

A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 2016; 387: 2312–22). Individuals with incipient TB have not yet developed induced clinical symptoms, radiographic abnormalities, or microbiologic evidence consistent with active TB.

Currently, treatment of LTBI is the main treatment intervention available to prevent development of active TB disease in those already infected with Mtb. TB preventive treatment for LTBI is expanding, but most of those for whom it would be beneficial are not yet accessing care. One reason for this is under-diagnosis of people with LTBI, for multiple reasons including lack of or subclinical symptoms meaning people do not seek out health care, and failure to accurately test for TB when people do present to health professionals.

There is a need therefore for a rapid, simple, accurate test which allows for the detection of incipient TB at a time point early enough to permit effective intervention and treatment. It is against this background that the present invention was developed.

Summary

In an aspect of the present disclosure, there is provided a method of diagnosis of a disease in an asymptomatic human subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

admixing Mycobacteria-specific bacteriophage D29 with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

isolating DNA from the admixture;

determining if a Mycobacterial DNA sequence is present or absent in the DNA isolated from the admixture by one or more of: performing PCR on the DNA isolated from the admixture using forward and reverse primers specific for the Mycobacterial DNA sequence, and/or performing sequencing suitable to identify the Mycobacterial DNA sequence, and optionally determining homology between the Mycobacterial DNA sequence, if present, and known Mycobacterial DNA sequences;

optionally, performing a Mycobacterium-specific cell-mediated immune (CMI) response test on a sample isolated from the subject to generate a positive or negative CMI response test outcome;

wherein the asymptomatic human subject is diagnosed as having incipient tuberculosis (TB)

when the Mycobacterial DNA sequence is present, with either a positive or negative CMI response test outcome, where present; and

wherein the asymptomatic human subject is diagnosed as having latent tuberculosis infection (LTBI) when the Mycobacterial DNA sequence is absent and a positive CMI response test outcome is present.

In another aspect of the present disclosure, there is provided a method of predicting if a subject is at risk of developing or having incipient tuberculosis (TB), the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

5 isolating DNA from the admixture;

identifying if Mycobacterial DNA sequences are present in the DNA;

wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of developing or having incipient TB.

10 In a further aspect of the present disclosure, there is provided a method of assessing the tuberculosis (TB) disease prognosis for a subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

15 isolating DNA from the admixture;

identifying if Mycobacterial DNA sequences are present in the DNA isolated from the admixture;

wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of having incipient TB and/or developing active TB disease.

20 In another aspect of the present disclosure, there is provided a use of one or more anti-TB medicament or therapy in the manufacture of a medicament for a method of treatment of tuberculosis (TB) in a subject in need thereof, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of incipient TB in the subject; and, where incipient TB is identified, and

25 wherein one or more anti-TB medicament or therapy are to be administered to the subject.

In a further aspect of the present disclosure, there is provided a use of one or more anti-incipient TB medicament or therapy in the manufacture of a medicament for a method of treatment of incipient tuberculosis (TB) in a subject in need thereof, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

30 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

isolating DNA from the admixture;

identifying the presence of Mycobacterial sequences in the DNA; and

35 wherein one or more anti-incipient TB medicament or therapy are to be administered to the subject.

In another aspect of the present disclosure, there is provided a use of one or more anti-TB medicament or therapy in the manufacture of a medicament for a method of improving tuberculosis (TB) prognosis in a subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
5 using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of incipient TB in the subject; and, where incipient TB is identified, and
wherein one or more anti-TB medicament or therapy are to be administered to the subject.

In a further aspect of the present disclosure, there is provided a use of a bacteriophage for measuring in a blood sample isolated from a human subject the presence of one or more Mycobacterial biomarker in
10 the manufacture of a kit for assessing if said human subject is at risk of developing or has incipient tuberculosis (TB) or active tuberculosis disease.

In a first example of the present disclosure there is provided:

a method of diagnosis of a disease in an asymptomatic human subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

15 admixing Mycobacteria-specific bacteriophage D29 with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

isolating DNA from the admixture;

determining if a Mycobacterial DNA sequence is present or absent in the DNA isolated from the
20 admixture by one or more of: performing PCR on the DNA isolated from the admixture using forward and reverse primers specific for the Mycobacterial DNA sequence, and/or performing sequencing suitable to identify the Mycobacterial DNA sequence, and optionally determining homology between the Mycobacterial DNA sequence, if present, and known Mycobacterial DNA sequences;

optionally, performing a Mycobacterium-specific cell-mediated immune (CMI) response test on a
25 sample isolated from the subject to generate a positive or negative CMI response test outcome;

wherein the asymptomatic human subject is diagnosed as having incipient tuberculosis (TB) when the Mycobacterial DNA sequence is present, with either a positive or negative CMI response test outcome, where present; and

wherein the asymptomatic human subject is diagnosed as having latent tuberculosis infection
30 (LTBI) when the Mycobacterial DNA sequence is absent and a positive CMI response test outcome is present.

In another example of the present disclosure there is provided:

a method of diagnosing incipient tuberculosis (TB) in a human subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;
 incubating the admixture under conditions that permit lysis of Mycobacteria;
 5 isolating DNA from the admixture;
 determining if one or more Mycobacterial DNA sequence is present in the DNA isolated from the
 admixture;
 wherein the presence of a Mycobacterial DNA sequence indicates that the subject has incipient
 tuberculosis.

10 In one example the human subject is an asymptomatic human subject, i.e. the subject is not
 displaying any symptoms of active tuberculosis disease.

In another example of the present disclosure there is provided:

a method of predicting if a subject is at risk of developing or having incipient tuberculosis (TB), the
 method comprising:

15 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;
 incubating the admixture under conditions that permit lysis of Mycobacteria;
 isolating DNA from the admixture;
 20 identifying if Mycobacterial DNA sequences are present in the DNA;
 wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of
 developing or having incipient TB.

In another example of the present disclosure there is provided:

a method of predicting if a subject is at risk of developing active tuberculosis (TB), the method
 25 comprising:
 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;
 incubating the admixture under conditions that permit lysis of Mycobacteria;
 30 isolating DNA from the admixture;
 identifying if Mycobacterial DNA sequences are present in the DNA;
 wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of
 developing active TB.

In another example of the present disclosure there is provided:

35 a method of assessing the tuberculosis (TB) disease prognosis for a subject, the method comprising:
 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

isolating DNA from the admixture;

- 5 identifying if Mycobacterial DNA sequences are present in the DNA isolated from the admixture;
wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of having incipient TB and/or developing active TB disease.

In another example of the present disclosure there is provided:

a method of treatment of tuberculosis (TB) in a subject in need thereof, the method comprising:

- 10 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of incipient TB in the subject; and, where incipient TB is identified,
administering one or more anti-TB medicament or therapy to the subject.

- 15 A bacteriophage-mediated Mycobacterial DNA release assay means any assay or method as described herein whereby a Mycobacterium-specific bacteriophage (i.e. a bacteriophage that will selectively lyse mycobacteria or a particular mycobacterium species or strain) is used to lyse Mycobacteria in a sample in order to release the Mycobacterial DNA, following which the presence of Mycobacterial DNA is confirmed in the sample by any suitable method, for example PCR using Mycobacterium-specific
20 primers, or by next-generation sequencing.

In another example of the present disclosure there is provided:

a method of treatment of incipient tuberculosis (TB) in a subject in need thereof, the method comprising:

- obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
25 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an admixture;
incubating the admixture under conditions that permit lysis of Mycobacteria;
isolating DNA from the admixture;
identifying the presence of Mycobacterial sequences in the DNA; and
30 administering one or more anti-incipient TB medicament or therapy to the subject.

In another example of the present disclosure there is provided:

a method of improving tuberculosis (TB) prognosis in a subject, the method comprising:

- obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of
35 incipient TB in the subject; and, where incipient TB is identified,
administering one or more anti-TB medicament or therapy to the subject.

Suitable anti-TB medicaments or therapies in accordance with any example of the present disclosure will be known to those skilled in the art, and may be selected based on clinical or other indication. Suitable anti-TB

medicaments or therapies may include rifampicin, ethambutol hydrochloride, pyrazinamide, and isoniazid (with pyridoxine hydrochloride), which may be administered to a subject alone (one anti-TB medicament or therapy) or in any suitable combination of more than one anti-TB medicament or therapy.

In an example of the present disclosure, the sample of peripheral blood mononuclear cells (PBMCs) may be isolated from the subject by any suitable method, for example density centrifugation (e.g. Ficoll-Paque), isolation by cell preparation tubes (CPT™), isolation by SepMate™ tubes, or isolation using an erythrocyte aggregation agent e.g. HetaSep™.

In another example of the present disclosure there is provided:

use of a bacteriophage for measuring in a blood sample isolated from a human subject the presence of one or more Mycobacterial biomarker in the manufacture of a kit for assessing if said human subject is at risk of developing or has incipient tuberculosis (TB) or active tuberculosis disease.

In one example of the present disclosure, determining if one or more Mycobacterial DNA sequence is present in the DNA may comprise performing polymerase chain reaction, for example using Mycobacterium-specific primers.

In one example of the present disclosure, the one or more Mycobacterial DNA sequence may comprise e.g. the IS6110 element and/or the IS900 element.

In one example of the present disclosure, the method may comprise sequencing the one or more Mycobacterial DNA sequence, and optionally determining homology between the Mycobacterial DNA sequence and known Mycobacterial DNA sequences. Known mycobacterial DNA sequences may comprise those identified from published sequence databases, or they may comprise DNA sequences identified from TB-infected individuals that the subject is known or is suspected to have come into contact with.

In one example of the present disclosure, the subject may be a human subject. In one example of the present disclosure, the subject may be a mammal, such as but not limited to a primate a great ape or a human. In one example of the present disclosure, the subject may be selected from at least one of: an asymptomatic subject and/or a TB-contact subject. In one example of the present disclosure, the subject may be selected from at least one of: an asymptomatic human subject and/or a TB-contact human subject. An asymptomatic subject means any subject who is not displaying any symptoms of active tuberculosis disease that are typically used to confirm diagnosis. Such symptoms may be determined by clinical or radiological assessment. Additionally the subject may give a negative test result by sputum smear microscopy and/or mycobacterial culture. A TB-contact subject means any subject who has come into contact with another individual known or suspected to have a tuberculosis infection. A TB-contact subject may be identified by TB contact tracing.

In one example of the present disclosure, the subject may be immunocompromised and/or immunosuppressed. Both immunocompromise and immunosuppression may be considered states of immunodeficiency. Immunocompromise in a subject may be caused by a disease that directly or indirectly causes immunosuppression. Examples of such diseases include particular cancers (e.g. leukaemia, lymphoma, multiple myeloma, cancers of bone marrow, cancers of blood cells), chronic infections e.g. acquired immunodeficiency syndrome (AIDS) caused by infection with the human immunodeficiency virus (HIV), and various hormonal and metabolic disorders including anaemia, hypothyroidism and hyperglycaemia. In one example, the subject may be infected with HIV. Immunosuppression in a subject may also be caused by malnutrition, aging, the administration of an immunosuppressive agent, medication or immunosuppressant (for example chemotherapy, a disease-modifying anti-rheumatic drug (a DMARD), an immunosuppressive drug administered in relation to organ transplant (an antirejection medication), a steroid including a corticosteroid e.g. a glucocorticoid), exposure to environmental toxins, or the abuse of alcohol, illegal drugs or nicotine. In one example, the subject may be taking or have taken immunosuppressive medication, particularly one or more medications selected from the group consisting of: chemotherapy, a DMARD, an antirejection medication and a glucocorticoid.

In one example of the present disclosure, the subject may be a TB-contact subject, and suitably the method is performed on a sample of PBMCs obtained from the subject within 12 months, 6 months, 5 months, 4 months, 3 months, 2 months or 1 month of the subject coming into contact with a TB-infected individual.

In one example of the present disclosure, the bacteriophage may be (myco)bacteriophage D29 or TM4, preferably D29. Other bacteriophages suitable for lysing Mycobacteria will be known to those skilled in the art, e.g. see Hatfull GF. (2018) Mycobacteriophages. *Microbiol Spectr.* 2018;6(5):10.1128/microbiolspec.GPP3-0026-2018. doi:10.1128/microbiolspec.GPP3-0026-2018.

In one example of the present disclosure, incubating the admixture under conditions that permit lysis of Mycobacteria may comprise incubation at approximately 37 °C for approximately or less than 6 hours, 5 hours, 4 hours, 3.5 hours, 3 hours, 2.5 hours, 2 hours, 1.5 hours or 1 hour, preferably for approximately 3.5 hours.

In one example of the present disclosure, the optional CMI response test may comprise an interferon-gamma release assay (IGRA) test. An example of a suitable IGRA test is the QuantiFERON-TB Gold Plus ((QFT) Qiagen Inc) assay. Other Mycobacteria-specific CMI response tests will be known to those skilled in the art.

Various further aspects and examples of the present disclosure will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification are incorporated herein by reference in their entirety.

“and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or example of the present disclosure and apply equally to all aspects and examples which are described.

It will further be appreciated by those skilled in the art that although the present disclosure has been described by way of example with reference to several examples. It is not limited to the disclosed examples and that alternative examples could be constructed without departing from the scope of the present disclosure as defined in the appended claims.

Unless specifically stated, a process comprising steps may be performed in any suitable order. Thus steps can be performed in any appropriate order.

Sequence homology can be measured using known methods. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or to line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) *J Mol Evol* 36:290-300; Altschul, S, F *et al* (1990) *J Mol Biol* 215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Brief Description of the Figures

Figure 1 is a graph showing the results of a Mycobacterium-specific cell-mediated immune (CMI) response test (an IGRA test: the QuantiFERON-TB Gold Plus ((QFT) Qiagen Inc) assay) at baseline for a number of asymptomatic subjects determined to be in group 2 during the course of the study. All the subjects included in Fig. 1 showed a positive QFT test outcome at one or more timepoint during the study, and all were asymptomatic at baseline. The Y axis shows an arbitrary assigned participant number for each subject, the X axis shows the ratio between Antigen-1 and Mitogen (positive control antigen) as determined using the QFT kit. Subjects corresponding to assigned participant numbers 455, 493 and 494 tested positive by bacteriophage-mediated Mycobacterial DNA release assay (Actiphage™) at baseline.

CLAIMS

1. A method of diagnosis of a disease in an asymptomatic human subject, the method comprising:
 - obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 - admixing Mycobacteria-specific bacteriophage D29 with the sample of PBMCs to produce an
 - 5 admixture;
 - incubating the admixture under conditions that permit lysis of Mycobacteria;
 - isolating DNA from the admixture;
 - determining if a Mycobacterial DNA sequence is present or absent in the DNA isolated from the admixture by one or more of: performing PCR on the DNA isolated from the admixture using forward and
 - 10 reverse primers specific for the Mycobacterial DNA sequence, and/or performing sequencing suitable to identify the Mycobacterial DNA sequence, and optionally determining homology between the Mycobacterial DNA sequence, if present, and known Mycobacterial DNA sequences;
 - optionally, performing a Mycobacterium-specific cell-mediated immune (CMI) response test on a sample isolated from the subject to generate a positive or negative CMI response test outcome;
 - 15 wherein the asymptomatic human subject is diagnosed as having incipient tuberculosis (TB) when the Mycobacterial DNA sequence is present, with either a positive or negative CMI response test outcome, where present; and
 - wherein the asymptomatic human subject is diagnosed as having latent tuberculosis infection (LTBI) when the Mycobacterial DNA sequence is absent and a positive CMI response test outcome is
 - 20 present.
2. The method according to claim 1, wherein the Mycobacterial DNA sequence comprises the IS6110 element or the IS900 element.
3. The method according to claim 1 or claim 2, wherein the asymptomatic human subject is a TB-contact
- 25 subject.
4. The method according to claim 3, wherein the method is performed on a sample of PBMCs obtained from the asymptomatic human subject within 12 months, 6 months, 5 months, 4 months, 3 months, 2 months or 1 month of the asymptomatic human subject coming into contact with a TB-infected individual.
5. The method according to any preceding claim, wherein incubating the admixture under conditions that
- 30 permit lysis of Mycobacteria comprises incubation at approximately 37 °C for approximately or less than 6 hours, 5 hours, 4 hours, 3.5 hours, 3 hours, 2.5 hours, 2 hours, 1.5 hours or 1 hour, preferably for approximately 3.5 hours.
6. The method according to any preceding claim, wherein the optional CMI response test comprises an interferon-gamma release assay (IGRA) test.
7. A method of predicting if a subject is at risk of developing or having incipient tuberculosis (TB), the
- 35 method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

5 isolating DNA from the admixture;

identifying if Mycobacterial DNA sequences are present in the DNA;

wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of
 developing or having incipient TB.

8. A method of assessing the tuberculosis (TB) disease prognosis for a subject, the method comprising:

10 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;
 admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

isolating DNA from the admixture;

15 identifying if Mycobacterial DNA sequences are present in the DNA isolated from the admixture;

wherein the presence of Mycobacterial DNA sequences indicates that the subject is at risk of
 having incipient TB and/or developing active TB disease.

9. Use of one or more anti-TB medicament or therapy in the manufacture of a medicament for a method
 of treatment of tuberculosis (TB) in a subject in need thereof, the method comprising:

20 obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of
 incipient TB in the subject; and, where incipient TB is identified, and

wherein one or more anti-TB medicament or therapy are to be administered to the subject.

10. Use of one or more anti-incipient TB medicament or therapy in the manufacture of a medicament for
 25 a method of treatment of incipient tuberculosis (TB) in a subject in need thereof, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

admixing a Mycobacteria-specific bacteriophage with the sample of PBMCs to produce an
 admixture;

incubating the admixture under conditions that permit lysis of Mycobacteria;

30 isolating DNA from the admixture;

identifying the presence of Mycobacterial sequences in the DNA; and

wherein one or more anti-incipient TB medicament or therapy are to be administered to the
 subject.

11. Use of one or more anti-TB medicament or therapy in the manufacture of a medicament for a method
 35 of improving tuberculosis (TB) prognosis in a subject, the method comprising:

obtaining a sample of peripheral blood mononuclear cells (PBMCs) isolated from the subject;

using a bacteriophage-mediated Mycobacterial DNA release assay to identify the presence of
 incipient TB in the subject; and, where incipient TB is identified, and

wherein one or more anti-TB medicament or therapy are to be administered to the subject.

12. Use of a bacteriophage for measuring in a blood sample isolated from a human subject the presence of one or more Mycobacterial biomarker in the manufacture of a kit for assessing if said human subject is at risk of developing or has incipient tuberculosis (TB) or active tuberculosis disease.