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

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(54) Title: TREATMENT OF ANGIOGENESIS DISORDERS

(57) Abstract: This invention concerns pathological angiogenesis and cancer, related treatment methods, and related compositions. Also disclosed are related diagnosis kits and methods.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24697

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/113; A61K 31/7088; C07H 21/00 (2012.01)
USPC - 514/44A; 536/24.5
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): C12N 15/113; A61K 31/7088; C07H 21/00 (2012.01)
USPC: 514/44A; 536/24.5

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)
PubWEST (DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents
Search Terms Used: IGFBP2, MERTK, PITPNC1, metastat$, inhibit$, endothelial recruitment, apoptosis, angiogenesis, carinoma$, tumor$, glioblatoma$, mi-RNA, miR-126

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0286287 A1 (RUSSO et al.) 20 November 2008 (20.11.2008) abstract; para [0016]; [0019]; [0020]; [0008]; [0171]	1-4
Y	BAYES-GENIS et al. "The Insulin-Like Growth Factor Axis: A Review of Atherosclerosis and Restenosis" Circ. Res.; 2000; Vol. 86; pg. 125-130. Entire document, especially abstract; pg. 127, col 2, para 2-3	1-4
A	US 2006/0178329 A1 (Gleave et al.) 10 August 2006 (10.08.2006) esp: abstract, para [0004], [0036], [0075].	1-4
A	Fukushima et al. "Roles of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) in Glioblastoma" ANTICANCER RESEARCH (2007) 27: 3685-3692. entire document.	1-4
X,P	PNG et al. "A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells" Nature; 14 December 2011; Vol. 481 (7380); pg. 190-194. Entire document	1-4
L	PNG et al. "A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells" Nature; 14 December 2011; Vol. 481 (7380); pg. 190-194. Abstract only [online]: available at <http://www.ncbi.nlm.nih.gov/pubmed/22170610> downloaded on 7 August 2012.	1-4

☐ 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
7 August 2012 (07.08.2012)	05 SEP 2012
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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24697

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.: 7, 12, 13, 17, 30-33, 38-42, 51 and 65-67
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.

Groups I+: claims 1-6, directed to a method for inhibiting endothelial recruitment in a subject in need thereof, comprising administering to the subject a first agent that inhibits expression or activity of a first protein selected from the group consisting of: IFGBP2, MERTK and PITPNC1; wherein the first invention is limited to IGFBP2 (claims 1-4) (applicants may opt for additional inhibited proteins to be searched by specifying the protein and paying an additional invention search fee for each elected protein).

- Please see extra sheet for continuation -

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.:
1-4, limited to IGFBP2

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.

Continuation of Box III: Lack of Unity of Invention

Groups II+: claims 8-11, directed to a method for treating metastatic cancer in a subject in need thereof, comprising administering to the subject a first agent that inhibits expression or activity of a first protein selected from the group consisting of: IGFBP2, MERTK and PITPNC1; wherein the first invention is limited to IGFBP2 (claims 8 and 9) (applicants may opt for additional inhibited proteins to be searched by specifying the protein and paying an additional invention search fee for each elected protein).

Groups III+: claims 14 and 15, directed to an isolated nucleic acid comprising a sequence encoding an RNAi agent capable of inhibiting expression of a protein selected from the group consisting of IGFBP2, MERTK, and PITPNC1; wherein the first invention is limited to an RNAi agent to IGFBP2, and its associated sequences (applicants may opt for additional inhibited proteins to be searched by specifying the protein and paying an additional invention search fee for each elected protein).

Groups IV+: claims 16, 18, and 53-64, directed to a composition comprising an agent that inhibits expression or activity of a protein selected from the group consisting of: IGFBP2, MERTK, and PITPNC1, wherein the agent is an antibody, a nucleic acid, a polypeptide, or a small molecule compound and wherein the first agent inhibits angiogenesis; wherein the first invention is limited to IGFBP2. (applicants may opt for additional inhibited proteins to be searched by specifying the protein and paying an additional invention search fee for each elected protein).

Groups V+, claims 19-23, directed to a method and kit for diagnosing a metastatic potential of cancer in a subject, comprising obtaining a first expression level for a first gene of the subject selected from the group consisting of IGFBP2, MERTK, and PITPNC1, and comparing the first expression level with a first predetermined level for the selected first gene, wherein the subject is determined to have or be prone to develop metastatic cancer if the first expression level is greater than the first predetermined level; wherein the first invention is limited to IGFBP2 (claims 19, 20 and 22). (applicants may opt for additional genes to be searched by specifying the gene and paying an additional invention search fee for each elected gene).

Group VI: claims 24-29, directed to a method for inhibiting endothelial recruitment in a subject in need thereof, comprising administering to the subject a first agent that increases expression or activity of GAS6.

Group VII: claims 34-37, directed to a method for treating metastatic cancer in a subject in need thereof, comprising administering to the subject a first agent that increase expression or activity of GAS6, wherein the first agent inhibits angiogenesis.

Group VIII: claims 43-46, directed to a composition comprising an agent that increases expression or activity of GAS6.

Group IX: claim 47, directed to a method for generating a population of mammalian cancer cells with increased metastatic tissue colonization potential, comprising performing serial rounds of transplantation, isolation, and repeat transplantation of a population of labeled or unlabeled cancer cells into a living tissue.

Group X: claims 48, 49 and 52, directed to a method for identifying a gene or a non-coding RNA that regulates metastatic cancer colonization of a body tissue, comprising:
a) introducing one or more shRNA, RNAi, microRNA, or non-coding RNA molecules into a population of starting cancer cells to generate a population of engineered cancer cells;
b) transplanting said population of engineered cancer cells into a tissue of the body;
c) isolating transplanted cells to obtain a population of isolated cancer cells; and
d) assessing the quantity of the shRNA, RNAi, micro RNA, or non-coding RNA molecule or protein encoding gene in the population of isolated cancer cells, wherein a decrease in the quantity of said shRNA, RNAi, microRNA, or non-coding RNA in the population of isolated cells relative to its quantity prior to transplantation indicates that the target gene or genes of said shRNA, RNAi, microRNA, or non-coding RNAs represents a gene required for metastatic colonization of said tissue.

Groups XI: claim 50, directed to a composition comprising a population of metastatic human colon cancer cells with an increased potential to colonize the liver.

The inventions listed as Groups I+ to XI 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:

The special technical features of the claims of Groups I+ to XI are indicated in the Group descriptions, above.

There is no common technical element shared by all of the above Groups.

- Please see second extra sheet for continuation -

Continuation of first extra sheet: Lack of Unity of Invention

Groups I+ to IV+ share the common technical element of being related to an agent that inhibits expression or activity of a first protein selected from the group consisting of: IFGBP2, MERTK and PITPNC1. The claims of Groups II+ are related to a method for treating metastatic cancer in a subject in need thereof, comprising administering to the subject a first agent that inhibits expression or activity of a first protein selected from the group consisting of: IGFBP2, MERTK and PITPNC1. The claims of Groups III+ are related to an isolated nucleic acid comprising a sequence encoding an RNAi agent capable of inhibiting expression of a protein selected from the group consisting of IGFBP2, MERTK, and PITPNC1. The claims of Groups IV+ are related to a composition comprising an agent that inhibits expression or activity of a protein selected from the group consisting of: IGFBP2, MERTK, and PITPNC1, wherein the agent is an antibody, a nucleic acid, a polypeptide, or a small molecule compound and wherein the first agent inhibits angiogenesis. These common technical elements do not represent an improvement over the prior art of the article entitled "Roles of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) in Glioblastoma" by Fukushima et al., which discloses wherein IGFBP2 is upregulated in glioblastoma multiforme (abstract), concomitantly with VEGF, and is linked to angiogenesis, as well as wherein inhibition of expression of IGFBP2 using RNAi directed thereto reduces invasion of GBM cells, suggesting an inhibition of angiogenic activity thereby (pg. 3688, col 1, para 1). Although Fukushima does not specifically refer to the inhibition of the invasiveness of the GBM cells as being inhibition of metastasis, it would have been obvious to a person skilled in the art to use the method taught by Fukushima to inhibit metastases or metastatic cancer, since metastases were well known in the art to be associated with cancer cell invasiveness. Further, although Fukushima does not specifically recite a composition comprising the inhibitory nucleic acid, it would have been obvious to a person skilled in the art to formulate an appropriate composition comprising the inhbitory nucleic acid taught by Fukushima for administration to an animal or human for therapeutic purposes.

Groups I+ and VI are related to endothelial cell recruitment, wherein the claims of Groups I+ are further related to a method for inhibiting endothelial recruitment in a subject in need thereof, comprising administering to the subject a first agent that inhibits expression or activity of a first protein. These common technical elements do not represent an improvement over the prior art of US 2005/0202008 A1 to Williams et al., which discloses wherein "Another potential PRO364 or PRO175 polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation...The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the PRO364 or PRO175 polypeptide (para [0293]). Further wherein The PRO364 or PRO175 polypeptides or agonists or antagonists thereto may also be employed...to stimulate or inhibit migration of endothelial cells (para [0301]) related to angiogenesis (para [0300], [0301]).

The claims of Groups VI-VIII are related to increasing the expression or activity of GAS6. This common technical element does not improve upon the prior art of US 2007/0224174 A1 to Kang et al., which teaches an agent (nucleic acid) for increasing the expression of GAS6 (murine gas6 expression vector; para [0041] - recombinant expression vector phGas6; para [0061]).

Groups II+, VII and IX-XI are related to metastatic cancer, wherein Groups IX-XI are further related to a population of mammalian cancer cells with increased metastatic tissue colonization potential, and transplantation of a population of cancer cells into living tissue. These common technical elements do not represent an improvement over the prior art of US 2005/0032101 A1 to Rio et al., which teaches a cell line demonstrating increased metastatic colonization potential following serial transplantation (para [0086]), wherein the metastatic cells demonstrate enhanced colonization potential for lung, or other tissues (kidney, ovary) in comparison to engineered cancer cells formed by altering the gene expression of a related, non-metastatic cell line derived from the same source as the metastatic cells (para [0113]).

Therefore, the inventions of Groups I+ to XI lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.