Tuberculosis is far from being controlled for different reasons: poor patient compliance with the prescribed standard-of-care in areas with limited resources, exacerbation of TB epidemics due to HIV coinfection, poor performance of BCG vaccination which is ineffective in protecting adults. In view of the increasing worldwide threat of TB and the inherent complexity of the *Mtb* infection and anti-mycobacterial immune response, there remains a need for improved vaccine strategies for diagnosing, preventing and treating tuberculosis, especially in endemic regions.

The present invention fulfils this and other needs by providing an immunogenic combination of *Mtb* antigens that is tailored for all phases of the natural course of infection.

This technical problem is solved by the provision of the embodiments as defined in the claims.

Other and further aspects, features and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

BRIEF SUMMARY

The present invention relates generally to an immunogenic combination comprising at least 5 different antigens or nucleic acid molecules encoding the at least 5 antigens, wherein said antigens are independently obtained from a Mycobacterium species, wherein the at least 5 mycobacterial antigens are selected from the group consisting of from the group consisting of ESAT-6 (Rv3875), TB10.4 (Rv0288), Ag85B (Rv1886), RpfB, RpfD, Rv0111, Rv0569, Rv1733c, Rv1807, Rv1813, Rv2029c, Rv2626, Rv3407 and Rv3478, and particularly from a Mycobacterium species of the tuberculosis complex such as Mycobacterium tuberculosis (*Mtb*). A data mining scoring system was developed and used to classify a panel of *Mtb* antigens based on their immunogenicity and protection properties. Upon sequence alignment, biochemical and bioinformatics prediction studies, 14 *Mtb* antigens were selected and combined into antigen/vector combination and fusion polypeptides.

In one embodiment, the antigen combination of the invention is multiphasic where the at least 5 mycobacterial antigens are from two or the three phases of the natural course of a Mycobacterium infection, namely the active, latent and resuscitation phases. The mycobacterial antigens can be employed/expressed in the form of mixture or in one or more fusion polypeptide(s) as described herein.

The present disclosure also concerns fusion polypeptides of specific mycobacterial antigens, nucleic acid molecules and vectors encoding/expressing such fusion polypeptides and compositions comprising or encoding said fusion polypeptides as well as methods of

preparing said fusions, vectors and compositions. The present disclosure also relates to antibodies against such mycobacterial antigens and fusion polypeptides. It further relates to the use of such immunogenic combinations, fusion polypeptides, nucleic acid molecules, vectors, compositions or antibodies for the purpose of the diagnosis, prevention, or treatment of a Mycobacterium infection or ameliorating a condition associated with a Mycobacterium infection.

A further embodiment includes a method of treating, preventing or inhibiting a Mycobacterium infection or ameliorating a condition associated with Mycobacterium infection in a subject in need thereof, comprising providing or administering this immunogenic combination, fusion polypeptide, nucleic acid molecule, vector or composition.

Still a further embodiment concerns a method of eliciting an immune response in a subject in need thereof, comprising providing or administering this immunogenic combination, fusion polypeptide, nucleic acid molecule, vector or composition, for the purpose of inducing or stimulating an immune response in this subject or for preventing or treating a Mycobacterium infection.

Still yet an embodiment provides a kit of parts comprising a plurality of containers and instructions for providing or administering to a subject this immunogenic combination, fusion polypeptide, nucleic acid molecule, vector or composition.

Another embodiment provides a kit of reagents for antibody assay for diagnosis of a Mycobacterium infection (e.g. tuberculosis) comprising this immunogenic combination, fusion polypeptide, nucleic acid molecule, vector or composition.

The disclosed antigen combination offers improved and unexpected immunogenic properties (e.g. level, quality and/or scope of the immunogenic response) as compared to the individual antigens.

~~In another aspect, t~~The present ~~invention~~ disclosure also relates to a fusion polypeptide comprising two or more mycobacterial antigens comprised or encoded by the immunogenic combination disclosed herein.

In yet another aspect, there is provided a nucleic acid molecule encoding said at least 5 mycobacterial antigens comprised in the immunogenic combination ~~or the fusion polypeptide~~ as disclosed herein.

Yet another aspect relates to a vector comprising one or more nucleic acid molecule(s) disclosed herein.

Still another aspect relates to a process of producing an infectious virus particle comprising the steps of (i) introducing the viral vector disclosed herein into a suitable cell line, (ii) culturing said cell line under suitable conditions so as to allow the production of said infectious viral particle, (iii) recovering the produced viral particle from the culture of said cell line, and (iv) optionally purifying said recovered viral particle.

Another aspect relates to a host cell comprising the immunogenic combination, ~~the fusion polypeptide,~~ the nucleic acid molecule or the vector as disclosed herein.

Still another aspect relates to a method for recombinant production of the mycobacterial antigens comprised or encoded by the immunogenic combination ~~or in the fusion polypeptide~~ disclosed herein, which comprises the steps of (i) introducing a vector into a suitable host cell to produce a transfected or infected host cell, (ii) culturing *in-vitro* said transfected or infected host cell under conditions suitable for growth of the host cell, (iii) recovering the cell culture, and (iv) optionally, purifying the mycobacterial antigen(s) or the fusion polypeptide from the recovered cell and/or culture supernatant.

Yet another aspect relates to a composition comprising at least one of the immunogenic combination, ~~the fusion polypeptide,~~ the nucleic acid molecule, the vector or the host cell as disclosed herein or any combination thereof.

Still yet another aspect relates to the use of the immunogenic combination, ~~the fusion polypeptide,~~ the nucleic acid molecule, the vector, the host cell or the composition as disclosed herein, in the manufacture of a medicament, for preventing or treating a Mycobacterium infection or any disease and pathologic condition caused by or associated with such a Mycobacterium infection.

~~Yet another aspect relates to~~Also disclosed is an antibody which selectively binds to at least one of the mycobacterial antigens comprised in or encoded by the immunogenic combination or the fusion polypeptide, the nucleic acid molecule, the vector, the host cell or the composition as disclosed herein.

~~A f~~Further ~~aspect relates to~~ disclosed is a method for the detection and/or quantification a mycobacterial antigen in a biological sample taken from a subject infected or susceptible to be infected by a Mycobacterium which comprises the steps of bringing said biological sample into contact with an antibody disclosed herein as reagent under conditions allowing the

formation of a complex between the mycobacterial antigen and the antibody reagent and detecting and/or quantifying the formation of said complex by any appropriate means.

Still ~~a further aspect relates to~~ disclosed is a kit of reagents for diagnosis a Mycobacterium infection for antigen assay comprising the antibody disclosed above and for antibody assay comprising the immunogenic combination or the fusion polypeptide, the nucleic acid molecule, the vector, the host cell or the composition disclosed herein.

The present disclosure is particularly useful in the context of immunotherapy as stand alone or as BCG booster for preventive or therapeutic purposes in the Mycobacterium infection field, e.g. preventing *Mtb* infection and/or prevention of primary TB and/or prevention of reactivation in latently infected subjects. It can also be used in association with standard (e.g. antibiotic-therapy) or any other novel treatment that is currently developed (e.g. small direct or indirect inhibitor molecules; antibodies or immunotherapeutics, etc). The present disclosure is also useful in the veterinary field, for example to reduce or abolish the

Claims

1. An immunogenic combination comprising at least 5 different antigens or nucleic acid molecules encoding the at least 5 antigens, wherein said antigens are independently obtained from a Mycobacterium species, wherein the at least 5 mycobacterial antigens are selected from the group consisting of from the group consisting of ESAT-6 (Rv3875), TB10.4 (Rv0288), Ag85B (Rv1886), RpfB, RpfD, Rv0111, Rv0569, Rv1733c, Rv1807, Rv1813, Rv2029c, Rv2626, Rv3407 and Rv3478.

2. The immunogenic combination according to claim 1, wherein the mycobacterial antigens are obtained from a Mycobacterium species of the tuberculosis complex selected from the group consisting of *M. tuberculosis* (*Mtb*), *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. canetti*, *M. caprae*, and *M. microti*.

3. The immunogenic combination according to claim 1 or 2, wherein said immunogenic combination comprises or encodes mycobacterial antigens from at least 2 different infection phases selected from the group consisting of active, resuscitation and latent phases.

4. The immunogenic combination according to claim 3, wherein said immunogenic combination is multiphasic comprising or encoding at least one antigen from the active infection phase, at least one antigen from the resuscitation infection phase and at least one antigen from the latent infection phase.

5. The immunogenic combination according to claim 3 or 4, wherein said antigen(s) of the active phase is/are selected from the group consisting of ESAT-6 (Rv3875), CFP-10 (Rv3874), TB10.4 (Rv0288), Ag85A (Rv3804), Ag85B (Rv1886), Rv3619, Rv3620 and PPE family proteins Rv3478 and Rv2608.

6. The immunogenic combination according to claim 5, wherein said immunogenic combination comprises or expresses at least ESAT-6 (Rv3875), Ag85B (Rv1886) and TB10.4 (Rv0288).

7. The immunogenic combination according to claim 3 or 4, wherein said antigen(s) of the latent phase is/are selected from the group consisting of Rv0081, Rv0111, Rv0198, Rv0569,

Rv1733c, Rv1735, Rv1737, Rv1806, Rv1807, Rv1813, Rv2005c, Rv2029c, Rv2032, Rv2626, Rv2627, Rv2628, Rv2660c, Rv3407 and Rv3812.

8. The immunogenic combination according to claim 7, wherein said antigen(s) of the latent phase is/are selected from the group consisting of Rv0111, Rv0569, Rv1733c, Rv1807, Rv1813, Rv2029c, Rv2626, and Rv3407.

9. The immunogenic combination according to claim 3 or 4, wherein said antigen(s) of the resuscitation phase is/are selected from the group consisting of RpfA, RpfB, RpfC, RpfD and RpfE.

10. The immunogenic combination according to claim 9, wherein said immunogenic combination comprises or expresses at least RpfB and RpfD.

~~11. The immunogenic combination according to any one of claims 1 to 10, wherein said immunogenic combination comprises or encodes at least 5 mycobacterial antigens selected from the group consisting of ESAT-6 (Rv3875), CFP-10 (Rv3874), TB10.4 (Rv0288), Ag85A (Rv3804), Ag85B (Rv1886), Rv3619, Rv3620, RpfA, RpfB, RpfC, RpfD, RpfE, Rv0081, Rv0111, Rv0198, Rv0569, Rv1733c, Rv1735, Rv1737, Rv1806, Rv1807, Rv1813, Rv2005c, Rv2029c, Rv2032, Rv2626, Rv2627, Rv2628, Rv2660c, Rv3407 Rv3478, and Rv3812.~~

~~12~~11. The immunogenic combination according to claim ~~11~~1, wherein the at least 5 mycobacterial antigens are selected ~~from the group consisting of from the group consisting of ESAT-6 (Rv3875), TB10.4 (Rv0288), Ag85B (Rv1886), RpfB, RpfD, Rv0111, Rv0569, Rv1733c, Rv1807, Rv1813, Rv2029c, Rv2626, Rv3407 and Rv3478; and preferably~~ from the group of polypeptides comprising an amino acid sequence at least 80% homologous or identical to any of SEQ ID NO: 1-24.

~~13~~12. The immunogenic combination according to anyone of claims 1 to ~~12~~11, wherein said immunogenic combination comprises or encodes the mycobacterial antigens in the form of

separate polypeptides or in the form of one or more fusion polypeptides or both in the form of separate antigen(s) and fusion(s).

~~14. A fusion polypeptide comprising two or more mycobacterial antigens comprised or encoded by the immunogenic combination according to anyone of claims 5 to 12.~~

~~15. The fusion polypeptide according to claim 14 comprising at least 2 polypeptides selected from the group of polypeptides comprising an amino acid sequence at least 80% identical to any of SEQ ID NO: 1-24.~~

~~16. The fusion polypeptide according to claim 14 or 15, wherein said fusion polypeptide comprises mycobacterial antigens of the same infection phase.~~

~~17. The fusion polypeptide according to claim 14 or 15, wherein said fusion polypeptide comprises mycobacterial antigens from two different infection phases or even from the active, resuscitation and latent phases.~~

~~18. The fusion polypeptide according to claim 16 or 17, wherein said fusion polypeptide is selected from the group consisting of:~~

- ~~(i) A fusion polypeptide comprising *M. tuberculosis* antigens Rv2029c, Rv2626, Rv1733c and Rv0111;~~
- ~~(ii) A fusion polypeptide comprising *M. tuberculosis* antigens Rv0569, Rv1813, Rv3407, Rv3478 and Rv1807;~~
- ~~(iii) A fusion polypeptide comprising *M. tuberculosis* antigens Ag85B, TB10.4 and ESAT6;~~
- ~~(iv) A fusion polypeptide comprising *M. tuberculosis* antigens RpfB and RpfD;~~
- ~~(v) A fusion polypeptide comprising *M. tuberculosis* antigens RpfB, RpfD, Ag85B, TB10.4 and ESAT6;~~
- ~~(vi) A fusion polypeptide comprising *M. tuberculosis* antigens Ag85B, Rv2626, RpfB, RpfD andRv1733c;~~
- ~~(vii) A fusion polypeptide comprising *M. tuberculosis* antigens Rv2029, TB10.4, ESAT6 and Rv0111; and~~

(viii) A fusion polypeptide comprising *M. tuberculosis* antigens Ag85B, Rv2626 and Rv1733c.

19. The fusion polypeptide according to claim 18, wherein said fusion polypeptide is selected from the group of polypeptides comprising an amino acid sequence which exhibits at least 80% identity with anyone of the following amino acid sequences

- the amino acid sequence shown in SEQ ID NO: 28 from approximately position 32 to approximately position 506;
- the amino acid sequence shown in SEQ ID NO: 29 from approximately position 10 to approximately position 484;
- the amino acid sequence shown in SEQ ID NO: 30 from approximately position 32 to approximately position 380;
- the amino acid sequence shown in SEQ ID NO: 31 from approximately position 10 to approximately position 358;
- the amino acid sequence shown in SEQ ID NO: 32 from approximately position 32 to approximately position 855;
- the amino acid sequence shown in SEQ ID NO: 33 from approximately position 10 to approximately position 833;
- the amino acid sequence shown in SEQ ID NO: 34 from approximately position 32 to approximately position 1115;
- the amino acid sequence shown in SEQ ID NO: 35 from approximately position 10 to approximately position 1093;
- the amino acid sequence shown in SEQ ID NO: 36 from approximately position 32 to approximately position 956;
- the amino acid sequence shown in SEQ ID NO: 37 from approximately position 32 to approximately position 607;
- the amino acid sequence shown in SEQ ID NO: 38 from approximately position 37 to approximately position 932; or
- the amino acid sequence shown in SEQ ID NO: 39 from approximately position 37 to approximately position 831; and

wherein said amino acid sequence further comprises an initiator Met.

~~20. The fusion polypeptide according to claim 18 or 19, wherein said fusion polypeptide further comprises appropriate targeting peptide(s) such as signal and/or trans-membrane peptides.~~

~~21~~13. A nucleic acid molecule encoding said at least 5 mycobacterial antigens comprised in the immunogenic combination according to anyone of claims 5 to ~~13~~ 12~~or the fusion polypeptide according to anyone of claims 14 to 20~~.

~~22~~14. The nucleic acid molecule according to claim ~~21~~13, which exhibits at least 80% identity, with the nucleotide sequence shown in any of SEQ ID NO: 40-51 or a fragment thereof.

~~23~~15. A vector comprising one or more nucleic acid molecule(s) according to claim ~~21~~ 13 or ~~22~~14.

~~24~~16. The vector of claim ~~23~~15, wherein said vector is a plasmid or a viral vector selected from the group consisting of retrovirus, adenovirus, adenovirus-associated virus (AAV), poxvirus, herpes virus, measles virus, foamy virus, alphavirus, vesicular stomatis virus.

~~25~~17. The vector of claim ~~24~~16, wherein said vector is a E1-defective adenoviral vector.

~~26~~18. The vector of claim ~~24~~16, wherein said vector is a poxvirus vector selected from the group consisting of fowlpox, canarypox and vaccinia virus vector.

~~27~~19. The vector of claim ~~26~~18, wherein said vaccinia virus vector is Copenhagen, Wyeth, NYVAC and modified Ankara (MVA) strains.

~~28~~20. The vector of claim ~~23~~15, wherein said vector is a bacterium cell selected from the group consisting of *M. bovis* BCG, Lactobacillus, Listeria, Salmonella and Pseudomonas.

~~29~~21. The vector according to anyone of claims ~~23~~ 15 to ~~28~~ 20 which is selected from the group consisting of:

i. A vector encoding a fusion polypeptide comprising Rv2029c, Rv2626, Rv1733c and Rv0111 and a fusion polypeptide comprising RpfB, RpfD, Ag85B, TB10.4 and ESAT-6;

ii. A vector encoding a fusion polypeptide comprising Rv2029c, Rv2626, Rv1733c and Rv0111, a fusion polypeptide comprising RpfB, RpfD, Ag85B, TB10.4 and ESAT-6 and a fusion polypeptide comprising Rv0569, Rv1813, Rv3407, Rv3478 and Rv1807;

iii. A vector encoding a fusion polypeptide comprising Rv2029c, Rv2626, Rv1733c and Rv0111, a fusion polypeptide comprising RpfB, RpfD, Ag85B, TB10.4 and ESAT-6 and a fusion polypeptide comprising Rv0569, Rv1813, Rv3407, Rv3478 and Rv1807;

iv. A vector encoding a fusion polypeptide comprising Ag85B, Rv2626 RpfB, RpfD and Rv1733 and a fusion polypeptide comprising Rv2029c, TB10.4, ESAT-6 and Rv0111;

v. A vector encoding a fusion polypeptide comprising Ag85B, Rv2626 RpfB, RpfD and Rv1733, a fusion polypeptide comprising Rv2029c, TB10.4, ESAT-6 and Rv0111 and a fusion polypeptide comprising Rv0569, Rv1813, Rv3407, Rv3478 and Rv1807; and

vi. A vector encoding a fusion polypeptide comprising RpfB, RpfD, Ag85B, TB10.4 and ESAT-6 and a fusion polypeptide comprising Rv0569, Rv1813, Rv3407, Rv3478 and Rv1807.

~~30~~22. The vector according to anyone of claims ~~23~~15 to ~~29~~21 which is in the form of infectious virus particles.

~~31~~23. A process of producing an infectious virus particle comprising the steps of (i) introducing the viral vector of anyone of claims ~~24~~16 to ~~27~~19 and ~~29~~21 to ~~30~~22 into a suitable cell line, (ii) culturing said cell line under suitable conditions so as to allow the production of said infectious viral particle, (iii) recovering the produced viral particle from the culture of said cell line, and (iv) optionally purifying said recovered viral particle.

~~32~~24. A host cell comprising the immunogenic combination of anyone of claims 1 to ~~13~~12, ~~the fusion polypeptide of anyone of claims 14 to 20,~~ the nucleic acid molecule of anyone of claims ~~21~~ 13 to ~~22~~ 14 or the vector of anyone of claims ~~23~~ 15 to ~~30~~22.

~~33~~25. A method for recombinant production of the mycobacterial antigens comprised or encoded by the immunogenic combination of anyone of claims 1 to ~~13~~ 12~~or in the fusion polypeptide of anyone of claims 14 to 20~~, which comprises the steps of (i) introducing a vector into a suitable host cell to produce a transfected or infected host cell, (ii) culturing *in-vitro* said transfected or infected host cell under conditions suitable for growth of the host cell, (iii) recovering the cell culture, and (iv) optionally, purifying the mycobacterial antigen(s) or the fusion polypeptide from the recovered cell and/or culture supernatant.

~~34~~26. The method of claim ~~33~~25, wherein said suitable host cell is an *E coli* host cell and in particular a *E. coli* strain carrying the D13 prophage in its genome.

~~35~~ 27. A composition comprising at least one of the immunogenic combination of anyone of claims 1 to ~~13~~12, ~~the fusion polypeptide of anyone of claims 14 to 20,~~ the nucleic acid molecule of anyone of claims ~~21~~ 13 to ~~22~~14, the vector of anyone of claims ~~23~~ 15 to ~~30~~ 22 or the host cell of claim ~~32~~ 24 or any combination thereof.

~~36~~28. The composition of claim ~~35~~ 27 which further comprises a pharmaceutically acceptable vehicle.

~~37~~29. Use of the immunogenic combination of anyone of claims 1 to ~~13~~12, ~~the fusion polypeptide of anyone of claims 14 to 20,~~ the nucleic acid molecule of anyone of claims ~~21~~ 13 to ~~22~~14, the vector of anyone of claims ~~23~~ 15 to ~~30~~22, the host cell of claim ~~32~~ 24 or the composition of anyone of claims ~~35~~ 27 or ~~36~~ 28 in the manufacture of a medicament for preventing or treating a Mycobacterium infection or any disease and pathologic condition caused by or associated with such a Mycobacterium infection.

~~38~~30. The use according to claim ~~37~~29, for preventing infection or delaying the

risk of infection with a Mycobacterium in a subject in need thereof, especially a subject who has been in close contact with an infected individual having developed an active disease.

~~39~~31. The use according to claim ~~37~~29, for treating an active disease in a subject infected with a Mycobacterium species and especially *M. tuberculosis*.

~~40~~32. The use according to claim ~~37~~29, for preventing or treating reactivation in a subject latently infected with a Mycobacterium species and especially *M. tuberculosis*.

~~41~~33. The use according to claim ~~37~~29, as a BCG booster.

~~42~~34. The use according to anyone of claims ~~37~~ 29 to ~~41~~ 33 wherein the medicament is used in association with one or more chemotherapeutic drug(s) effective against a Mycobacterium infection, and especially one or more antibiotic chemotherapy.

~~43~~35. The use according to anyone of claims ~~37~~ 29 to ~~42~~34, for inducing or enhancing an immune response in the administered subject.

~~44~~36. The use according to claim ~~43~~35, wherein said induced or stimulated immune response is a CD4+ and/or CD8+-mediated T cell response directed to a mycobacterial antigen/epitope.

~~45. An antibody which selectively binds to at least one of the mycobacterial antigens comprised in or encoded by the immunogenic combination of anyone of claims 1 to 13 or the fusion polypeptide of anyone of claims 14 to 20, the nucleic acid molecule of anyone of claims 21 to 22, the vector of anyone of claims 23 to 30, the host cell of claim 32 or the composition of anyone of claims 35 or 36.~~

~~46. A method for the detection and/or quantification a mycobacterial antigen in a biological sample taken from a subject infected or susceptible to be infected by a Mycobacterium which comprises the steps of bringing said biological sample into contact with the antibody of claim 45 as reagent under conditions allowing the formation of a complex between the mycobacterial antigen and the antibody reagent and detecting and/or quantifying the formation of said complex by any appropriate means.~~

~~47. A kit of reagents for diagnosis a Mycobacterium infection for antigen assay comprising the antibody of claim 45 and for antibody assay comprising the immunogenic combination of anyone of claims 1 to 13 or the fusion polypeptide of anyone of claims 14 to 20, the nucleic acid molecule of anyone of claims 21 to 22, the vector of anyone of claims 23 to 30, the host cell of claim 32 or the composition of anyone of claims 35 or 36.~~