easily distinguished from infected individuals (this is particularly important in light of the fact that BCG does not protect against latent infection). In general, individuals who have received BCG but are not infected by M. *tuberculosis* show a PPD reaction below 10 mm in diameter whereas people who have a PPD reaction above 10 mm in diameter are considered to have been infected by M. *tuberculosis*. However, this rule is not applicable to individuals with immunosuppression due to HIV infection, which may result in a PPD reaction below 10 mm in diameter); or in endemic countries, where people infected by non-tuberculosis mycobacteria can show a PPD reaction above 10 mm in diameter.

Progress over recent years has seen the development of *in vitro* T cell based assays, based on interferon-gamma release and using antigens which are more specific to M. *tuberculosis* than PPD, namely ESAT-6 and CFP-10. These high specificity tests appear to be at least as sensitive as the tuberculin skin test and also demonstrate less cross-reactivity due to BCG vaccination. See Pai M et al *Expert Rev. Mol. Diagn.* 2006 6(3):413-422 for a recent review of latent TB diagnosis. However, since ESAT-6/CFP-10 are early stage antigens, assays based on ESAT-6/CFP-10 may only perform optimally in recently infected people. Consequently, the identification of new antigens specifically associated with latent tuberculosis may aid the development of more sensitive assays that could detect longer-term latent infections.

There remains a need for effective strategies for the treatment and prevention of tuberculosis, in particular the treatment and prevention of latent TB and the prevention of reactivation of TB.

## ~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure relates generally to the identification of Rv2386c as a TB antigen (in particular an antigen associated with latent TB) and to related methods and uses in the prevention and treatment of TB, especially the prevention and treatment of latent TB and the prevention or delay of TB reactivation.

The present ~~invention~~disclosure provides an isolated polypeptide which comprises:

(i) an Rv2386cprotein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence.

The present ~~invention~~disclosure also provides a polypeptide which comprises:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence;

for use as a medicament.

A further aspect of the ~~invention~~disclosure relates to a method for the treatment or prevention of TB comprising the administration of a safe and effective amount of a polypeptide comprising:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence;

to a subject in need thereof, wherein said polypeptide induces an immune response, in particular an immune response against *Mycobacterium tuberculosis*.

The use of a polypeptide comprising:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence;

in the manufacture of a medicament for the treatment or prevention of TB, represents another aspect of the invention.

The present ~~invention~~disclosure provides an isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide which comprises:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence.

Also provided is a polynucleotide comprising a nucleic acid sequence encoding a polypeptide which comprises:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence;

for use as a medicament.

A further aspect of the ~~invention~~disclosure relates to a method for the treatment or prevention of TB comprising the administration of a safe and effective amount of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence.

Host cells, transformed with said expression vector, form a further aspect of the ~~invention~~disclosure.

Additionally provided is a host cell which recombinantly expresses a polypeptide which comprises:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence.

Further, there is provided a method for the production of a polypeptide comprising:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence; said method comprising the step of recombinantly expressing said polypeptide within a host cell.

Additionally provided is an antibody or fragment thereof which specifically binds to a polypeptide comprising:

(i) an Rv2386c protein sequence;

(ii) a variant of an Rv2386c protein sequence; or

(iii) an immunogenic fragment of an Rv2386c protein sequence.

The use of said antibodies in diagnosis is also provided (such as methods for the diagnosis of tuberculosis comprising determining the presence of an antibody or fragment thereof which specifically binds to the polypeptides of the invention in a biological sample from a test subject).

Also provided are diagnostic kits comprising:

(a) a polypeptide of the present invention;

(b) apparatus sufficient to contact said polypeptide with a sample (e.g. whole blood or more suitably PBMC) from an individual; and

(c) means to quantify the T cell response of the sample.

Another aspect of the ~~invention~~disclosure relates to a diagnostic kit comprising:

(a) a polypeptide of the present invention; and

(b) apparatus sufficient to contact said polypeptide with the dermal cells of a patient.

In one embodiment the subject receiving a polypeptide, polynucleotide or composition of the invention may have active tuberculosis (e.g. active infection by *M. tuberculosis*). In a second embodiment the subject may have latent tuberculosis (e.g. dormant infection by *M. tuberculosis*). In a third embodiment the subject may be free from tuberculosis (e.g. free from infection by *M. tuberculosis*).

A subject receiving a polypeptide, polynucleotide or composition of the invention may have previously been vaccinated for tuberculosis (e.g. vaccinated against infection by *M. tuberculosis*), such as having been vaccinated with Bacillus Calmette-Guerin (BCG).
Alternatively, a subject receiving a polypeptide, polynucleotide or composition of the invention may not have previously been vaccinated for tuberculosis (e.g. not vaccinated against infection by *M. tuberculosis*), such as not having been vaccinated with *Bacillus* Calmette-Guerin (BCG).

According to one aspect, there is provided an isolated polypeptide which comprises: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1, for use as a medicament.

According to another aspect, there is provided a pharmaceutical composition comprising a polypeptide which comprises: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; and a pharmaceutically acceptable carrier or excipient.

According to another aspect, there is provided an immunogenic composition comprising a polypeptide which comprises: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from an Rv2386c protein sequence of SEQ ID No: 1; and a non-specific immune response enhancer.

According to another aspect, there is provided a composition comprising an Rv2386c component and an M72 component, wherein said Rv2386c component is a polypeptide which comprises: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; and said M72 component is: (i) a polypeptide which comprises an amino acid sequence having at least 90% identity to SEQ ID No: 25; or (ii) a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID No: 25: and wherein when said M72 component is provided in the form of a polypeptide, the Rv2386c component and an M72 component may be provided as either two individual polypeptide components or as a fusion protein comprising both polypeptide components.

According to another aspect, there is provided a composition comprising an Rv2386c component and an M72 component, wherein said Rv2386c component is a polynucleotide comprising a nucleic acid sequence encoding a polypeptide which comprises: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; and said M72 component is: (i) a polypeptide which comprises an amino acid sequence having at least 90% identity to SEQ ID No: 25; or (ii) a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID No: 25: and wherein when said M72 component is provided in the form of a polynucleotide, the Rv2386c component and an M72 component may be provided as two individual polynucleotide components, as a single polynucleotide encoding two individual polypeptide components or as a single polynucleotide encoding a fusion protein comprising both polypeptide components.

According to another aspect, there is provided use of a polypeptide comprising: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; in the manufacture of a medicament for the treatment or prevention of tuberculosis.

According to another aspect, there is provided use of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3 7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; in the manufacture of a medicament for the treatment of tuberculosis.

According to another aspect, there is provided use of a polypeptide comprising: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence; in the manufacture of a medicament for the treatment of tuberculosis with one or more chemotherapeutic agents effective against tuberculosis.

According to another aspect, there is provided use of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3 7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1; in the manufacture of a medicament for the treatment of tuberculosis with one or more chemotherapeutic agents effective against tuberculosis.

According to another aspect, there is provided a fusion protein comprising a polypeptide comprising:

(i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1.

According to another aspect, there is provided a polynucleotide comprising a nucleotide sequence encoding a fusion protein comprising a polypeptide comprising: (i) an Rv2386c protein sequence of SEQ ID No: 1 or 3-7; (ii) a variant of an Rv2386c protein sequence having at least 90% identity to SEQ ID No: 1; or (iii) an immunogenic fragment of at least 10 amino acid residues from the Rv2386c protein sequence of SEQ ID No: 1.

## DESCRIPTION OF THE FIGURES

Figure 1: Percentage of CD4 and CD8 cells from immunised CB6F1 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 21 (i.e. 7 days post second immunisation).

Figure 2: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD4 response in immunised CB6F1 mice.

Figure 3: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD8 response in immunised CB6F1 mice.

Figure 4: Percentage of CD4 and CD8 cells from immunised CB6F1 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 35 (i.e. 7 days post third immunisation).

Figure 5: Cytokine profile at day 35 (i.e. 7 days post third immunisation) of the antigen specific CD4 response in immunised CB6F1 mice.

Figure 6: Cytokine profile at day 35 (i.e. 7 days post third immunisation) of the antigen specific CD8 response in immunised CB6F1 mice.

Figure 7: Percentage of CD4 and CD8 cells from immunised C57BL/6 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 21 (i.e. 7 days post second immunisation).

Figure 8: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD4 response in immunised C57BL/6 mice.

Figure 9: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD8 response in immunised C57BL/6 mice.