

## (51) International Patent Classification:

A61K 39/00 (2006.01) C07K 14/40 (2006.01)

C07K 7/04 (2006.01)

## (21) International Application Number:

PCT/EP2012/002310

## (22) International Filing Date:

31 May 2012 (31.05.2012)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

11004486.4 1 June 2011 (01.06.2011) EP

## (71) Applicant (for all designated States except US): ETH Zurich [CH/CH]; Raemistrasse 101 / ETH transfer, CH-8092 Zurich (CH).

## (72) Inventors; and

## (75) Inventors/Applicants (for US only): LEIBUNDGUT-LANDMANN, Salomé [CH/CH]; Imbisbühlstrasse 106, CH-8049 Zurich/ Zurich (CH). BÄR, Eva [DE/CH]; Üetlibergstrasse 29, CH-8045 Zurich (CH).

## (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

## Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

## (54) Title: T CELL EPITOPE OF CANDIDA ALBICANS

Fig. 1

|        | SEQ ID No. 1         | 1   | KGLNDWNYPVSSSESFSYT | 18  |        |
|--------|----------------------|-----|---------------------|-----|--------|
| ALS1   | <i>C. albicans</i>   | 236 | KGLNDWNYPVSSSESFSYT | 253 | P46590 |
| ALS3   | <i>C. albicans</i>   | 236 | KGLNDWNYPVSSSESFSYT | 253 | O74623 |
| ALS9   | <i>C. albicans</i>   | 236 | KGLNDWNFPVSSSESFSYT | 253 | Q9C471 |
| ALS4   | <i>C. albicans</i>   | 237 | GLNDWNYPISSESFSYT   | 253 | O74660 |
| ALS5   | <i>C. albicans</i>   | 236 | KGVDWNHPTVSESFSYT   | 253 | Q874K9 |
| ALS2   | <i>C. albicans</i>   | 236 | KGLNDWNFPVSSDSLST   | 252 | O74657 |
| ALS6   | <i>C. albicans</i>   | 238 | GLNSWNMPVSSSESFSYT  | 254 | Q9Y8F2 |
| ALS9   | <i>C. dubliensis</i> | 235 | KGLNDWNFPVSSSESFSYT | 252 | B9WJ72 |
| ALSD2* | <i>C. dubliensis</i> | 237 | GVNDWNFPVSSSESFSYT  | 253 | Q9HF70 |
| ALSD3* | <i>C. dubliensis</i> | 237 | GLNDWNYPISSEKSFST   | 253 | Q9HF69 |
| ALS2   | <i>C. dubliensis</i> | 235 | KGVDWNFPVSYDSFSY    | 251 | B9WJD2 |
| ALSD1* | <i>C. dubliensis</i> | 238 | GLNSWNMPVSSDSFSYT   | 254 | Q9HF72 |
| *      | <i>C. tropicalis</i> | 240 | LNDWNQPMNSEFSYT     | 255 | C5MDW9 |
|        | <i>C. tropicalis</i> | 240 | LNDWNQPTNSETFSYT    | 255 | C5M4Q1 |
|        | <i>C. tropicalis</i> | 240 | LNSWNYPTSDSFSYT     | 255 | C5MDN9 |
|        | <i>C. tropicalis</i> | 240 | LNPWNQPMNAESFSYT    | 255 | C5M4P0 |

(57) Abstract: The invention provides an epitope of T helper cells. The peptide comprises the amino acid sequence of SEQ ID No. 1 or a derivative thereof. Said peptide is useful for the preparations of compositions or vaccines for fungal infections, in particular Candidiasis.

## T cell epitope of *Candida albicans*

### Field of the invention

- 5 The present invention relates to T cell epitopes derived from *Candida albicans* ALS protein, useful for the production of anti-fungal vaccines and in the treatment of fungal infections.

### Background of the invention

10

Fungi have emerged as major causes of human disease, especially among those who are immunocompromised or hospitalized with serious underlying diseases. The frequency of invasive fungal infections has clearly increased in recent decades and opportunistic fungal pathogens are among the first four causes of hospital-acquired blood stream infections.

15

*Candida albicans* is the most important fungal pathogen. However, the spectrum of clinically relevant fungal pathogens is widening. At the same time, the frequency of fungi resistant to antifungal drugs is increasing and the number of patients at risk for opportunistic infections is rising.

20

*C. albicans* is a commensal fungal organism that colonizes the skin and the mucosal surfaces of normal individuals, without causing disease. However, when normal host defence mechanisms are impaired, *C. albicans* can become pathogenic. The clinical spectrum of *C. albicans* infections ranges from mucocutaneous and vulvovaginal to disseminated life threatening infections. Invasive candidiasis is a consequence of the

25

haematogenous spread of *C. albicans* to multiple organs, including the kidneys, brain, heart, liver and lungs. Invasive candidiasis has an incidence of up to 30 cases per 100'000 individuals, and an associated mortality of more than 30% and a high morbidity in those who survive. Systemic *Candida* infections are most often seen in patients who have taken broad spectrum antibiotics, have had a central venous catheter implanted or

30

prolonged hospital and intensive care unit stay. Oropharyngeal candidiasis (thrush) is frequent problem in HIV infected individuals. Conventional chemotherapy as treatment strategy has proven to be unsatisfactory because of fungal resistances and its high toxicity.

The rising incidence of these different fungal diseases and the increase of resistance against classical antifungal drugs necessitate the development of novel preventive and therapeutic strategies.

- 5 To date, no anti-fungal vaccine is available. Most fungal diseases typically occur in immunocompromised individuals which are not prone to vaccination (at least to active immunisation). Nevertheless, a high number of at-risk patients could benefit from a vaccine: candidate patients for hematopoietic stem cell transplant, candidate patients for solid organ transplant, patients with acute myeloid leukemia or solid tumors, IBD patients  
10 before the use of immunosuppressive corticosteroids, patients undergoing deep surgery (especially gastrointestinal surgery), patients admitted to intensive care units, etc.

- WO07/081896 describes a vaccine including the N-terminal end of the ALS1 protein (Agglutinin-like protein 1) from *C. albicans*, in particular the protein encoded by the  
15 nucleotide stretch 52-1296, and the homologous region of the ALS3 protein. The protection induced by the vaccine is mediated by T cells in mice and in human probably also by antibodies. However, the use of a large protein for vaccination instead of a small peptide may elicit a mixed response and lymphocytes of various specificities may be primed. The fraction of protective lymphocytes may thus be diluted by cells with non-  
20 protective specificities and the efficiency of the vaccine may thereby be reduced. Furthermore, the preparation of a large protein by recombinant technology may contain contaminating substances (e.g. endotoxin) which may cause a risk of uncontrolled, adverse or toxic reactions.

## 25 **Summary of the invention**

- A *C. albicans*-derived T cell epitope was identified. The peptide is derived of the N-terminal part of the *C. albicans* ALS1 protein and covers amino acids 236-253. The same sequence stretch is also found in the *C. albicans* ALS3 protein. A vaccine comprising said  
30 epitope will elicit a well-defined immune response characterized by T cells of defined and protective specificity. Furthermore, by the choice of a suitable adjuvant the T cells can be skewed towards a well-defined effector type that is known to mediate protection from fungal infections. In addition, selective B cell epitopes can be introduced. The risk of inducing undefined and potentially non-protective or even self-reactive responses with the  
35 identified peptide is strongly reduced compared to responses induced with the larger fragment of the ALS1/ALS3 protein available in the art. Because the said peptide is small and can be synthesized synthetically and with a high degree of purity, a vaccine

comprising said epitope will generate less ill-defined, adverse or toxic reactions than the larger subunit available in the art.

5 A central part of said epitope has been identified as represented by SEQ ID No. 7. An epitope derived from SEQ ID No. 1 should ideally comprise (or at least consist of) SEQ ID No. 7.

10 Thus, in a first aspect, the invention provides a peptide having T helper cell stimulating activity, i.e. a T cell epitope; said peptide comprises the amino acid sequence of SEQ ID No. 1 or a derivative thereof wherein 1, 2, 3 or several amino acids are substituted, lacking and/or added.

15 In another aspect, the invention provides a T- helper cell epitope of a *C. albicans* ALS protein comprising or consisting of a peptide sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7.

20 Further, the invention provides a composition comprising at least one T cell epitope being comprised within a peptide sequence, wherein the epitope has a sequence selected from the group consisting of SEQ ID No. 1 or a derivative thereof, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7. Said composition may be used in the treatment, prevention or alleviation of symptoms of fungal infections and/or as vaccine and/or as immunomodulating agent.

25 In a further aspect, the invention provides a method of inducing an immune response in a subject, wherein the peptide disclosed herein is administered to the subject. In another embodiment, the method involves the administration of the epitope disclosed herein to the subject. In still another embodiment, the method involves the administration of the composition disclosed herein to the subject.

30 The invention also contemplates a method for enriching a T helper cell population in vitro by employing a peptide immunogen comprising the T cell epitope disclosed herein.

35 In still another aspect, the invention provides a method of treating, preventing or alleviating symptoms of fungal infection in a subject, comprising administering to the subject a vaccine comprising a peptide disclosed herein or an immunologically active

fragment of the peptide thereof. In another embodiment of said method, the composition disclosed herein is administered to the subject.

Also described herein is a method for isolating T cells being specific for the peptide

5 disclosed herein comprising the steps of

- (i) obtaining T cells from a suitable donor;
- (ii) screening the donor T-cells for their specificity for said peptide;
- (iii) selecting those T-cell clones which recognize the peptide; and
- (iv) clonally expanding the selected T-cells.

10

Specific preferred embodiments of the invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

#### Brief description of the figures

15

The invention will be better understood and objects other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such description makes reference to the annexed drawings, wherein:

- 20 Figure 1 shows the sequence alignments of SEQ ID No. 1 to the most homologous sequence stretches from *C. albicans*, *C. dubliniensis* and *C. tropicalis*, as identified by a database search. Underlined letters indicate amino acids which differ from SEQ ID No. 1. Asterix marked: *C. dubliniensis* proteins ALSD2, ALSD3 and ALSD1 are putative agglutinin-like proteins; listed *C. tropicalis* proteins are conserved hypothetical proteins.
- 25 Digits in column 3 and 5 indicate the first and last amino acid shown. Column 6: SwissProt accession number.

- Figure 2 shows the response of T helper cells isolated from the lymph nodes of *C. albicans*-infected mice (infected once [(1) in figure 2A, open triangles in figure 2B] or twice consecutively [(2) in figure 2A, filled triangles in figure 2B]) to the entire fungus (*C. albicans*) or SEQ ID No. 1 (peptide pALS<sub>236-253</sub>). The response of T helper cells is reflected by their IL-17A production which was measured either by intracellular cytokine staining and flow cytometry (figure 2A, numbers indicate the % of IL-17A-producing CD4<sup>+</sup>CD3<sup>+</sup>Vβ<sup>+</sup> cells) or by ELISA (figure 2B) after re-stimulation of the cells in vitro with *C. albicans* or peptide pALS<sub>236-253</sub> (5 µg/ml in figure 2A, 5 µg/ml to 5 ng/ml in figure 2B) or medium (M).
- 30
- 35

Figure 3 shows that the response of *C. albicans*-specific T helper cells to SEQ ID No. 1 is dependent on MHC class II. Draining lymph nodes of *C. albicans*-infected mice were re-stimulated in vitro with the indicated concentrations of peptide of SEQ ID No. 1 (pALS<sub>236-253</sub>) in the presence (+ anti-MHC II in figure 3A, filled circles in figures 3B and 3C) or absence of a blocking antibody specific for MHC class II (- anti-MHC II in figure 3A, filled squares in figures 3B and 3C). IL-17A production by T helper cells was measured by intracellular cytokine staining and flow cytometry (figure 3A, numbers indicate the % of IL-17A-producing CD4<sup>+</sup>CD3<sup>+</sup>Vβ<sup>+</sup> cells) or by ELISA (figures 3B and 3C). Data in figures 3A and 3C are from mice infected twice consecutively, data in figure 3B are from mice infected once.

Figure 4 shows a comparison of the T helper cell response to SEQ ID No. 1 and previously described T cell epitopes from *C. albicans*. Lymph nodes of *C. albicans*-infected mice were re-stimulated in vitro with SEQ ID No. 1 (peptide pALS<sub>236-253</sub>, filled circles in figures 4C and 4D), pATG<sub>227-242</sub> (filled triangles in figures 4C and 4D), MP65 (open squares in figures 4C and 4D) or p41 (open diamonds in figures 4C and 4D) and IL-17A production by CD4<sup>+</sup>CD3<sup>+</sup>Vβ<sup>+</sup> T helper cells was measured by intracellular cytokine staining and flow cytometry (figures 4A and 4B). Representative data are shown and the % of IL-17A producing cells is indicated. Figures 4C and 4D show secreted IL-17A in the culture supernatant as measured by ELISA. Data in figures 4A and 4C are from mice infected once, data in figures 4B and 4D are from mice infected twice consecutively.

Figure 5 shows the T helper cell response to immunisation with the peptide of SEQ ID No. 1 with CpG (condition 2) or with CpG alone (condition 3); immunisation with the peptide of SEQ ID No. 1 with curdlan (condition 4) or with curdlan alone (condition 5); or without immunisation (condition 1). Draining lymph nodes were isolated 7 days after immunisation, re-stimulated in vitro with the peptide pALS<sub>236-253</sub> (striped bars), *C. albicans* (white bars) or medium (black bars); and IL-17A (figure 5A) or IFN-γ levels (figure 5B) in the culture supernatant were measured by ELISA.

Figure 6 shows the survival of mice challenged with *C. albicans* 3 weeks after they were immunized with either curdlan in incomplete Freund's adjuvant (filled squares) or the peptide of SEQ ID No. 1 and curdlan in incomplete Freund's adjuvant (filled circles). The x-axis indicates days after challenge, whereas the y-axis shows % of surviving mice.

Figure 7 shows the response of human memory T helper cells to the peptide of SEQ ID No. 1. Peripheral blood leukocytes were re-stimulated with *C. albicans*, the peptide

pALS<sub>236-253</sub> or medium (M) and cytokine production (IL-2, IL-17A and IFN- $\gamma$ ) by CD4<sup>+</sup>CD45RA<sup>-</sup> cells was analyzed by intracellular cytokine staining and flow cytometry. Data from one representative blood donor is shown in figure 7A. The results from a selection of individuals are shown in figures 7B and 7C, whereby each symbol represents one donor. Re-stimulation with *C. albicans* is shown on the left; re-stimulation with peptide pALS<sub>236-253</sub> is shown on the right. The percentage of IL-2 producing (figure 7B) and IL-17A-producing (figure 7C) cells within the CD4<sup>+</sup>CD45RA<sup>-</sup> memory T helper cell population is shown.

## 10 Detailed description of the invention

It is a general object of the invention to provide T cell epitope derived from *C. albicans*-ALS1 or ALS3. Preferably, said epitope comprises at least SEQ ID No. 7 or at least consists of SEQ ID No. 7.

15

The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

20

## 25 Definitions

In order that the present invention may be more readily understood, certain terms will be defined as follows. Additional definitions are set forth throughout the detailed description. Unless otherwise defined, scientific and technical terms used in connection with the invention described herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

30

The term "peptide" as used herein refers to organic compounds consisting of two or more amino acids joined covalently by peptide bonds. Preferably, the molecule has a linear structure. Molecules small enough to be synthesized synthetically from the constituent amino acids are, by convention, called peptides rather than proteins. The dividing line is at

35

about 50 amino acids. Peptides dialyse through natural membranes, while proteins do not. Peptides may or may not comprise non-amino acid constituents.

The term "T helper cell" ("T<sub>H</sub> cell") refers to a subset of lymphocytes which complete  
5 maturation in the thymus and have various roles in the immune system, including the  
identification of specific foreign antigens in the body and the activation and deactivation of  
other immune cells. By this, T helper cells are involved in almost all adaptive immune  
responses. Mature T<sub>H</sub> cells are believed to always express the surface protein CD4 and  
are therefore also termed CD4+ T cells. However, not all CD4+ T cells are T helper cells:  
10 there are some exceptions such as regulatory T cells and subgroups of natural killer cells  
and cytotoxic T cells which also express CD4, but they can be distinguished by their  
expression of other specific markers known in the art.

The term "T cell epitope" refers to antigenic peptides which can be presented on the  
15 surface of antigen-presenting cells where they are bound to MHC molecules and  
recognized by T cells. T helper cells recognize peptide-bound MHC class II molecules via  
their T cell receptor. Pathogens are taken up by antigen presenting cells, killed and  
digested in the phagolysosomal compartment. The proteins of the pathogens are  
processed and degraded into peptides which are loaded onto MHC molecules and  
20 transported to the cell surface. Despite the large number of potential antigens available in  
each pathogen, T cell responses are usually restricted to a few epitopes. For most  
pathogens, including fungi, these epitopes remain largely unknown. The recognition of a  
peptide-MHC complex by a T cell leads to its activation and the induction of an adaptive  
immune response specific for the pathogen from which the peptide epitope is derived.

25 Accordingly, a peptide has "T helper cell stimulating activity" when it is able to activate T  
helper cells and induce said adaptive immune response.

The term "ALS protein" refers to a protein of the agglutinin-like family of *C. albicans* which  
30 consists of 8 conserved proteins. Proteins conserved to the *C. albicans* ALS proteins are  
also found in *C. dubliniensis* and *C. tropicalis*.

The term "immunomodulating agent" refers to agents being capable of enhancing and  
adjusting immune responses including cytotoxic T cell (CTL) responses, antibody  
35 responses, and/or T helper cell responses, but may also affect innate immune cell  
pouulations.

The term "subject" in the context of the methods of this invention refers to a mammal, such as a mouse, a rabbit, a monkey or a human being. In preferred embodiment of the methods, the subject is a human being.

- 5 The term "antibody" as used herein includes full-lengths immunoglobulins and any antigen binding fragment thereof. Examples of antigen binding fragments encompass Fab fragments, F(ab')<sub>2</sub> fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; scFv fragments; single domains or dAb fragments; isolated complementarity determining regions (CDR) or a combination of two or more  
10 isolated CDRs which may optionally be joined by a synthetic linker. The antibody is optionally glycosylated.

- The term "linked" as used herein means physically attached. This may be in the form of a covalent bond, an aromatic bond, an ionic bond or van der Waals bonds; preferably, the  
15 linkage is via a covalent bond.

- In a first embodiment, the invention provides a peptide which is presented abundantly on MHC class II molecules of antigen-presenting cells during *C. albicans* infection. Said peptide comprises the amino acid sequence of SEQ ID No. 1 or a derivative thereof  
20 wherein 1, 2, 3 or several amino acids are substituted or lacking. Preferably, the peptide has T helper cell stimulating activity.

- Up to 25% of all *C. albicans*-specific T helper cells which are primed during infection in mice are responsive to SEQ ID No. 1 indicating that the peptide functions as or contains a  
25 strongly immunodominant epitope. In addition, T helper cells from healthy human individuals carrying different alleles of MHC class II molecules recognize the same peptide at a high frequency. This cross-species and cross-allelic reactivity is extremely rare given the strict sequence requirement for a peptide to be bound to a given MHC class II molecule. In a mouse model, vaccination with this peptide induces protection from  
30 candidiasis.

- The peptide typically has a length of at least 9-30 amino acid residues; it may have any length of at least 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10 or 9 amino acid residues. In a preferred embodiment, said peptide has 18 amino  
35 acid residues. In another preferred embodiment, the peptide has 9 amino acid residues.

In the most preferred embodiment of the instant invention, the peptide comprises or consists of SEQ ID No. 1.

Alternatively, the peptide may be a derivative of SEQ ID No. 1 wherein one or more amino acids may be substituted, lacking and/or added. In one embodiment, one, two, three, four or five amino acids are substituted and/or one, two, three, up to nine amino acids are lacking and/or one, two, three, up to twelve amino acids are added. Preferably, the derivative comprises or is selected from the group consisting of peptides of SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 and SEQ ID No. 6. The derivative is preferably immunologically active, i.e. it still retains T helper cell stimulating activity. In a preferred embodiment, the peptide is an immunological active fragment of SEQ ID No. 1; preferably, said immunological active fragment comprises SEQ ID No. 7. In another preferred embodiment, the peptide comprises at least SEQ ID No. 7.

In another embodiment, a T cell epitope derived from a *C. albicans* ALS protein is provided which comprises or consists of a peptide sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7.

The peptides and/or epitopes disclosed herein may be fused to at least one second entity, such as a carbohydrate or glycolipid (e.g. of a fungal cell wall, in particular of *C. albicans*) and/or a peptide or a protein, such as an antibody.

In another aspect, the peptide and/or the epitope disclosed herein can be used as vaccine or as immunomodulating agent.

In another aspect, the invention provides a composition, said composition comprising at least one T cell epitope within a peptide sequence, wherein the epitope has a sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7. Accordingly, the peptide sequence may be longer than the indicated epitope sequences. In another embodiment, the peptide sequence is derived from SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7 and comprises additional amino acids, e.g. at the N-terminal and/or C-terminal end.

Said peptide comprised in the composition is preferably the peptide and/or epitope disclosed herein. Accordingly, as defined above, it has typically a length of at least 9-30

amino acid residues; such as at least 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10 or 9 amino acid residues.

In one embodiment, the peptide has 18 amino acid residues. Preferably, said peptide  
5 consists of SEQ ID No. 1.

In another preferred embodiment, the peptide comprises at least the sequence of SEQ ID No. 7 and has at least 9 amino acids.

10 Also, the composition may comprise one or more other components. Said components are preferably pharmaceutically acceptable. If they have a pharmaceutical effect and said effect is desired, then the component is shall be present in a pharmaceutically effective amount. In one embodiment, the component is an adjuvant. In another embodiment, the component is an excipient. Suitable adjuvants and/or excipients are known in the art.

15

The composition may further comprise at least one B cell epitope (such as an antibody epitope) and/or at least one CTL (cytotoxic T-lymphocyte) epitope. Said at least one B cell epitope and/or at least one CTL epitope may be linked to the at least one T cell epitope.

20 Alternatively or additionally, the composition may further comprise one or more T cell epitopes. Said T cell epitopes may stem for example from *C. albicans* or from another fungus.

The peptide of the composition may be linked to at least one second entity. In one  
25 embodiment, the second entity is an antibody epitope. Such antibody epitope, without being limited to, may be a carbohydrate, such as a carbohydrate of a fungal cell wall, e.g. a carbohydrate (e.g.  $\beta$ -glucan, mannan, glycolipid, phospholipomannan) of *C. albicans*. Such glycoconjugates are seen as promising candidates for subunit vaccines. Anti-mannan and anti- $\beta$ -glucan antibodies have protective effects against fungi including  
30 *C. albicans* because they bind to the fungal cell wall and can act through mechanisms involving direct neutralization, opsonisation, complement activation or direct antifungal activity. Conjugation of a carbohydrate (which is by itself not very immunogenic) with a strong T cell epitope enhances its immunogenicity multifold. The resulting antibody response is much stronger and characterized by high-affinity antibodies of a switched  
35 isotype (IgG and IgA instead of IgM).

Alternatively or additionally, the peptide may be linked to an antibody. Such antibody may e.g. be useful to target the epitope disclosed herein to a specific site to which said antibody selectively binds, e.g. within a subject's body, thereby accumulating the epitope at the target site for effective treatment. Examples of suitable antibodies include

5 antibodies targeting dendritic cells (DC) such as CD11c, DEC205, Clec9a or Dectin-1.

The peptide may be linked in several ways to the antibody epitope and/or antibody.

The disclosed composition may be used for the treatment, prevention or alleviation of

10 symptoms of fungal infections. As such, the composition may e.g. be used as vaccine or as immunomodulating agent.

The peptide (or epitope) disclosed herein and/or the composition disclosed herein can be used for the treatment, prevention or alleviation of symptoms of fungal infections. Such

15 fungal infection may be, without being limited to, an infection with *Candida* spp. (including *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*), *Aspergillus* spp. (including *A. fumigatus*, *A. flavus*, *A. niger*), *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatidis*, Zygomycetes. *Candida* infections include, without being limited to, vaginal candidiasis, oropharyngeal candidiasis

20 (thrush), *Candida* Onychomycosis, cutaneous candidiasis, systemic candidemia. Typically, the treatment, prevention or alleviation of symptoms of fungal infections involves the administration of an effective amount of the peptide and/or composition to a subject in need thereof. Said subject is preferably a mammal, more preferable a human being. The peptide (or epitope) and/or the composition disclosed herein can be administered to the

25 subject via the typical routes of administration, such as ID (intradermal), IM (intramuscular) or PO (oral).

In another aspect, a method of inducing an immune response in a subject is provided, which comprises the step of administering to the subject the peptide disclosed herein, the

30 epitope disclosed herein or the composition disclosed herein.

Preferably, the immune response involves the activation and the priming of T helper cells which may mediate a protective effect directly via effector cytokines (leading e.g. to enhanced recruitment of neutrophils and macrophages) and/or exert their role as helper

35 cells for B cells and/or CTLs. T helper cells can strongly enhance B cell responses which are characterized by high affinity antibodies of the IgG and/or IgA isotype. Most importantly they also induce B cell memory for long term protection.

In another aspect, a method for enriching a T helper cell population in vitro is provided wherein a peptide immunogen comprising the T cell epitope disclosed herein is employed. This method is particularly useful for adoptive antifungal immunotherapy.

5

In still another aspect, the present invention provides a method of treating, preventing or alleviating symptoms of fungal infection in a subject, comprising administering to the subject a vaccine comprising a peptide disclosed herein or a derivative thereof which is still immunologically active. In a preferred embodiment, the vaccine is the composition disclosed herein.

10

Different fungal infections and subjects have been outlined above and also apply here to the method.

Here again, in a preferred embodiment of said method, the peptide is fused to a second entity; preferably, an antibody epitope, such as a carbohydrate. Said carbohydrate may e.g. be a carbohydrate of the fungal cell wall.

15

In still another aspect, a method for isolating T cells being specific for the peptide disclosed herein is provided. Said method comprises the steps of

20

- (i) obtaining T cells from a suitable donor;
- (ii) screening the donor T-cells for their specificity for said peptide;
- (iii) selecting those T-cell clones which recognize said peptide; and
- (iv) clonally expanding the selected T-cells.

25

The steps are preferably conducted in the indicated order; steps (ii) to (iv) and are typically done in vitro.

The selection step (iii) may e.g. be made by cell-sorting techniques or limiting-dilution cloning and the specificity of the cells may be identified e.g. by tetramer staining or by measuring their secretion of cytokines or expression of activation markers in response to the said peptide.

30

Optionally, the method may further comprise step (v) adoptively transferring the expanded T cells into a subject. Accordingly, the method is a method of adoptive immunotherapy. Such method of adoptively transferring antifungal immune responses might improve the outcome in recipients suffering e.g. from invasive fungal infection.

35

The subject may have been treated before the transfer of the expanded T cells by radiotherapy or chemotherapy and transplanted e.g. with hematopoietic stem cells from a suitable donor, e.g. in order to treat specific types of leukaemia. The transfer of the  
5 clonally expanded T cells may magnify the endogenous response of the subject towards the fungal infection.

The subject receiving the transferred T cells may be or not be the donor of step (i), i.e. the donor of the T cells. In one embodiment, the donor and the recipient are different  
10 individuals of the same species, i.e. an allogeneic transplantation is made. In such cases, the donor of the T cells is preferentially the donor of the hematopoietic stem cells transplant. In order to reduce unwanted side effects, the step of clonally expanding the selected T cells is particularly important to avoid the transfer of potential alloreactive T cells. In a preferred embodiment, the subject is the donor of the T cells such that an  
15 autologous transplant is made. The transfer may be made by methods known in the art, such as infusion.

Further disclosed is a nucleic acid encoding the peptide sequences disclosed herein. The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those  
20 containing modified backbones. Preferably, the nucleic acid is an expression vector or a cloning vector. In some embodiment, the nucleic acid is used for the treatments, prevention or alleviation of symptoms of a fungal infection.

The nucleic acid may be introduced into a suitable host cell by known molecular biology  
25 techniques. Said host cell may e.g. be an *E. coli* cell, a yeast cell such as a *S. cerevisiae* cell, an insect cell, a plant cell or a mammalian cell. Further provided is a host cell comprising said nucleic acid.

The host cell may be cultivated under suitable conditions so that the peptides or larger  
30 polypeptides comprising the herein disclosed peptides are produced and preferably secreted into the culture medium. Alternatively, the peptides of the present invention can be synthesised in vitro or generated by enzymatic digestion of larger polypeptides or proteins.

35 The peptide disclosed herein, in particular SEQ ID No. 1, may be combined with a suitable adjuvant and used as a vaccine. Such vaccine could be useful to prevent infections with *C. albicans*. Alternatively, the peptide may be fused to an antibody epitope and also be

used as vaccine. Using said peptide as the T cell inducing component may be particularly useful as humans contain a high frequency memory T cells specific for SEQ ID No.1 which can be readily reactivated. Such vaccine could then be useful to prevent infections with fungi other than *C. albicans* which do not express the ALS protein as many of the fungal cell wall carbohydrates are preserved, including most importantly endemic fungal species such as Histoplasma and Blastomyces which cause disease in normal healthy individuals. Furthermore, women suffering from vaginal candidiasis which affects otherwise healthy individuals should be an important target population for such a vaccine.

10 The peptide may further be used in adoptive immunotherapy.

### Examples

#### Example 1: Method of epitope isolation

15

*C. albicans* (strain SC5314) was grown in medium where the only ammonium source was either  $^{14}\text{N}$  ammonium or  $^{15}\text{N}$  ammonium. DC have been infected with a 1:1 mixture of both types of *C. albicans* cells in vitro. A cell lysate of the infected DC was prepared and MHCII molecules were purified by affinity chromatography using an anti-MHCII-coupled BrCN sepharose column. MHCII-bound peptides were isolated by acidic elution and separated from the MHCII molecules by size filtration. Eluted peptides were separated by size and sequenced using an MS/MS-HPLC method. *Candida*-specific peptide sequences were identified with the help of *C. albicans* sequence databases.

20

25 The Mascot search algorithm was used to analyse the peptide mass fingerprint. Three overlapping peptides derived from a single protein obtained the highest index. These are SEQ ID No. 1 KGLNDWNYPVSSSESFSY;  
SEQ ID No. 8 KGLNDWNYPVSSSESFSY;  
SEQ ID No. 9 KGLNDWNYPVSSSESFS.

30 The longest 18aa peptide, i.e. SEQ ID No. 1, was used for the future experiments. It was synthesized synthetically at EMC Microcollections GmbH.

#### Example 2: In silico affinity prediction

35 The Immune epitope database analysis resource (<http://tools.immuneepitope.org>) was used to predict the MHC class II binding affinity for the identified peptides. The consensus prediction method used calculates a score for every possible 15-mer from the given

peptide. Table 1 shows the scores calculated for the identified peptides with I-A<sup>b</sup>, i.e. the MHCII molecule in C57Bl/6 mice. The core sequence (SEQ ID No. 7) is highlighted in bold letters.

5 Table 1:

|                          | score |
|--------------------------|-------|
|                          |       |
| KGLNDWNYPV <b>SSE</b> SF | 3.2   |
| GLNDWNYPV <b>SSE</b> SFS | 2.98  |
| LNDWNYPV <b>SSE</b> SFSY | 3.14  |
| NDWNYPV <b>SSE</b> SFSYT | 3.94  |

High affinity is typically reflected by a score of 0-5; intermediate affinity of a score of 5-500.

- 10 The consensus method applied to predict the peptide binding to MHC class molecules is based on a combination of available computational prediction tools. This method provides the currently best performance. It was published by P. Wang et al. (PLOS Comput. Biol., 2008) and is available on the IEDB analysis resource website (<http://tools.immuneepitope.org>).

15

#### Example 3: Conservation of the SEQ ID No. 1 peptide epitope

- Subsequently, the SEQ ID No. 1 was subjected to a sequence identity search using the Basic Local Alignment Search Tool (BLAST) which finds regions of similarity between biological sequences (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). SEQ ID No. 1 was compared to the genome sequence of *C. albicans* and found to be 100% identical to amino acids 236-253 of the agglutinin-like ALS1 and ALS3 proteins. Furthermore, a high similarity of SEQ ID No. 1 (with a stretch of amino acids of) the other members of the ALS gene family was found, namely ALS9, ALS4, ALS5, ALS2 and ALS6, whereby one, two or three amino acids were dissimilar from SEQ ID No. 1 in each case. These results are shown in figure 1.

- The ALS gene family consists of 8 conserved GPI-linked cell surface proteins that mediate adhesion to and invasion into host tissues. ALS3 (and maybe other ALS proteins as well) binds to cadherins on endothelial and epithelial cells and this binding induces endocytosis of the fungus. ALS3 acts as a virulence factor of *C. albicans*.

A sequence identity search with non-albicans species of *Candida* identified a high sequence homology of SEQ ID No. 1 with stretches of ALS-related proteins from *C. dubliniensis* and *C. tropicalis*, two species closely related to *C. albicans* (figure 1). These results suggest that T helper cells specific for SEQ ID No. 1 may also be primed during infection with *C. dubliniensis* and *C. tropicalis* and that they may also be important for protection against those species.

Example 4: Validation of the identified SEQ ID No. 1

C57Bl/6 mice were infected orally with *C. albicans* by placing a small cotton ball soaked with a suspension of  $5 \times 10^7$  cfu/ml *C. albicans* strain SC5314 under the tongue of anesthetized mice for 90 minutes. Mice were infected either once (1° infection) or twice consecutively (2° infection, 3 weeks interval between infections). The 2° infection elicited a memory response characterized by a more rapid kinetic of the response and an approximately 10-fold higher frequency of *C. albicans*-specific T helper cells.

Draining lymph nodes from *C. albicans*-infected or naïve control mice were removed and restimulated in vitro with heat-killed *C. albicans* ( $2.5 \times 10^5$  cfu/ml) or pALS<sub>236-253</sub> peptide (SEQ ID No. 1, 5 µg/ml or a titration from 5 µg/ml to 5 ng/ml) or left untreated (medium). No response is expected from naïve lymph node cells or after control restimulation with medium. The T helper cell response was evaluated by measuring secreted IL-17A levels in the culture supernatant by ELISA or by quantifying IL-17A production by CD4<sup>+</sup>CD3<sup>+</sup>Vβ<sup>+</sup> cells with intracellular cytokine staining and flow cytometry analysis. Results are shown in figure 2. These results show that the peptide of SEQ ID No. 1 is a strong T cell epitope of *C. albicans* which is recognized by up to 25% of all *C. albicans*-specific T helper cells. The T helper cells specific for this epitope secrete large amounts of IL-17A when primed in a mouse model of oropharyngeal candidiasis.

In order to show that the effect of the pALS<sub>236-253</sub> peptide was dependent on MHC class II and the T cell receptor (TCR), the binding of the peptide to MHC class II was blocked with an anti-MHC class II antibody. For this, draining lymph nodes from *C. albicans*-infected mice were removed and re-stimulated in vitro with different concentrations of the pALS<sub>236-253</sub> peptide in the presence or absence of 10 µg/ml anti-MHC class II antibody. The response of the T helper cells to the peptide was evaluated by measuring intracellular IL-17A production by flow cytometry (figure 3A) or secreted IL-17A in the culture supernatant by ELISA (figure 3B and 3C). A strong inhibition of the response was observed in the

conditions where the antibody was added. These results confirm that the response of T helper cells to the pALS<sub>236-253</sub> peptide is specific and mediated via MHC class II.

Example 5: Functional comparison of the peptide of SEQ ID No. 1 with other previously described T cell epitopes

In order to evaluate the relative importance of the peptide of SEQ ID No. 1 with peptides that have been proposed previously to function as T cell epitopes from *C. albicans* (MP65 and p41, see Petrella et al, 2008, Infection & Immunity, 76: 4359; Stuehler et al, 2011, Blood), draining lymph nodes from *C. albicans*-infected mice (1<sup>st</sup> or 2<sup>nd</sup> infection) were restimulated in vitro with the pALS<sub>236-253</sub>, pATG<sub>227-242</sub>, MP65 or p41 peptides (5 µg/ml to 5 ng/ml each). The pATG<sub>227-242</sub> peptide was identified in our isolation procedure as the second best candidate. The reactivity of T cells to the different peptides was evaluated by measuring IL-17A production from CD4<sup>+</sup>CD3<sup>+</sup>Vβ<sup>+</sup> T helper cells by intracellular cytokine staining and flow cytometry (figure 4A and 4B) or secreted IL-17A in the culture supernatant by ELISA (figure 4C and 4D). The most prominent response was the one against the peptide of SEQ ID No. 1. This is reflected in the fraction of T cells that responded to the peptide (figure 4A and 4B) as well as in the absolute levels of secreted cytokine (figure 4C and 4D). The effect was most pronounced after the 2<sup>nd</sup> infection (figure 4B and 4D). These data confirm that the peptide of SEQ ID No. 1 is a very prominent and currently the most important T cell epitope of *C. albicans*.

Example 6: Immunization of mice with the peptide of SEQ ID No. 1

In order to elicit a T cell response specific for the pALS<sub>236-253</sub> peptide, mice were immunized in the hind footpad with SEQ ID No. 1 mixed with adjuvant (CpG or curdlan) or with adjuvant alone. 1 week later, the draining lymph nodes containing the activated T cells were removed and restimulated in vitro with the pALS<sub>236-253</sub> peptide (5 µg/ml) or with heat-inactivated *C. albicans* (2.5\*10<sup>5</sup> cfu/ml) for 48 hours. Cytokine production was quantified by ELISA, see figures 5 A and B.

As published before (e.g. Joffre et al., EJI 2010, 40: 1255), CpG induces a Th1 response, whereas curdlan induces a Th17 response when used as adjuvant. Curdlan is a high-molecular weight β-glucan which represents a major component of the fungal cell wall.

Immunization with the pALS<sub>236-253</sub> peptide, leads to the priming of T helper cells which are specific not only for the peptide but also for *C. albicans* (see figure 6). Furthermore, they

acquire effector cell function, characterized by their IFN- $\gamma$  production if CpG is used as adjuvant, or their IL-17A production in case of curdlan. These results confirm what is expected. However, they are an important prerequisite for the subsequent vaccination experiments (example 7, see below).

5

Example 7: Vaccination with the peptide of SEQ ID No. 1 mediates protection from *C. albicans* infection

In order to test whether the pALS<sub>236-253</sub> peptide mediates protection from *C. albicans* infection, mice were immunized subcutaneously with the pALS<sub>236-253</sub> peptide mixed with curdlan in IFA or curdlan in IFA as control (figure 6). Curdlan served as an adjuvant to induce Th17 priming and IFA was used to enhance the response because of its property to generate a depot of the co-injected peptide and adjuvant and thus to boost the response. 3 weeks later, mice were challenged with  $2 \times 10^5$  *C. albicans* intravenously and survival of the mice was monitored. The group immunized with the peptide of SEQ ID No. 1 was much less susceptible to *C. albicans* and a larger proportion of the mice survived longer than in the control group.

These results show that immunization of mice with the peptide of SEQ ID No. 1 confers protection from *C. albicans* infection (Figure 6).

Example 8: CD4<sup>+</sup> memory T cells specific for the SEQ ID No. 1 are present in healthy human individuals

A large number of human HLA alleles show a good affinity score for the pALS<sub>236-253</sub> peptide. They include DRB1\*04 and DRB3\*01. Furthermore, a high binding affinity is also predicted for DQB1\*03, DQB1\*04, DPB1\*04 and DPB1\*02.

In order to evaluate whether human *Candida*-specific T cells do also recognize the pALS<sub>236-253</sub> peptide, peripheral blood lymphocytes (PBLs) from healthy human individuals (which are exposed to *C. albicans* constitutively because the fungus is part of the normal human gut flora) have been re-stimulated with the pALS<sub>236-253</sub> peptide. The T cell response was evaluated by measuring intracellular cytokine production (IL-2, IL-17A and IFN- $\gamma$ ) in CD4<sup>+</sup>CD45RA<sup>+</sup> memory T cells. The data from one representative individual is shown in figure 7A. All so far tested individuals display peptide-specific memory T cells and their proportion constitutes a significant fraction of the pool of all *C. albicans*-specific memory T cells (figure 7B and 7C). The selective production of IL-2 and IL-17A in the

absence of any IFN- $\gamma$  production by pALS<sub>236-253</sub> peptide-specific T cells is in line with what was shown for *C. albicans*-specific human T cells before (Acosta-Rodriguez et al., 2007, Nat.Immunol., 8: 639). The finding that the peptide of SEQ ID No. 1 is recognized not only by mouse but also human T helper cells in the majority of all individuals indicates that it is a useful vaccine candidate. The strength of the response that is elicited to the peptide is remarkable.

### *Equivalents*

Numerous modifications and alternative embodiments of the present invention will be apparent to those skilled in the art in view of the foregoing description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the best mode for carrying out the present invention. Details of the structure may vary substantially without departing from the spirit of the invention, and exclusive use of all modifications that come within the scope of the appended claims is reserved. It is intended that the present invention be limited only to the extent required by the appended claims and the applicable rules of law.

All literature and similar material cited in this application, including, patents, patent applications, articles, books, treatises, dissertations, web pages, figures and/or appendices, regardless of the format of such literature and similar materials, are expressly incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including defined terms, term usage, described techniques, or the like, this application controls.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described in any way.

While the present inventions have been described in conjunction with various embodiments and examples, it is not intended that the present teachings be limited to such embodiments or examples. On the contrary, the present inventions encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

The claims should not be read as limited to the described order or elements unless stated to that effect. It should be understood that various changes in form and detail may be

made without departing from the scope of the appended claims. Therefore, all embodiments that come within the scope and spirit of the following claims and equivalents thereto are claimed.

## Claims

1. A peptide comprising the amino acid sequence of SEQ ID No. 1 or a derivative thereof wherein 1, 2, 3 or several amino acids are substituted, lacking or added.
- 5 2. The peptide of claim 1, having a length of at least 9 amino acid residues.
3. The peptide of claim 1 or 2, having T helper cell stimulating activity.
- 10 4. The peptide of anyone of the preceding claims, having SEQ ID No. 1.
5. The peptide of claims 1 or 2, selected from the group consisting of SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 and SEQ ID No. 6.
- 15 6. The peptide of claims 1 or 2, comprising SEQ ID No. 7.
7. The peptide of anyone of the preceding claims, being an isolated peptide.
8. A T cell epitope of a *C. albicans* ALS protein comprising a peptide sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7.
- 20 9. The peptide or epitope of anyone of claims 1-8 for the treatment, prevention or alleviation of symptoms of fungal infections.
- 25 10. The peptide or epitope of anyone of claims 1-8 for the treatment, prevention or alleviation of symptoms of *Candida* infection.
11. The peptide or epitope of anyone of claims 1-8 for the treatment, prevention or alleviation of symptoms of vaginal candidiasis.
- 30 12. The peptide or epitope of anyone of claims 1-8 for use as vaccine.
13. The peptide or epitope of anyone of claims 1-8 for use as immunomodulating agent.
- 35 14. A composition comprising at least one T cell epitope being comprised within a peptide sequence, wherein the epitope has a sequence selected from the group consisting of

SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7.

15 15. The composition of claim 14, wherein said peptide sequence has a length of at least 9 amino acid residues.

16. The composition of claims 14 or 15, wherein said peptide sequence has 18 amino acid residues.

10 17. The composition of anyone of claims 14-16, wherein said peptide sequence is SEQ ID No. 1.

15 18. The composition of anyone of claims 14-17, wherein the peptide comprises at least the sequence of SEQ ID No. 7.

19. The composition of anyone of claims 14-18, further comprising a pharmaceutically acceptable adjuvant.

20 20. The composition of anyone of claims 14-19, wherein the peptide is fused to an antibody epitope.

21. The composition of claim 20, wherein the antibody epitope is a carbohydrate of the fungal cell wall.

25 22. The composition of anyone of claims 14-21, wherein the epitope is fused to an antibody.

30 23. The composition of anyone of claims 14-22, further comprising at least one B cell epitope and/or at least one CTL epitope.

24. The composition of anyone of claims 14-22, further comprising one or more T cell epitopes.

35 25. The composition of anyone of claims 14-24 for the treatment, prevention or alleviation of symptoms of fungal infections.

26. The composition of claim 25 for the treatment, prevention or alleviation of symptoms of a *Candida* infection.
27. The composition of claim 26 for the treatment, prevention or alleviation of symptoms of vaginal candidiasis.
28. The composition of anyone of claims 14-24 for use as vaccine.
29. The composition of anyone of claims 14-24 for use as immunomodulating agent.
30. A method of inducing an immune response in a subject, the method comprising administering to the subject the peptide of anyone of claims 1-7, the epitope of claim 8 or the composition as claimed in anyone of claims 14-24.
31. The method of claim 30, wherein the subject is a human being.
32. The method of claim 30 or 31, wherein the immune response involves the production of T helper cells.
33. The method of claim 32, wherein the immune response does not involve the production of antibodies.
34. The method of claim 32, wherein the immune response further involves the production of antibodies.
35. A method for enriching a T helper cell population *in vitro* by employing a peptide immunogen comprising the T cell epitope of anyone of claims 1-8.
36. A method of treating, preventing or alleviating symptoms of fungal infection in a subject, comprising administering to the subject
- (i) a vaccine comprising a peptide of anyone of claims 1 to 8 or an immunologically active fragment of the peptide; or
  - (ii) the composition of anyone of claims 14-24.
37. The method of claim 36, wherein the fungal infection is a *Candida* infection.
38. The method of claims 36 or 37, wherein the fungal infection is vaginal candidiasis.

39. The method of anyone of claims 36-38, wherein the peptide is fused to an antibody epitope.

5 40. The method of claim 39, wherein the peptide is fused to a carbohydrate.

41. The method of claim 40, wherein the carbohydrate is a carbohydrate of the fungal cell wall.

10 42. A method for isolating T cells being specific for the peptide of anyone of claims 1-8 comprising the steps of

(i) obtaining T cells from a suitable donor;

(ii) screening the donor T-cells for their specificity for the peptide of anyone of claims 1-8;

(iii) selecting those T-cell clones which are specific for the peptide of anyone of claims 1-8;

15 and

(iv) clonally expanding said selected T-cells.

43. The method of claim 42, being a method of adoptive immunotherapy and further comprising the step of adoptively transferring the expanded T cells into a subject.

20

44. The method of claim 43, wherein the subject has been treated by radiotherapy or chemotherapy before said adoptive transfer of the expanded T cells.

45. The method of anyone of claims 42 to 44, wherein the subject is the donor of step (i).

25

46. The method of anyone of claims 42 to 44, wherein the subject is different from the donor of step (i).

30 47. The method of anyone of claims 42 to 46, wherein the selection step is made by cell-sorting techniques, tetramer staining or limiting-dilution cloning.

Fig. 1

|        |                      |     |                      |     |        |
|--------|----------------------|-----|----------------------|-----|--------|
|        | SEQ ID No. 1         | 1   | KGLNDWNYPVSSSESFSYT  | 18  |        |
| ALS1   | <i>C. albicans</i>   | 236 | KGLNDWNYPVSSSESFSYT  | 253 | P46590 |
| ALS3   | <i>C. albicans</i>   | 236 | KGLNDWNYPVSSSESFSYT  | 253 | O74623 |
| ALS9   | <i>C. albicans</i>   | 236 | KGLNDWNEFPVSSSESFSYT | 253 | Q9C471 |
| ALS4   | <i>C. albicans</i>   | 237 | GLNDWNYPISSSESFSYT   | 253 | O74660 |
| ALS5   | <i>C. albicans</i>   | 236 | KGVDWNHPVTSESFSYT    | 253 | Q874K9 |
| ALS2   | <i>C. albicans</i>   | 236 | KGLNDWNEFPVSSDSLST   | 252 | O74657 |
| ALS6   | <i>C. albicans</i>   | 238 | GLNSWNMPVSSSESFSYT   | 254 | Q9Y8F2 |
| ALS9   | <i>C. dubliensis</i> | 235 | KGLNDWNEFPVSSSESFSYT | 252 | B9WJ72 |
| ALSD2* | <i>C. dubliensis</i> | 237 | GVNDWNEFPVSSSESFSYT  | 253 | Q9HF70 |
| ALSD3* | <i>C. dubliensis</i> | 237 | GLNDWNYPIESKSFSYT    | 253 | Q9HF69 |
| ALS2   | <i>C. dubliensis</i> | 235 | KGVDWNEFPVSYDSFSY    | 251 | B9WJD2 |
| ALSD1* | <i>C. dubliensis</i> | 238 | GLNSWNMPVSSDSFSYT    | 254 | Q9HF72 |
| *      | <i>C. tropicalis</i> | 240 | LNDWNQPMNSESFST      | 255 | C5MDW9 |
|        | <i>C. tropicalis</i> | 240 | LNDWNQPTNSETFST      | 255 | C5M4Q1 |
|        | <i>C. tropicalis</i> | 240 | LNSWNYPTSADESFSY     | 255 | C5MDN9 |
|        | <i>C. tropicalis</i> | 240 | LNPWNQPMNAESFSY      | 255 | C5M4P0 |

Fig. 2A

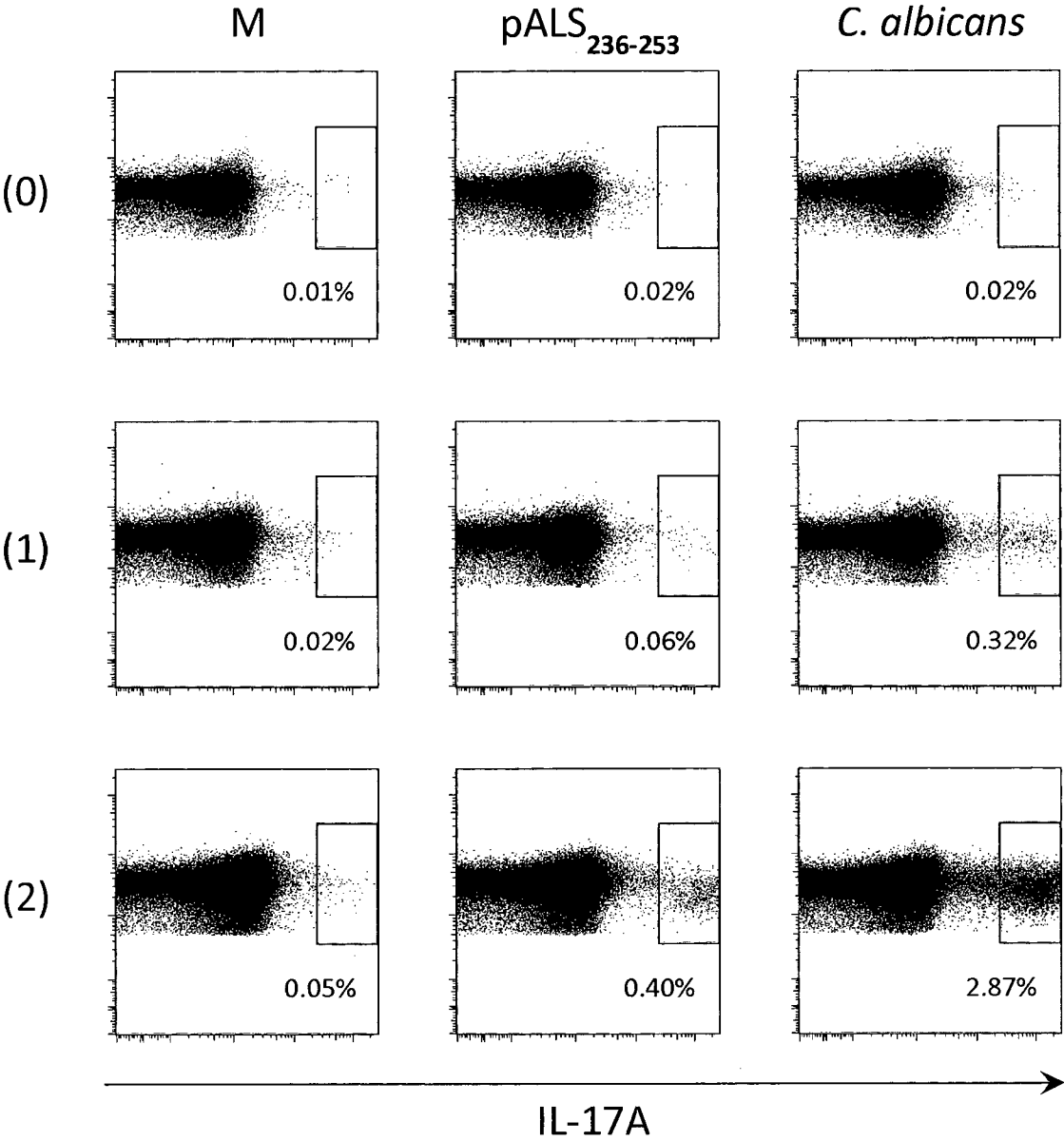


Fig. 2B

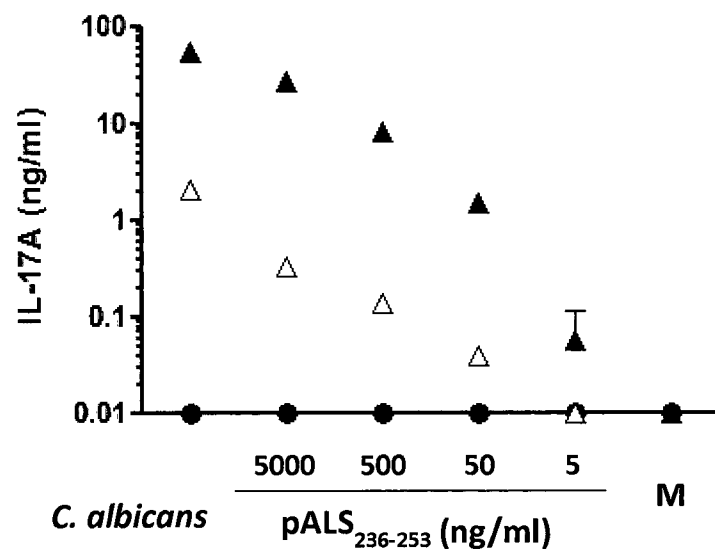


Fig. 3A

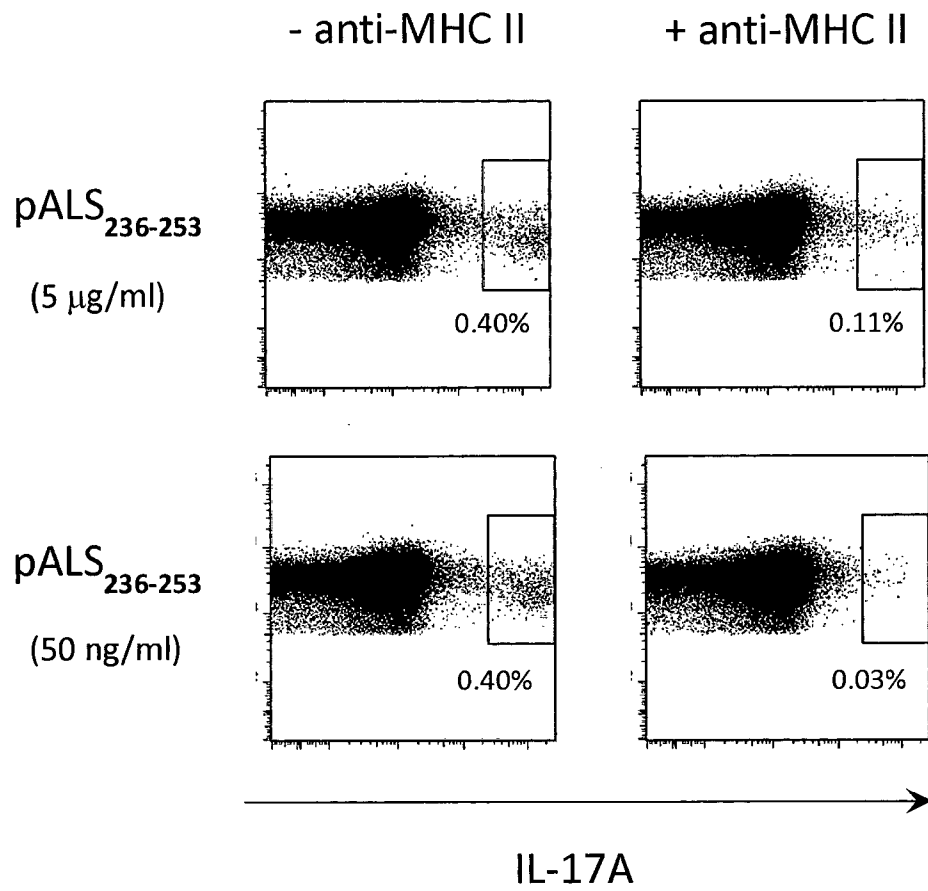


Fig. 3B

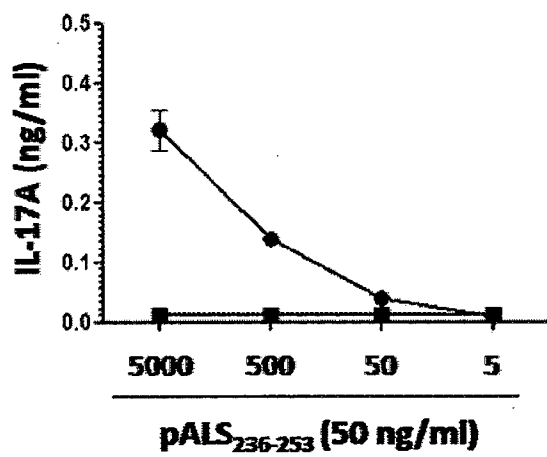
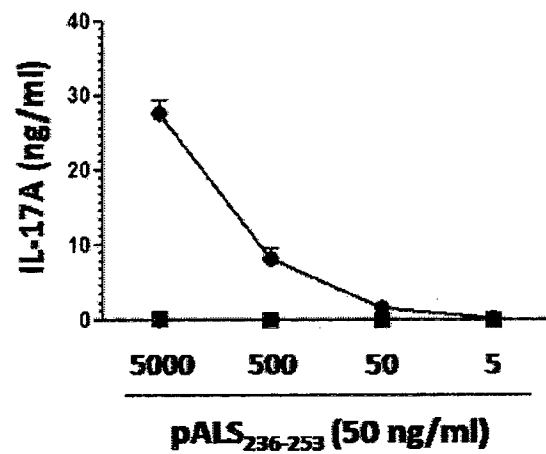


Fig. 3C



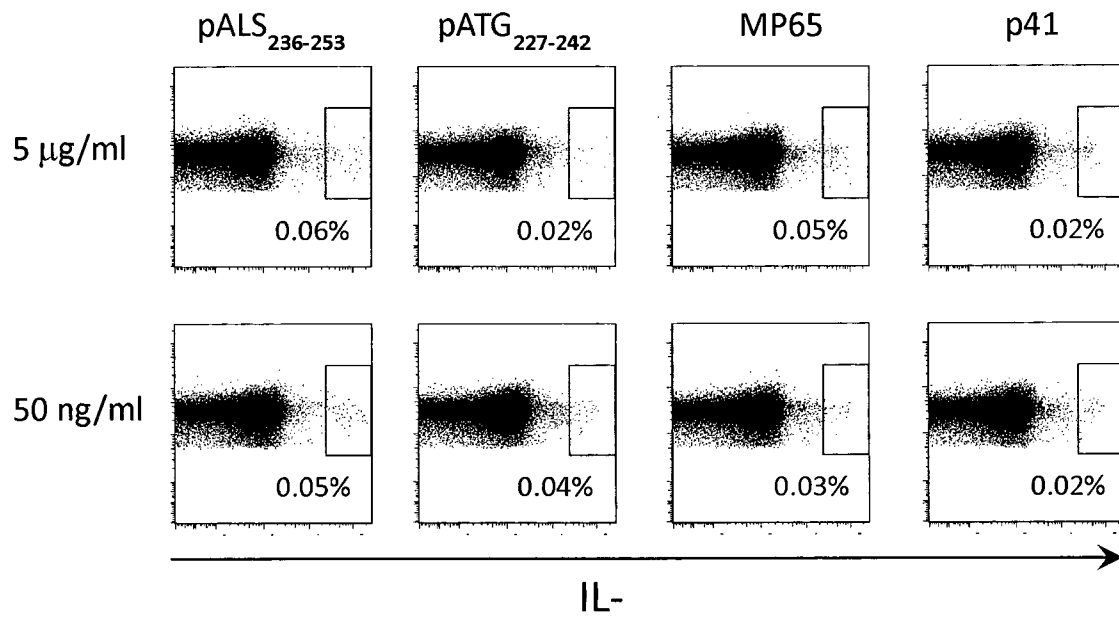
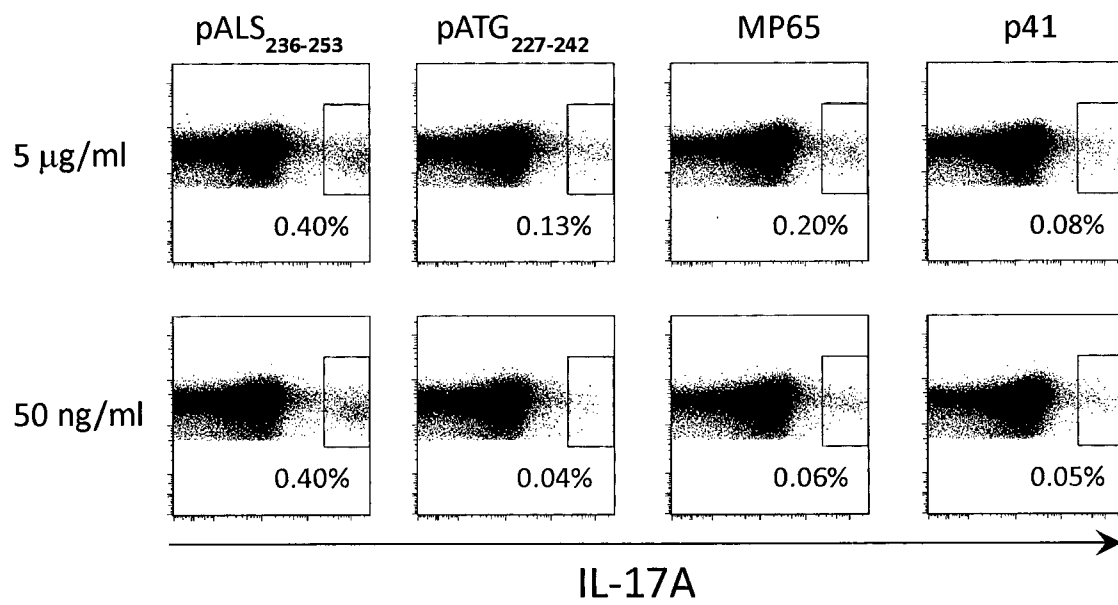
**Fig. 4A****Fig. 4B**

Fig. 4C

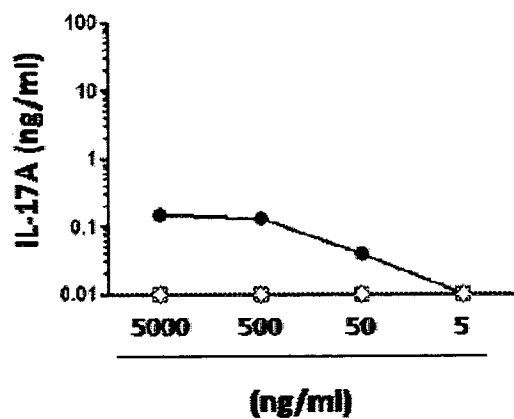


Fig. 4D

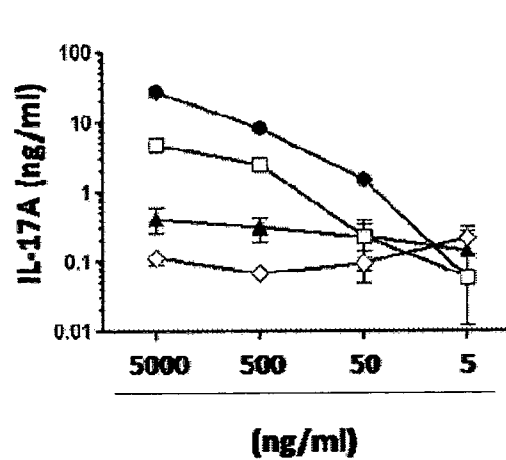


Fig. 5A

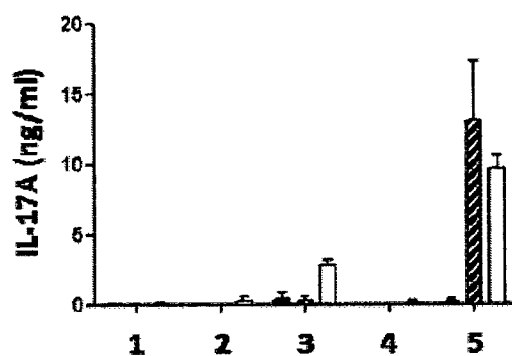


Fig. 5B

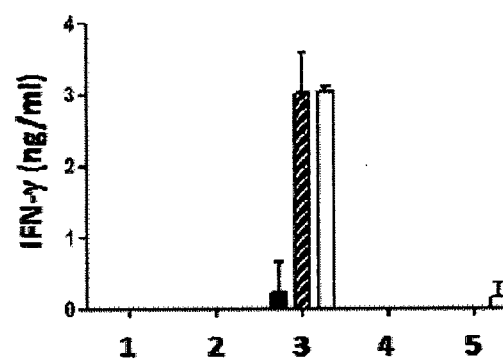


Fig. 6

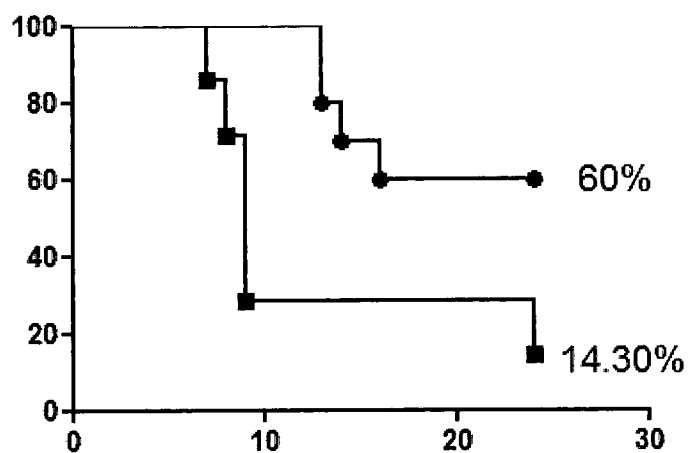


Fig. 7A

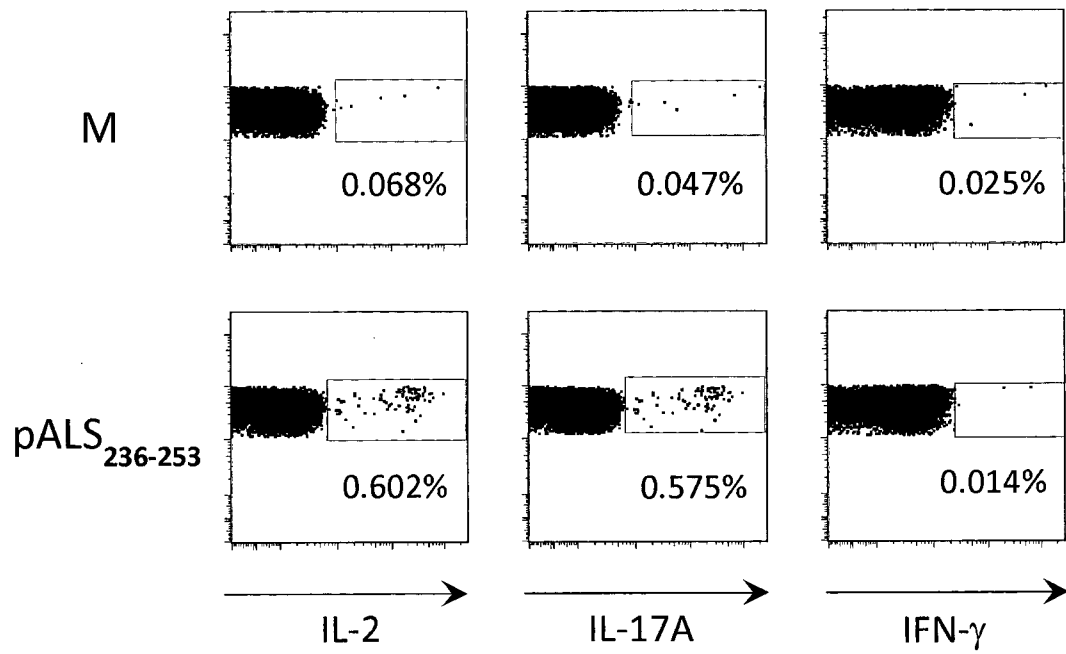


Fig. 7B

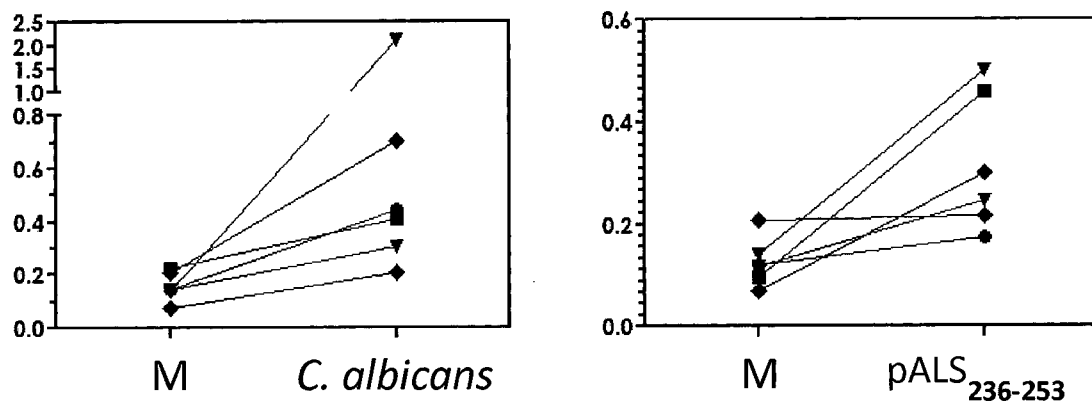
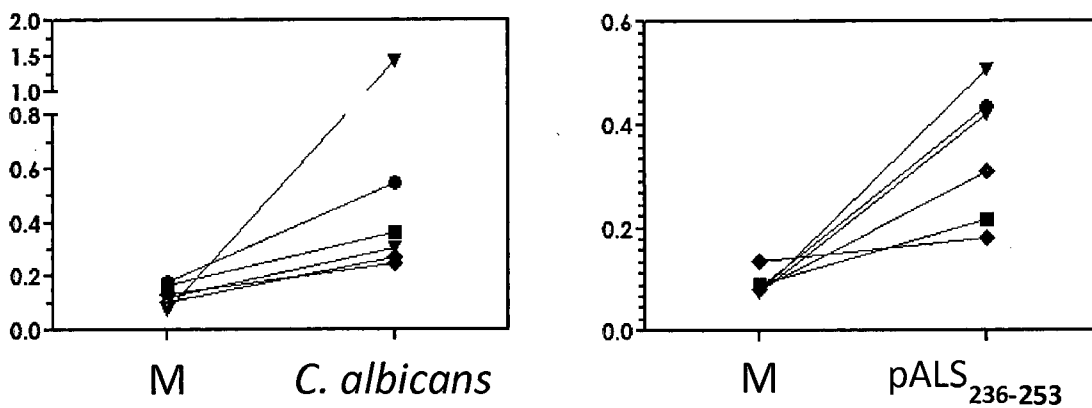


Fig. 7C



## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2012/002310

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00 C07K7/04 C07K14/40  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No.                                                       |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| X         | <p>WO 2008/109833 A2 (UNIV FLORIDA [US];<br/>CLANCY CORNELIUS JOSEPH [US]; NGUYEN<br/>MINH-HONG THI)<br/>12 September 2008 (2008-09-12)</p> <p>page 7 - page 40<br/>page 152; table 18<br/>claim 35<br/>page 20, line 10 - line 16<br/>-----<br/>-/--</p> | <p>1-4,<br/>6-10,<br/>12-15,<br/>18-26,<br/>28-34,<br/>36,37,<br/>39-41</p> |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

5 November 2012

Date of mailing of the international search report

19/11/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Voigt-Ritzer, Heike

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/002310

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                             |
|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                         | Relevant to claim No.                                                       |
| X                                                    | <p>SPELLBERG BRAD ET AL: "Antibody titer threshold predicts anti-candidal vaccine efficacy even though the mechanism of protection is induction of cell-mediated immunity",<br/>JOURNAL OF INFECTIOUS DISEASES, UNIVERSITY OF CHICAGO PRESS, CHICAGO, IL, vol. 197, no. 7, 1 April 2008 (2008-04-01), pages 967-971, XP002546593, ISSN: 0022-1899, DOI: 10.1086/529204 the whole document</p> <p>-----</p> | <p>1-4,<br/>6-10,<br/>12-15,<br/>18-26,<br/>28-34,<br/>36,37,<br/>39-47</p> |
| X                                                    | <p>WO 2007/081896 A2 (LOS ANGELES BIOMED RES INST [US]; EDWARDS JOHN E JR [US]; FILLER SCOTT) 19 July 2007 (2007-07-19)</p> <p>figure 37<br/>example 11<br/>page 2, line 27 - page 3, line 12</p> <p>-----</p>                                                                                                                                                                                             | <p>1-4,<br/>6-15,<br/>18-34,<br/>36-47</p>                                  |
| A                                                    | <p>NISINI ROBERTO ET AL: "Antigenic properties and processing requirements of 65-kilodalton mannoprotein, a major antigen target of anti-Candida human T-cell response, as disclosed by specific human T-cell clones",<br/>INFECTION AND IMMUNITY, vol. 69, no. 6, June 2001 (2001-06), pages 3728-3736, XP002685738, ISSN: 0019-9567 the whole document</p> <p>-----</p>                                  | <p>1-47</p>                                                                 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/002310

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)                                                                                                                                                              | Publication<br>date                                                                                                                      |
|-------------------------------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| WO 2008109833 A2                          | 12-09-2008          | US 2010119533 A1<br>WO 2008109833 A2                                                                                                                                                    | 13-05-2010<br>12-09-2008                                                                                                                 |
| WO 2007081896 A2                          | 19-07-2007          | AU 2007205065 A1<br>CA 2636277 A1<br>DK 1982190 T3<br>EP 1982190 A2<br>EP 2428800 A1<br>JP 2009524601 A<br>US 2007077256 A1<br>US 2009297562 A1<br>US 2012014995 A1<br>WO 2007081896 A2 | 19-07-2007<br>19-07-2007<br>22-10-2012<br>22-10-2008<br>14-03-2012<br>02-07-2009<br>05-04-2007<br>03-12-2009<br>19-01-2012<br>19-07-2007 |