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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))*

— *of inventorship (Rule 4.17(iv))*

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:
24 December 2014

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING ALBUMIN-BINDING ARGININE DEIMINASE FOR CANCER TARGETING TREATMENT

(57) Abstract: The present invention provides a pharmaceutical composition containing albumin-binding arginine deiminase fusion protein (AAD) for treating cancer or other arginine-dependent diseases. The AAD fusion protein can be purified from both soluble and insoluble fractions of crude proteins, it binds to human serum albumin (HSA) and has its high activity with longer half life for efficient depletion of arginine in cancer cells. The specific activities of wild-type ADI and AAD in the present invention are 8.4 and 9.2 U/mg (at physiological pH 7.4), respectively. The AAD used in the present invention can be used in the treatment of various cancers (e.g. pancreatic cancer, leukemia, head and neck cancer, colorectal cancer, lung cancer, breast cancer, liver cancer, nasopharyngeal cancer, esophageal cancer, prostate cancer, stomach cancer & brain cancer) and curing arginine-dependent diseases. The composition can be used alone or in combination with at least one chemotherapeutic agent to give a synergistic effect on cancer treatment and/or inhibiting metastasis.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/20943

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/50 (2014.01)
USPC - 424/94.3
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 9/14, 9/127, 38/50; A61P 35/00, 35/04; C12N 11/08 (2014.01)
USPC: 424/94.3, 450, 484; 435/180; CPC: C12N 9/78, 9/96, 11/08

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)
MicroPatent (US-Granted, US-Applications, EP-A, EP-B, WO, JP, DE-G, DE-A, DE-T, DE-U, GB-A, FR-A); ProQuest; IP.com; Google; Google Scholar; 'arginine deiminase fusion protein,' 'albumin-binding domain,' bind, 'serum albumin'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2010/0303893 A1 (LUO, Y et al.) December 2, 2010; paragraphs [0014], [0022]-[0024], [0028], [0030], [0033], [0046], [0047], [0049], [0051]-[0053], [0059], [0061], [0064], [0065], [0070], [0074]; Claim 61	1-3, 7, 9, 12, 13, 18/1-18/3, 18/7, 18/9, 18/7, 18/12, 18/13, 19/18/1-19/18/3, 19/18/7, 19/18/9, 19/18/12, 19/18/13, 20/19/18/1-20/19/18/3, 20/19/18/7, 20/19/18/9, 20/19/18/12, 20/19/18/13, 27-30 ----- 4-6, 8, 10, 11, 15, 16, 18/4-18/6, 18/8, 18/10, 18/11, 18/15, 18/16, 19/18/4-19/18/6, 19/18/8, 19/18/10, 19/18/11, 19/18/15, 19/18/16, 20/19/18/4-20/19/18/6, 20/19/18/8, 20/19/18/10, 20/19/18/11, 20/19/18/15, 20/19/18/16, 21-26 ----- 14

☒ 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
26 September 2014 (26.09.2014)	22 OCT 2014
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: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/20943

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.:
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:

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.:

Groups I+: Claims 1-4, 4 (in-part), 5 (in-part), 6 (in-part), 7, 8 (in-part), 9-13, 14 (in-part), 15, 16, 18 (in-part), 19 (in-part), 20 (in-part), 21-30, SEQ ID NOs: 36, 40, 46, 47, 50, 52

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.:

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.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0305982 A1 (Jensen, K et al.) Dec. 10, 2009; paragraphs [0118], [0120], [0130], [0163], [0332]	4-6, 18/4-18/6, 19/18/4-19/18/6, 20/19/18/4-20/19/18/6
Y	US 2004/0039179 A1 (MCAULIFFE, J et al.) February 26, 2004; paragraphs [0017], [0019], [0024], [0027], [0033]	8, 18/8, 19/18/8, 20/19/18/8
Y	US 2003/0157091 A1 (HOOGENBOOM, HRJM) August 21, 2003; paragraphs [0279], [0319], [0321]	10, 11, 15, 16, 18/10, 18/11, 18/15, 18/16, 19/18/10, 19/18/11, 19/18/15, 19/18/16, 20/19/18/10, 20/19/18/11, 20/19/18/15, 20/19/18/16, 26
Y	US 2004/0001827 A1 (DENNIS, MS et al.) January 1, 2004; abstract; paragraphs [0108], [0109], [0112]	21-25
A	US 7569384 B2 (ROSEN, CA et al.) August 4, 2009; column 37, Table 2, fusion no. 72	14
A	VAN DEN BERG, MA et al. Genome Sequencing And Analysis Of The Filamentous Fungus Penicillium Chrysogenum. Nature Biotechnology. October 2008, Vol. 26, No. 10; pages 1161-1168. DOI 10.1038/nbt.1498	14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/20943

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

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.

Groups I+: Claims 1-30 are directed toward an albumin-binding arginine deiminase fusion protein, use of the fusion protein in preparation of a medicament, and methods of making the fusion protein.

The albumin-binding arginine deiminase fusion protein will be searched to the extent that the protein encompasses an albumin binding peptide, protein or domain including SEQ ID NO: 46 (albumin-binding domain, ABD without linker amino acid sequence), or an active portion thereof; a linker molecule including SEQ ID NO: 50 (linker 1 amino acid sequence); and an arginine deiminase domain including SEQ ID NO: 36 (arginine deiminase, ABD1 amino acid sequence). It is believed that Claims 1-3, 4 (in-part), 5 (in-part), 6 (in-part), 7, 8 (in-part), 9-13, 14 (in-part), 15, 16, 18 (in-part), 19 (in-part), 20 (in-part) and 21-30 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 36 (arginine deiminase, ABD1 amino acid sequence), 46 (albumin-binding domain, ABD without linker amino acid sequence) and 50 (linker 1 amino acid sequence). Applicant is invited to elect additional albumin binding sequences, linker sequences and/or arginine deiminase sequences to be searched. Additional sequences will searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected albumin binding sequences, linker sequences, and/or arginine deiminase sequences. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NOs: 37 (arginine deiminase, ABD1 amino acid sequence), 47 (albumin-binding domain, ABD without linker amino acid sequence), 51 (linker 1 amino acid sequence).

The inventions of Groups I+ share the technical features including an albumin-binding arginine deiminase fusion protein comprising a first portion comprising one or two components selected from an albumin-binding domain, an albumin-binding peptide or an albumin-binding protein(s) fused to a second portion comprising arginine deiminase to form the albumin-binding arginine deiminase fusion protein such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase and is also able to bind serum albumin; use of the albumin-binding arginine deiminase fusion in preparation of a medicament for treating a cancer, or for treating arginine-dependent diseases in a patient, comprising administering a clinically effective amount of the fusion protein to the patient to reduce the availability of circulating arginine; a method of making the albumin-binding arginine deiminase fusion protein, comprising constructing a fusion gene coding for the albumin-binding arginine deiminase fusion protein, inserting the fusion gene into a vector, inserting the vector into a host organism and expressing a protein including the albumin-binding arginine deiminase fusion protein; a method of making the albumin-binding deiminase fusion protein by intein-mediated protein ligation between the albumin-binding domain, albumin-binding peptide or albumin-binding protein(s) and the second portion comprising arginine deiminase; and a pharmaceutical composition comprising the albumin-binding arginine deiminase fusion protein in a pharmaceutically-acceptable carrier.

-***-Continued on Next Supplemental Page-***-

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

However, these shared technical features are previously disclosed by US 2010/0303893 A1 to Luo, et al. (hereinafter 'Luo') and further in view of US 2004/0001827 A1 (DENNIS) and US 2003/0157091 A1 (HOOGENBOOM). Luo discloses an albumin-conjugated (albumin-conjugated; paragraph [0022]) arginine deiminase (arginine deiminase; paragraph [0014]) fusion protein (fusion protein; paragraph [0023]) comprising a first portion comprising an albumin domain (conjugate proteins including albumin (a first portion an albumin domain); paragraph [0022]) fused to a second portion comprising arginine deiminase (fused to a second portion comprising arginine deiminase; paragraphs [0014], [0023]) to form the albumin-conjugated arginine deiminase fusion protein (to form the albumin-conjugated arginine deiminase fusion protein; paragraphs [0022], [0023]) such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase (the conjugate includes an active arginine deiminase fragment (such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase); paragraph [0030]); use of the albumin-conjugated arginine deiminase fusion in preparation of a medicament (use of the albumin-conjugated arginine deiminase fusion in preparation of a medicament; paragraph [0049]) for treating a cancer (anti-tumor medicament (for treating cancer); paragraph [0049]), or for treating arginine-dependent diseases in a patient (or for treating arginine-dependent diseases in a patient; paragraph [0070]), comprising administering (administering; paragraph [0071]) a clinically effective amount of the fusion protein to the patient (preventing or treating tumors or diseases (a clinically effective amount of the fusion protein to the patient); Claims 61, 63) to reduce the availability of circulating arginine (to reduce the arginine content of a body or tissue (to reduce the availability of circulating arginine); Claim 63); a method of making the albumin-conjugated arginine deiminase fusion protein (a method of obtaining (making) the albumin-conjugated arginine deiminase fusion protein; paragraphs [0022], [0023]), comprising constructing a fusion gene coding for the albumin-conjugated arginine deiminase fusion protein (fusion expression (constructing a fusion gene coding for the albumin-conjugated arginine deiminase fusion protein); paragraphs [0023]), and expressing a protein including the albumin-binding arginine deiminase fusion protein (expressing a protein including the albumin-binding arginine deiminase fusion protein; paragraphs [0022], [0023]); and a pharmaceutical composition (a pharmaceutical composition; paragraph [0065]) comprising the albumin-conjugated arginine deiminase fusion protein (comprising the albumin-conjugated arginine deiminase fusion protein; paragraphs [0022], [0023], [0065]) in a pharmaceutically-acceptable carrier (in a pharmaceutically acceptable carrier; paragraph [0065]); and wherein an active protein may be modified so as to increase its affinity for albumin (an active protein may be modified so as to increase its affinity for albumin; paragraph [0011]).

Luo does not disclose an albumin-binding arginine deiminase fusion protein comprising a first portion comprising one or two components selected from an albumin-binding domain, an albumin-binding peptide or an albumin-binding protein(s) fused to a second portion comprising arginine deiminase to form the albumin-binding arginine deiminase fusion protein such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase and is also able to bind serum albumin; use of the albumin-binding arginine deiminase fusion in preparation of a medicament for treating a cancer, or for treating arginine-dependent diseases in a patient, comprising administering a clinically effective amount of the fusion protein to the patient to reduce the availability of circulating arginine; a method of making the albumin-binding arginine deiminase fusion protein, comprising constructing a fusion gene coding for the albumin-binding arginine deiminase fusion protein, inserting the fusion gene into a vector, inserting the vector into a host organism and expressing a protein including the albumin-binding arginine deiminase fusion protein; a method of making the albumin-binding deiminase fusion protein by intein-mediated protein ligation between the albumin-binding domain, albumin-binding peptide or albumin-binding protein(s) and the second portion comprising arginine deiminase; and a pharmaceutical composition comprising the albumin-binding arginine deiminase fusion protein in a pharmaceutically-acceptable carrier.

Dennis discloses albumin binding peptides fused to biologically active molecules (albumin binding peptides fused to biologically active molecules; abstract), including enzymes (enzymes; paragraph [0024]), to increase the half-life of the product (to increase the half-life of the product; paragraph [0027]) including the use of a linker sequence between the protein and the peptide (including the use of a linker sequence between the protein and the peptide; paragraph [0049]), transfection of a host cell with an expression vector (transfection of a host cell with an expression vector; paragraph [0058]) for expression of the construct in hosts (expression of the construct in hosts; paragraphs [0108], [0109], [0112]).

Hoogenboom discloses generating functional target-binding proteins from at least two separate polypeptide chains by intein-mediated ligation (generating functional target-binding proteins from at least two separate polypeptide chains by intein-mediated ligation; abstract).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Luo regarding an albumin-conjugated or fused arginine deiminase, in order to have integrated an arginine deiminase fused to albumin binding peptides or polypeptides, as previously disclosed by Dennis, for enabling the industrial production of large volumes of the therapeutic protein, while retaining the capacity of the arginine deiminase to bind to albumin, for increasing the half-life of the enzyme in circulation. Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Luo, for producing an albumin-conjugated arginine deimidase, or an arginine deimidase modified with a domain which increases the affinity of the molecule for albumin, as previously disclosed by Luo, through utilization of the intein-mediated ligation methods previously disclosed by Hoogenboom, for producing an active bifunctional protein.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Luo, Dennis and Hoogenboom references, unity of invention is lacking.