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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

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))

(88) Date of publication of the international search report:

31 January 2013

(54) Title: MYCOBACTERIAL VACCINE VECTORS AND METHODS OF USING THE SAME

(57) Abstract: Mycobacterium bovis BCG is a potent stimulator of the cellular immune response and has potential as a recombinant vaccine vector. Disclosed are BCG strains that generate greater MHC class I presentation of a transgenic protein, relative to the unmutated parental strain, and that improve a recipient's CD8+ T cell response against the transgenic protein following vaccination. The mycobacterial constructs and their respective transposon mutant BCG strains exhibit increased immunogenicity that is several times greater than responses generated by the parental strain and other existing modified rBCG strains. Furthermore, upon introducing the SIV gag gene into these novel strains, we observed that these strains primed for increased CD8+ T cell responses in a heterologous prime/boost regimen, comparable to levels generated by plasmid DNA vaccines. Creation of a second generation rBCG vector that may be utilized as a vaccine vector for immunizing against a variety of pathogens.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/32164

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00, 39/04; C12N 1/20, 15/74 (2012.01)

USPC - 424/184.1; 248.1; 435/253.1, 471

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)

IPC(8) - A61K 39/00, 39/04; C12N 1/20, 15/74 (2012.01)

USPC - 424/184.1; 248.1; 435/253.1, 471

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)

WEST - DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ; Google Scholar

Search Terms: Mycobacterium, bovis, BCG, rBCG, Bacillus Calmette Guerin, isoniazid, induc\$, A, iniA, isoniazid inducible gene, iniA, isoniazid induced gene A, mutat\$, attenuat\$, mutan\$, delet\$, substitut\$, insertion, truncat\$, deletion, 1173P2, BCG0381, 0381

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLANGELI, R. et al. The Mycobacterium tuberculosis iniA gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. Molecular Microbiology. 2005, Volume 55(6), pages 1829-1840: Abstract; pg1830, col 1, para 2; pg 1832, col 1, para 2; pg 1832, col 2, para 1; pg 1838, col 1, para 2	1-5
A	US 2002/0151008 A1 (ALLAND et al.) 17 October 2002 (17.10.2002) para [0026]; [0030]; [0033]; [0036]; [0039]; [0048]	1-5
A	BROSCH, R. et al. Isoniazid inducible gene protein iniA. UniProtKB/TrEMBL Accession Number A1KFG4 [online]. 06 February 2007 [retrieved on 16 June 2012]. Retrieved from the Internet: <URL: http://www.uniprot.org/uniprot/A1KFG4.txt?version=1>, page 1	1-5
A	ZHENG, J. et al., Membrane subproteomic analysis of Mycobacterium bovis bacillus Calmette-Guerin. Proteomics. 2007, Vol 7, pages 3919-3931, entire document.	1-5

☐ Further documents are listed in the continuation of Box C.

* 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

11 September 2012 (11.09.2012)

Date of mailing of the international search report

24 SEP 2012

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/32164

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-34 and 39-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-5, drawn to a mycobacterium comprising one or more mutations that ablate or reduce expression of at least one gene selected from BCG_0381, BCG_0546c, BCG_0992, BCG_0993, BCG_1472c, BCG_1790, BCG_1964, BCG_2067c, BCG_2384c, BCG_2449c, BCG_2580, BCG_2588, BCG_2589, BCG_3231C, BCG_3297, BCG_3445, and BCG_3808c, or a homolog thereof, or an operon that includes said gene. The first invention encompasses the gene BCG_0381. Should an additional fee(s) be paid, Applicant is invited to elect an additional gene(s) to be searched.

-----continued on first blank sheet attached hereto-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, limited to the gene BCG_0381

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/32164

continuation of Box No III: Observations where unity of invention is lacking

Group II: Claims 35-38, drawn to a method of optimizing a mycobacterial vaccine vector comprising: a) mutagenizing a parental mycobacterial strain expressing an antigen by disruption of one or more genes of said parental mycobacterial strain using a transposon to produce a mutated mycobacterium; and b) assaying said mutated mycobacterium for MHC class I presentation of said antigen, wherein an increase in MHC class I presentation of said antigen relative to said parental mycobacterial strain indicates said mutated mycobacterium is an optimized mycobacterial vaccine vector.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I+ does not include the inventive concept of a method of optimizing a mycobacterial vaccine vector, as required by Group II.

Group II does not include the inventive concept of a mycobacterium comprising one or more mutations that ablate or reduce expression of at least one gene selected from BCG_0381, BCG_0546c, BCG_0992, BCG_0993, BCG_1472c, BCG_1790, BCG_1964, BCG_2067c, BCG_2384c, BCG_2449c, BCG_2580, BCG_2588, BCG_2589, BCG_3231C, BCG_3297, BCG_3445, and BCG_3808c, or a homolog thereof, or an operon that includes said gene, as required by Group I+.

Groups I+ and II share the technical feature of a mutated mycobacterium comprising one or more mutations that ablate or reduce expression of at least one gene.

Group I+ share the technical feature of a mycobacterium comprising one or more mutations that ablate or reduce expression of at least one gene.

However, this shared technical feature does not represent a contribution over the prior art as being anticipated by US 2009/0215031 A1 to Tyagi et al. (hereinafter 'Tyagi') (27 August 2009) that teaches a mutated mycobacterium comprising one or more mutations that ablate or reduce expression of at least one gene (abstract-'mutant Mycobacterium strains harboring a modified tyrosine phosphatase gene (mptpA or mptpB) wherein the mutant Mycobacterium strain is incapable of expressing the active tyrosine phosphatase', 'The mptpA or mptpB gene may be modified by replacing the internal sequences with an antibiotic resistance marker gene, which disrupts the expression of the active gene').

As said shared technical feature was known at the time of the invention, this cannot be considered special technical feature that would otherwise unify the Groups.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.