<211> 18

<212> PRT

<213> Betulaceae

<400> 29

Glu Lys Ala Val Gly Leu Leu Lys Ala Val Glu Ser Tyr Leu Leu Ala
1 5 10 15

His Ser

<210> 30

<211> 20

<212> PRT

<213> Betulaceae

<400> 30

Gly Glu Thr Leu Leu Arg Ala Val Glu Gly Tyr Leu Leu Ala His Ser
1 5 10 15

Asp Ala Tyr Asn
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<210> 31

<211> 15

<212> PRT

<213> Betulaceae

<400> 31

Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Pro Leu Ala His Ser
1 5 10 15

<210> 32

<211> 19

<212> PRT

<213> Betulaceae

<400> 32

Ala Gly Leu Phe Lys Ala Val Glu Asn Tyr Leu Val Ala His Pro Asn
1 5 10 15

Ala Tyr Asn

<210> 33
 <211> 19
 <212> PRT
 <213> Betulaceae

<400> 33

Glu Thr Leu Leu Arg Ala Val Glu Arg Tyr Leu Leu Ala His Ser Asp
 1 5 10 15

Ala Tyr Asn

<210> 34
 <211> 19
 <212> PRT
 <213> Betulaceae

<400> 34

Glu Ala Leu Phe Arg Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp
 1 5 10 15

Ala Tyr Asn

<210> 35
 <211> 18
 <212> PRT
 <213> Betulaceae

<400> 35

Val Asp Phe Phe Glu Phe Lys Asp Met Met Arg Ser Val Leu Val Arg
 1 5 10 15

Ser Ser

<210> 36
 <400> 36
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 <210> 37
 <211> 18
 <212> PRT
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<400> 37

Ala Glu Arg Glu Arg Ile Phe Lys Arg Phe Asp Ala Asn Gly Asp Gly
 1 5 10 15

Lys Ile

<210> 38
 <211> 15
 <212> PRT
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<400> 38

Phe Thr Asp Phe Gly Arg Ala Asn Arg Gly Leu Leu Lys Asp Val
 1 5 10 15

<210> 39
 <211> 15
 <212> PRT
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<400> 39

Phe Thr Asp Phe Ala Arg Ala Asn Arg Gly Leu Leu Lys Asp Val
 1 5 10 15

<210> 40
 <211> 21
 <212> PRT
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<400> 40

Pro Val Lys Gly Lys Leu Val Glu Lys Phe Lys Gly Leu Gly Val Thr
 1 5 10 15

Leu Leu His Gly Asp
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<210> 41
 <211> 24
 <212> PRT
 <213> Betulaceae

<400> 41

His Glu Ser Leu Val Lys Ala Phe Lys Gln Val Asp Val Val Ile Ser
 1 5 10 15

Thr Val Gly His Leu Gln Leu Ala
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<210> 42
 <211> 22
 <212> PRT
 <213> Betulaceae

<400> 42

Tyr Val Ser Ser Asn Phe Phe Ala Gly Tyr Phe Leu Pro Thr Leu Ala
 1 5 10 15

Gln Pro Gly Leu Thr Ser
 20

<210> 43
 <211> 17
 <212> PRT
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<400> 43

Pro Ile Asn Val Ile Leu Ala Ile Asn His Ser Val Phe Val Lys Gly
 1 5 10 15

Asp

<210> 44
 <211> 15
 <212> PRT
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<400> 44

Ala Glu Asn Phe Arg Ala Leu Cys Thr Gly Glu Lys Gly Asn Gly
 1 5 10 15

<210> 45
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 45

Phe Asn Tyr Glu Thr Glu Ala Thr Ser Val Ile Pro Ala Ala Arg Lys
 1 5 10 15

<210> 46
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 46

Phe Asn Tyr Glu Ile Glu Ala Thr Ser Val Ile Pro Ala Ala Arg Lys
 1 5 10 15

<210> 47
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 47

Phe Asn Tyr Glu Ile Glu Thr Thr Ser Val Ile Pro Ala Ala Arg Lys
 1 5 10 15

<210> 48
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 48

Phe Asn Tyr Glu Thr Glu Thr Thr Ser Val Ile Pro Ala Ala Arg Lys
1 5 10 15

<210> 49
<211> 19
<212> PRT
<213> Betulaceae

<400> 49

Pro Ala Ala Arg Met Phe Lys Ala Phe Ile Leu Asp Gly Asp Lys Leu
1 5 10 15

Val Pro Lys

<210> 50
<211> 19
<212> PRT
<213> Betulaceae

<400> 50

Pro Ala Ala Arg Leu Phe Lys Ala Phe Ile Leu Glu Gly Asp Thr Leu
1 5 10 15

Ile Pro Lys

<210> 51
<211> 19
<212> PRT
<213> Betulaceae

<400> 51

Pro Ala Ala Arg Leu Phe Lys Ala Phe Ile Leu Glu Gly Asp Asn Leu
1 5 10 15

Ile Pro Lys

<210> 52
<211> 12
<212> PRT
<213> Betulaceae

<400> 52

Pro Ala Ala Arg Met Phe Lys Ala Phe Ile Leu Asp
1 5 10

<210> 53
<211> 19
<212> PRT
<213> Betulaceae

<400> 53

Pro Ala Ala Arg Met Phe Lys Ala Phe Ile Leu Glu Gly Asp Lys Leu
Page 10

1 5 10 15

Val Pro Lys

<210> 54
<211> 18
<212> PRT
<213> Betulaceae

<400> 54

Pro Gly Thr Ile Lys Lys Ile Ser Phe Pro Glu Gly Phe Pro Phe Lys
1 5 10 15

Tyr Val

<210> 55
<211> 16
<212> PRT
<213> Betulaceae

<400> 55

Ser Pro Phe Lys Tyr Val Lys Glu Arg Val Asp Glu Val Asp His Ala
1 5 10 15

<210> 56
<211> 16
<212> PRT
<213> Betulaceae

<400> 56

Phe Pro Phe Lys Tyr Val Lys Asp Arg Val Asp Glu Val Asp His Thr
1 5 10 15

<210> 57
<211> 17
<212> PRT
<213> Betulaceae

<400> 57

Ala Asn Phe Lys Tyr Ser Tyr Ser Met Ile Glu Gly Gly Ala Leu Gly
1 5 10 15

Asp

<210> 58
<211> 17
<212> PRT
<213> Betulaceae

<400> 58

Thr Asn Phe Lys Tyr Ser Tyr Ser Val Ile Glu Gly Gly Pro Val Gly
1 5 10 15

Asp

<210> 59
 <211> 16
 <212> PRT
 <213> Betulaceae
 <400> 59

Thr Asn Phe Lys Tyr Asn Tyr Ser Val Ile Glu Gly Gly Pro Ile Gly
 1 5 10 15

<210> 60
 <211> 17
 <212> PRT
 <213> Betulaceae
 <400> 60

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Asp Gly Gly Ser Ile Leu
 1 5 10 15

Lys

<210> 61
 <211> 17
 <212> PRT
 <213> Betulaceae
 <400> 61

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Asn Gly Gly Ser Ile Leu
 1 5 10 15

Lys

<210> 62
 <211> 17
 <212> PRT
 <213> Betulaceae
 <400> 62

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Gln Gly Gly Ser Ile Leu
 1 5 10 15

Lys

<210> 63
 <211> 17
 <212> PRT
 <213> Betulaceae
 <400> 63

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Glu Gly Gly Ser Ile Leu
1 5 10 15

Lys

<210> 64
<211> 17
<212> PRT
<213> Betulaceae

<400> 64

Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Glu Gly Gly Ser Ile Leu
1 5 10 15

Lys

<210> 65
<211> 15
<212> PRT
<213> Betulaceae

<400> 65

Gly Ser Ile Leu Lys Ile Asn Asn Lys Tyr His Thr Lys Gly Asp
1 5 10 15

<210> 66
<211> 14
<212> PRT
<213> Betulaceae

<400> 66

Ser Ile Leu Lys Ile Ser Asn Lys Tyr His Thr Lys Gly Asp
1 5 10

<210> 67
<211> 18
<212> PRT
<213> Betulaceae

<400> 67

Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp
1 5 10 15

Ala Tyr

<210> 68
<211> 16
<212> PRT
<213> Betulaceae

<400> 68

Gly Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu Ala His Ser
 1 5 10 15

<210> 69
 <211> 19
 <212> PRT
 <213> Betulaceae

<400> 69

Lys Glu Met Gly Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu
 1 5 10 15

Ala His Ser

<210> 70
 <211> 19
 <212> PRT
 <213> Betulaceae

<400> 70

Lys Glu Lys Gly Glu Thr Leu Leu Arg Ala Val Glu Ser Tyr Leu Leu
 1 5 10 15

Ala His Ser

<210> 71
 <211> 14
 <212> PRT
 <213> Betulaceae

<400> 71

Gly Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys
 1 5 10

<210> 72
 <211> 14
 <212> PRT
 <213> Betulaceae

<400> 72

Phe Pro Gln Phe Lys Pro Gln Glu Ile Thr Gly Ile Met Lys
 1 5 10

<210> 73
 <211> 17
 <212> PRT
 <213> Betulaceae

<400> 73

Pro Thr Gly Met Phe Val Ala Gly Ala Lys Tyr Met Val Ile Gln Gly
 1 5 10 15

Arg

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

<210> 74
 <211> 17
 <212> PRT
 <213> Betulaceae

<400> 74

Ala Lys Tyr Met Val Ile Gln Gly Glu Pro Gly Arg Val Ile Arg Gly
 1 5 10 15

Lys

<210> 75
 <211> 18
 <212> PRT
 <213> Betulaceae

<400> 75

Gly Ile Lys Tyr Met Val Ile Gln Gly Glu Ala Gly Ala Val Ile Arg
 1 5 10 15

Gly Lys

<210> 76
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 76

Glu Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr
 1 5 10 15

<210> 77
 <211> 16
 <212> PRT
 <213> Betulaceae

<400> 77

Ser Leu Asn Thr Leu Arg Leu Arg Arg Ile Phe Asp Leu Phe Asp Lys
 1 5 10 15

<210> 78
 <211> 17
 <212> PRT
 <213> Betulaceae

<400> 78

Ala Glu Arg Glu Arg Ile Phe Lys Arg Phe Asp Ala Asn Gly Glu Gly
 1 5 10 15

Lys

<210> 79
 <211> 17
 <212> PRT
 <213> Betulaceae

<400> 79

Ala Glu Arg Glu Arg Ile Phe Lys Arg Phe Asp Ala Gly Gly Glu Gly
 1 5 10 15

Lys

<210> 80
 <211> 19
 <212> PRT
 <213> Betulaceae

<400> 80

Val Lys Gly Lys Leu Val Glu Lys Phe Lys Gly Leu Gly Val Thr Leu
 1 5 10 15

Leu His Gly

<210> 81
 <211> 15
 <212> PRT
 <213> Mycobacterium tuberculosis / leprae

<400> 81

Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1 5 10 15

<210> 82
 <211> 13
 <212> PRT
 <213> Influenza virus

<400> 82

Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
 1 5 10

<210> 83
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 83

Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
 1 5 10 15

<210> 84
 <211> 145
 <212> PRT

<213> Oleaceae

<400> 84

Glu Asp Ile Pro Gln Pro Pro Val Ser Gln Phe His Ile Gln Gly Gln
1 5 10 15

Val Tyr Cys Asp Thr Cys Arg Ala Gly Phe Ile Thr Glu Leu Ser Glu
20 25 30

Phe Ile Pro Gly Ala Ser Leu Arg Leu Gln Cys Lys Asp Lys Glu Asn
35 40 45

Gly Asp Val Thr Phe Thr Glu Val Gly Tyr Thr Arg Ala Glu Gly Leu
50 55 60

Tyr Ser Met Leu Val Glu Arg Asp His Lys Asn Glu Phe Cys Glu Ile
65 70 75 80

Thr Leu Ile Ser Ser Gly Arg Lys Asp Cys Asn Glu Ile Pro Thr Glu
85 90 95

Gly Trp Ala Lys Pro Ser Leu Lys Phe Lys Leu Asn Thr Val Asn Gly
100 105 110

Thr Thr Arg Thr Val Asn Pro Leu Gly Phe Phe Lys Lys Glu Ala Leu
115 120 125

Pro Lys Cys Ala Gln Val Tyr Asn Lys Leu Gly Met Tyr Pro Pro Asn
130 135 140

Met
145

<210> 85

<211> 133

<212> PRT

<213> Betulaceae

<400> 85

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
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65

70

75

80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 86

<211> 133

<212> PRT

<213> Betulaceae

<400> 86

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Gly Glu Glu Leu Ala Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65 70 75 80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 87

<211> 133

<212> PRT

<213> Betulaceae

<400> 87

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65 70 75 80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 88
<211> 133
<212> PRT
<213> Betulaceae

<400> 88

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65 70 75 80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 89
<211> 133
<212> PRT
<213> Betulaceae

<400> 89

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65 70 75 80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 90
<211> 133
<212> PRT
<213> Betulaceae

<400> 90

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
1 5 10 15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
20 25 30

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
35 40 45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
50 55 60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65 70 75 80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
85 90 95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
100 105 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
115 120 125

Ile Asp Gln Gly Leu
130

<210> 91
<211> 205
<212> PRT
<213> Betulaceae

<400> 91

Met Pro Cys Ser Thr Glu Ala Met Glu Lys Ala Gly His Gly His Ala
1 5 10 15

Ser Thr Pro Arg Lys Arg Ser Leu Ser Asn Ser Ser Phe Arg Leu Arg
20 25 30

Ser Glu Ser Leu Asn Thr Leu Arg Leu Arg Arg Ile Phe Asp Leu Phe
35 40 45

Asp Lys Asn Ser Asp Gly Ile Ile Thr Val Asp Glu Leu Ser Arg Ala
50 55 60

Leu Asn Leu Leu Gly Leu Glu Thr Asp Leu Ser Glu Leu Glu Ser Thr
65 70 75 80

Val Lys Ser Phe Thr Arg Glu Gly Asn Ile Gly Leu Gln Phe Glu Asp
85 90 95

Phe Ile Ser Leu His Gln Ser Leu Asn Asp Ser Tyr Phe Ala Tyr Gly
100 105 110

Gly Glu Asp Glu Asp Asp Asn Glu Glu Asp Met Arg Lys Ser Ile Leu
115 120 125

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Ser Gln Glu Glu Ala Asp Ser Phe Gly Gly Phe Lys Val Phe Asp Glu
130 135 140

Asp Gly Asp Gly Tyr Ile Ser Ala Arg Glu Leu Gln Met Val Leu Gly
145 150 155 160

Lys Leu Gly Phe Ser Glu Gly Ser Glu Ile Asp Arg Val Glu Lys Met
165 170 175

Ile Val Ser Val Asp Ser Asn Arg Asp Gly Arg Val Asp Phe Phe Glu
180 185 190

Phe Lys Asp Met Met Arg Ser Val Leu Val Arg Ser Ser
195 200 205

<210> 92
<211> 85
<212> PRT
<213> Betulaceae

<400> 92

Met Ala Asp Asp His Pro Gln Asp Lys Ala Glu Arg Glu Arg Ile Phe
1 5 10 15

Lys Arg Phe Asp Ala Asn Gly Asp Gly Lys Ile Ser Ala Ala Glu Leu
20 25 30

Gly Glu Ala Leu Lys Thr Leu Gly Ser Ile Thr Pro Asp Glu Val Lys
35 40 45

His Met Met Ala Glu Ile Asp Thr Asp Gly Asp Gly Phe Ile Ser Phe
50 55 60

Gln Glu Phe Thr Asp Phe Gly Arg Ala Asn Arg Gly Leu Leu Lys Asp
65 70 75 80

Val Ala Lys Ile Phe
85

<210> 93
<211> 24
<212> PRT
<213> Quercus alba

<220>
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<222> (5)..(5)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa can be any naturally occurring amino acid

<400> 93

Gly Val Phe Thr Xaa Glu Ser Gln Glu Thr Ser Val Ile Ala Pro Ala
1 5 10 15

Xaa Leu Phe Lys Ala Leu Phe Leu
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<210> 94

<211> 40

<212> PRT

<213> Carpinus betulus

<220>

<221> misc_feature

<222> (39)..(39)

<223> Xaa can be any naturally occurring amino acid

<400> 94

Gly Val Phe Asn Tyr Glu Ala Glu Thr Pro Ser Val Ile Pro Ala Ala
1 5 10 15

Arg Leu Phe Lys Ser Tyr Val Leu Asp Gly Asp Lys Leu Ile Pro Lys
20 25 30

Val Ala Pro Gln Ala Ile Xaa Lys
35 40

<210> 95

<211> 44

<212> PRT

<213> Alnus glutinosa

<400> 95

Gly Val Phe Asn Tyr Glu Ala Glu Thr Pro Ser Val Ile Pro Ala Ala
1 5 10 15

Arg Leu Phe Lys Ala Phe Ile Leu Asp Gly Asp Lys Leu Leu Pro Lys
20 25 30

Val Ala Pro Glu Ala Val Ser Ser Val Glu Asn Ile
35 40

<210> 96

<211> 110

<212> PRT

<213> Betula pendula

<220>

<223> Rubisco

<400> 96

Val Gln Cys Met Gln Val Trp Pro Pro Leu Gly Leu Lys Lys Phe Glu
1 5 10 15

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Thr Leu Ser Tyr Leu Pro Pro Leu Ser Ser Glu Gln Leu Ala Lys Glu
20 25 30

Val Asp Tyr Leu Leu Arg Lys Asn Leu Ile Pro Cys Leu Glu Phe Glu
35 40 45

Leu Glu His Gly Phe Val Tyr Arg Glu His Asn Arg Ser Pro Gly Tyr
50 55 60

Tyr Asp Gly Arg Tyr Trp Thr Met Trp Lys Leu Pro Met Phe Gly Cys
65 70 75 80

Asn Asp Ser Ser Gln Val Leu Lys Glu Leu Glu Glu Cys Lys Lys Ala
85 90 95

Tyr Pro Ser Ala Phe Ile Arg Ile Ile Gly Phe Asp Asp Lys
100 105 110

<210> 97
<211> 373
<212> PRT
<213> Pinaceae

<400> 97

Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe Val Ile
1 5 10 15

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
20 25 30

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
35 40 45

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
50 55 60

Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Pro Gly Thr Leu Arg Tyr
65 70 75 80

Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn Met
85 90 95

Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr Phe
100 105 110

Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys Val
115 120 125

Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu Tyr Leu Tyr
130 135 140

Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser Phe
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145 150 155 160

Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu Arg
 165 170 175

Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser Ser
 180 185 190

Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr Ile Ser
 195 200 205

Asn Asn Leu Phe Phe Asn His His Lys Val Met Ser Leu Gly His Asp
 210 215 220

Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe Asn
225 230 235 240

Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr Gly
 245 250 255

Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr Ala
 260 265 270

Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser Phe
 275 280 285

Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile Gly
 290 295 300

Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr Gln
305 310 315 320

Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu
 325 330 335

Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn Gly
 340 345 350

Asn Ala Thr Pro His Leu Thr Gln Asn Ala Gly Val Leu Thr Cys Ser
 355 360 365

Leu Ser Lys Arg Cys
 370

<210> 98
<211> 374
<212> PRT
<213> Pinaceae

<400> 98

Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Leu Ser Phe Val Ile
1 5 10 15

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
20 25 30

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
35 40 45

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
50 55 60

Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr Leu Arg
65 70 75 80

Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn
85 90 95

Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr
100 105 110

Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys
115 120 125

Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu His Leu
130 135 140

Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser
145 150 155 160

Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu
165 170 175

Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser
180 185 190

Ser Asp Gly Leu Val Asp Val Thr Leu Ser Ser Thr Gly Val Thr Ile
195 200 205

Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His
210 215 220

Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe
225 230 235 240

Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr
245 250 255

Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr
260 265 270

Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser
275 280 285

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile
290 295 300

Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr
305 310 315 320

Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr
325 330 335

Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn
340 345 350

Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr Cys
355 360 365

Ser Leu Ser Lys Arg Cys
370

<210> 99
<211> 514
<212> PRT
<213> *Cryptomeria japonica*

<400> 99

Met Ala Met Lys Leu Ile Ala Pro Met Ala Phe Leu Ala Met Gln Leu
1 5 10 15

Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp
20 25 30

Ser Val Val Glu Lys Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val
35 40 45

Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr
50 55 60

Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr
65 70 75 80

Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro
85 90 95

Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys
100 105 110

Gln Pro His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln
115 120 125

Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
130 135 140

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Leu Thr Gly Phe Thr Leu Met Gly Lys Gly Val Ile Asp Gly Gln Gly
 145 150 155 160
 Lys Gln Trp Trp Ala Gly Gln Cys Lys Trp Val Asn Gly Arg Glu Ile
 165 170 175
 Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr
 180 185 190
 Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His
 195 200 205
 Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
 210 215 220
 Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala
 225 230 235 240
 Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp
 245 250 255
 Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu
 260 265 270
 Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu
 275 280 285
 Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
 290 295 300
 Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser
 305 310 315 320
 Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser
 325 330 335
 Glu Asn Pro Ile Leu Ile Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala
 340 345 350
 Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys
 355 360 365
 Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
 370 375 380
 Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu
 385 390 395 400
 Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn
 405 410 415

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro
420 425 430

Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val
435 440 445

Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile
450 455 460

Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys
465 470 475 480

Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln
485 490 495

Glu Tyr Tyr Pro Gln Arg Trp Ile Cys Ser Cys His Gly Lys Ile Tyr
500 505 510

His Pro

<210> 100
<211> 514
<212> PRT
<213> Cryptomeria japonica
<400> 100

Met Ala Met Lys Phe Ile Ala Pro Met Ala Phe Val Ala Met Gln Leu
1 5 10 15

Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp
20 25 30

Ser Asp Ile Glu Gln Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val
35 40 45

Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr
50 55 60

Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr
65 70 75 80

Ala Trp Gln Ala Ala Cys Lys Lys Pro Ser Ala Met Leu Leu Val Pro
85 90 95

Gly Asn Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys
100 105 110

Gln Pro His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln
115 120 125

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt
 Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
 130 135 140

Leu Thr Gly Phe Thr Leu Met Gly Lys Gly Val Ile Asp Gly Gln Gly
 145 150 155 160

Lys Gln Trp Trp Ala Gly Gln Cys Lys Trp Val Asn Gly Arg Glu Ile
 165 170 175

Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr
 180 185 190

Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His
 195 200 205

Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
 210 215 220

Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala
 225 230 235 240

Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp
 245 250 255

Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu
 260 265 270

Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu
 275 280 285

Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
 290 295 300

Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser
 305 310 315 320

Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser
 325 330 335

Glu Asn Pro Ile Leu Ile Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala
 340 345 350

Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys
 355 360 365

Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
 370 375 380

Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu
 385 390 395 400

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn
405 410 415

Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro
420 425 430

Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val
435 440 445

Met Val Lys Asn Met Gly Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile
450 455 460

Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys
465 470 475 480

Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln
485 490 495

Glu Tyr Tyr Pro Gln Arg Trp Met Cys Ser Arg His Gly Lys Ile Tyr
500 505 510

His Pro

<210> 101
<211> 373
<212> PRT
<213> Cryptomeria japonica
<400> 101

Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Leu Ser Phe Val Ile
1 5 10 15

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
20 25 30

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
35 40 45

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
50 55 60

Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Pro Gly Thr Leu Arg Tyr
65 70 75 80

Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn Met
85 90 95

Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr Phe
100 105 110

Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys Val

Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu His Leu Tyr
130 135 140

Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser Phe
145 150 155 160

Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu Arg
165 170 175

Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser Ser
180 185 190

Asp Gly Leu Val Asp Val Thr Leu Ser Ser Thr Gly Val Thr Ile Ser
195 200 205

Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His Asp
210 215 220

Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe Asn
225 230 235 240

Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr Gly
245 250 255

Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr Ala
260 265 270

Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser Phe
275 280 285

Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile Gly
290 295 300

Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr Gln
305 310 315 320

Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu
325 330 335

Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn Gly
340 345 350

Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr Cys Ser
355 360 365

Leu Ser Lys Arg Cys
370

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

<211> 374

<212> PRT

<213> *Cryptomeria japonica*

<400> 102

Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe Val Ile
1 5 10 15

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
20 25 30

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
35 40 45

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
50 55 60

Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr Leu Arg
65 70 75 80

Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn
85 90 95

Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr
100 105 110

Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys
115 120 125

Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu Tyr Leu
130 135 140

Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser
145 150 155 160

Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu
165 170 175

Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser
180 185 190

Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr Ile
195 200 205

Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Ser Leu Gly His
210 215 220

Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe
225 230 235 240

Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr
245 250 255

N110180D GCW GML EMB seq listing for phasing - Jul 12.txt

Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr
260 265 270

Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser
275 280 285

Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile
290 295 300

Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr
305 310 315 320

Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr
325 330 335

Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn
340 345 350

Gly Asn Ala Thr Pro His Leu Thr Gln Asn Ala Gly Val Leu Thr Cys
355 360 365

Ser Leu Ser Lys Arg Cys
370