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(54) Title: ENGINEERED LISTERIA AND METHODS OF USE THEREOF

(57) Abstract: The invention provides a bacterium belonging to the genus *Listeria* containing a polynucleotide comprising a nucleic acid encoding a heterologous antigen, as well as fusion protein partners. Also provided are vectors for mediating site-specific recombination and vectors comprising removable antibiotic resistance genes.



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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/005457

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/74 A61K39/02

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 C12N

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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, 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	WO 2004/110481 A (CERUS CORP [US]; DUBENSKY THOMAS W JR [US]; BROCKSTEDT DIRK G [US]; CO) 23 December 2004 (2004-12-23) page 33 - page 45 tables 2,5	1-20, 29-62, 69,70, 75-134
X	WO 2005/071088 A (CERUS CORP [US]; DUBENSKY THOMAS W JR [US]; PORTNOY DANIEL A [US]; LUC) 4 August 2005 (2005-08-04) page 94 - page 103 page 111 figure 48C	1-20, 29-62, 69,70, 75-134
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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 document 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

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/005457

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>BROCKSTEDT DIRK G ET AL: "Recombinant listeria monocytogenes-based immunotherapy targeting mesothelin for the treatment of pancreatic and ovarian cancer." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 46, April 2005 (2005-04), page 1418, XP001536813 & 96TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-CANCER-RESEARCH; ANAHEIM, CA, USA; APRIL 16 -20, 2005 ISSN: 0197-016X abstract</p>	<p>1-20, 29-62, 69,70, 75-134</p>
X	<p>BROCKSTEDT D G ET AL: "Listeria-based cancer vaccines that segregate immunogenicity from toxicity" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 101, no. 38, 21 September 2004 (2004-09-21), pages 13832-13837, XP002305523 ISSN: 0027-8424 Last page</p>	<p>1-20, 29-62, 69,70, 75-134</p>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/005457

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:

Although claims 20, 30, 46-48, 59-62, 90, 98, 110, 116 and 132 are directed to a *method of treatment of the human/animal body*, the search has been carried out and based on the alleged effects of the compound/composition.
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.:
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:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. As all searchable claims could be searched without effort justifying an 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.:

1-20, 29-62, 69, 70 and 75-134 (completely)

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/US2007/005457

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. type of material
- a sequence listing
- table(s) related to the sequence listing
- b. format of material
- on paper
- in electronic form
- c. time of filing/furnishing
- contained in the international application as filed
- filed together with the international application in electronic form
- furnished subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20, 29-62, 69, 70 and 75-134 (completely)

Polynucleotide comprising a promoter and a nucleic acid operably linked to the promoter, wherein the nucleic acid encodes a fusion protein comprising a *Listeria monocytogenes* deletion mutant actA gene and a heterologous antigen like for example mesothelin or PSCA. *Listeria* bacterium comprising said polynucleotide, vaccine comprising said bacterium and use of the vaccine for cancer treatment.

2. claims: 21-28 (completely)

A *Listeria* bacterium comprising a genome, wherein the genome comprises a polynucleotide comprising a nucleic acid encoding a heterologous antigen, wherein the nucleic acid has been integrated into a virulence gene in the genome, wherein integration of the polynucleotide (a) disrupts expression of the virulence gene or (b) disrupts a coding sequence of the virulence gene.

3. claims: 63, 64 and 71 (completely)

A plasmid comprising a first nucleic acid encoding a phage integrase, a second nucleic acid encoding a phage attachment site (attP' site) and a third nucleic acid encoding a heterologous antigen or regulatory nucleic acid. Method of modifying a bacterial genome involving the use of said plasmid.

4. claims: 65, 66, 72 and 73 (completely)

A plasmid comprising: a) a first nucleic acid encoding a first region of homology to the bacterial genome, b) a second nucleic acid encoding a second region of homology to the bacterial genome, and c) a third nucleic acid comprising a bacterial attachment site (attBB'), wherein the third nucleic acid is flanked by the first and second nucleic acids and wherein the first and second nucleic acids are operably linked with each other and able to mediate homologous integration of the third nucleic acid into the bacterial genome. A *Listeria* bacterium modified by integration of said plasmid.

5. claim: 67 and 74 (completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A bacterium wherein the genome comprises a polynucleotide containing two operably linked heterologous recombinase binding sites flanking a first nucleic acid, wherein the two sites are two lox sites or two Frt sites and wherein the two lox sites are excisable by Cre recombinase and the the nucleic acid flanked by the two Frt sites is excisable by FLP recombinase.

6. claim: 68 (completely)

A method of excising a nucleic acid from a bacterial genome comprising contacting the genome with the Cre recombinase or FLP recombinase and allowing the recombinase to catalyze excision of the nucleic acid under conditions allowing or facilitating excision, wherein said nucleic acid is either flanked by lox sites (and the recombinase is Cre recombinase) or by Frt sites (and the recombinase is FLP recombinase).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/US2007/005457

Patent document cited in search report	A	Publication date	Patent family member(s)	Publication date
WO 2004110481	A	23-12-2004	AU 2004246999 A1	23-12-2004
			CA 2515369 A1	23-12-2004
			EP 1592441 A2	09-11-2005
			JP 2007503846 T	01-03-2007
			JP 2007125040 A	24-05-2007
			KR 20050105460 A	04-11-2005
			MX PA05008342 A	28-02-2006
WO 2005071088	A	04-08-2005	AU 2004314347 A1	04-08-2005
			CA 2551644 A1	04-08-2005
			EP 1708741 A2	11-10-2006