(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 September 2009 (11.09.2009)

PCT

(10) International Publication Number
WO 2009/111177 A3

(51) International Patent Classification:
C12N 15/00 (2006.01) *C12N 1/21* (2006.01)

(21) International Application Number:
PCT/US2009/034549

(22) International Filing Date:
19 February 2009 (19.02.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/034,111 5 March 2008 (05.03.2008) US

(71) Applicant *(for all designated States except US)*: **MOUNT SINAI SCHOOL OF MEDICINE OF NEW YORK UNIVERSITY** [US/US]; One Gustave L. Levy Place, New York, NY 10029 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WOO, Savio, L.C.** [US/US]; 279 Central Park West, Apt. 5B, New York, NY 10024 (US). **LI, Zhiyu** [US/US]; 8201 Britton Ave 4F, Elmhurst, NY 11373 (US). **WETMUR, James** [US/US]; 994 Post Road, Scarsdale, NY 10583 (US).

(74) Agent: **LUDWIG, Peter, S.**; Darby & Darby P.C., P.O. Box 770, Church Street Station, New York, NY 10008-0770 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:
7 January 2010

WO 2009/111177 A3

(54) Title: COMPOSITIONS AND METHODS COMPRISING GENETICALLY ENHANCED OBLIGATE AND FACULTATIVE ANAEROBIC BACTERIA FOR ONCOPATHIC CANCER THERAPY

(57) Abstract: Provided are genetically enhanced anaerobic bacteria for the oncopathic therapy of cancers, including pancreatic and other cancers that contain avascular, hypoxic regions. Within certain embodiments, genetically enhanced anaerobic bacteria contain one or more mutation(s). such as, for example, a deletion in one or more oxygen tolerance gene(s) such as a superoxide dismutase (*sod*), glutathione peroxidase (*gpo*), rubrerythrin (*rbr*), and/or an alcohol dehydrogenase family mernbei (*ydaD*) gene. Within further embodiments, genetically enhanced anaerobic bacteria contain one or more mutation(s), such as, for example, a deletion in one or more toxin gene(s) such as a phospholipase c (*plc*) gene. Within other embodiments, genetically enhanced anaerobic bacteria further express an inflammation suppressive gene such as, for example, a *Staphylococcus aureus* Panton-Valentine Leukocidin (*PVL*) gene and/or a *Yersinia enterocolitica* virulence factor (*LcrV*) gene. Genetically enhanced anaerobic bacteria disclosed herein, as exemplified by *Clostridium, Bifidobacterium,* and *Salmonella* species, are capable of selectively targeting hypoxic tumors.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/34549

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00, C12N 1/21 (2009.01)
USPC - 424/93.2; 435/252.1, 435/252.7, 435/252.8
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)
USPC - 424/93.2; 435/252.1, 435/252.7, 435/252.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/243, 252.3

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(DB=PGPB,USPT,USOC,EPAB,JPAB), Google scholar(clostridium perfringens sod mutation tumor, clostridium perfringens sod mutation, clostridium perfringens sod knockout, clostridium perfringens "sod knockout", clostridium perfringens "mutation in the sod gene", clostridium perfringens sod-, clostridium sod knockout)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRIOLAT et al., Identification of the Clostridium perfringens Genes Involved in the Adaptive Response to Oxidative Stress. Journal of Bacteriology, May 2002, Vol 184, No 9, pp 2333-2343; Abstract; pg 2333, left col, para 1, ln 1-15	1-4, 8
---		---
Y		5-7, 9-34, 46-47, 51-79
X	THEYS et al., Tumor-Specific Gene Delivery Using Genetically Engineered Bacteria. Current Gene Therapy, June 2003, Vol 3, No 3, pp 207-221; Abstract; pg 208, pg 209, right col, para 1, ln 1-16;para 2; pg 210, right col, para 2, ln 8-11; pg 211, right col, para 2, ln 3-7; pg 213, Table 1; pg 215, ATTENUATED SALMONELLA, Attenuation of Salmonella Typhimurium; pg 217, b. Attenuated Salmonella Typhimurium as a Transfer System for Suicide gene Therapy; pg 218, left col, para 2, ln 24-28	35-36, 40-41, 48, 80-81, 85-86, 93
---		---
Y		9-10, 14-16, 37-39, 42-47, 49-50, 82-84, 87-92, 94-95
Y	US 2003/0199035 A1 (ARNAU et al.), 23 October 2003 (23.10.2003); para [0023], [0107]	5
Y	DE GROOTE et al., Periplasmic superoxide dismutase protects Salmonella from products of phagocyte NADPH-oxidase and nitric oxide synthase. Proc. Natl. Acad. Sci. USA, 9 December 1997, Vol 94, No 25, pp. 13997-14001; Abstract	6-7, 43-44, 88-89
Y	AWAD et al, Virulence studies on chromosomal alpha-toxin and theta-toxin mutants constructed by allelic exchange provide genetic evidence for the essential role of alpha-toxin in Clostridium perfringens-mediated gas gangrene. Molecular Microbiology, January 1995, Vol 15, No 2, pp 191-202; Abstract	11-13, 15-16, 30-31, 46-47, 62-63, 71-72, 91-92
Y	RYAN et al., Use of bacteria in anti-cancer therapies. BioEssays, January 2006, Vol 28, No 1, pp 84-94; Abstract; pg 83, left col, para 2; pg 85, right col, para 2, ln 1-2; pg 86, left col, para 1, right col, para 2, ln 29-40, para 3; pg 87, Table 1; pg 88, left col, para 1-2	17-34, 51-79

☒ 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	Date of mailing of the international search report
5 May 2009 (05.05.2009)	21 MAY 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/34549

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GENESTIER et al., Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. The Journal of Clinical Investigation, November 2005, Vol 115, No 11, pp 3117-3127; Abstract; pg 3122, left col, para 1, ln 6-18	9-10, 14-16, 18-19, 21-22, 24-34, 37-39, 42, 45-47, 59-60, 67-70, 72-79, 82-84, 87, 90-92
Y	PAWELEK et al, Bacteria as tumor-targeting vectors. The Lancet Oncology, September 2003, Vol 4, pp 548-556; pg 551, right col, para 2	49, 94
Y	STASSEN et al., The Streptococcal Exotoxin Streptolysin O Activates Mast Cells To Produce Tumor Necrosis Factor Alpha by p38 Mitogen-Activated Protein Kinase- and Protein Kinase C-Dependent Pathways. Infection and Immunity, November 2003, Vol 71, No 11, pp 6171-6177	23-25, 34, 50, 64, 79, 95
Y	ELI LILLY AND COMPANY, GEMZAR (GEMCITABINE HCl) FOR INJECTION. Eli Lilly and Company, Indianapolis, IN 46285, USA, 7 May 2007; pg 8-10, Pancreatic Cancer	74
Y	ESCUDIER et al., Sorafenib in Advanced Clear-Cell Renal-Cell Carcinoma. N Engl J Med, 11 January 2007, Vol 356, No 2, pp 125-34; Abstract	75
Y	POYART et al., Characterization of superoxide dismutase Gram-positive bacteria by polymerase chain degenerate primers. FEMS Microbiology Letters, 15 August 1995, Vol 131, No 1, pp 41-45; Abstract; pg 44, right vol para 1, ln 19-20; pg 45, left col, para 1, ln 1-2	31, 72