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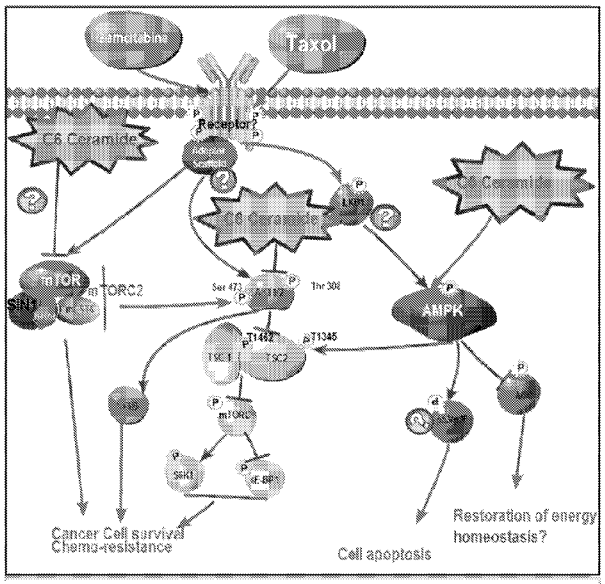
— *with international search report (Art. 21(3))*

[Continued on next page]

(54) **Title**: COMPOSITIONS AND METHODS FOR TREATING CANCER

Figure 6



C6 Ceramide sensitizes Taxol and Gemcitabine induced pancreatic cancer cell death by AMPK activation and PI3K/AKT/mTOR in-activation.

(57) **Abstract**: The disclosure provides compositions and methods for treating cancer using a combination of C6-ceramide and other anti-cancer agents, including a chemotherapeutic agent or an EGFR inhibitor. More specifically, the disclosure provides methods for treating cancer comprising an activating KRAS mutation. Further disclosed are the methods of using an enhancer of the AMPK signaling pathway, an inhibitor of the P13K/AKT signaling pathway, an inhibitor of mTOR signaling pathway, or an inhibitor of the MEK/ERK signaling pathway in the combination therapy.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/127; A61K 39/395; A61K 38/00 (2012.01)
USPC - 424/450; 424/141.1; 514/19.3
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 9/127; A61K 39/395; A61K 38/00; C12N 15/11; A61K 48/00; A61K 31/33; C12N 5/02 (2012.01)
USPC - 424/450; 424/141.1; 514/19.3; 514/44A; 514/44R; 514/183; 435/375

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 9/127; A61K 39/395; A61K 38/00; C12N 15/11; A61K 48/00; A61K 31/33; C12N 5/02 (2012.01) - see keyword below
USPC - 424/450; 424/141.1; 514/19.3; 514/44A; 514/44R; 514/183; 435/375 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: C6-ceramide, N-hexanoylsphingosine, kRAS, K-ras, mutation, G12C, G12A, G12D, G12R, G12S, G12V, G13C, G13D, apoptosis, taxol, paclitaxel, gemcitabine, oxaliplatin, cisplatin, delivery cremophore, alcohol, liposome, cancer, malignant, neoplastic, tumor, lymphoma, leukemia, carcinoma, sarcoma, mammal,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0246271 A1 (Wanebo) 01 October 2009 (01.10.2009), Abstract, para [0005], [0006], [0007], [0009], [0030], [0031], [0033], [0034], [0037], [0040], [0041], [0042], [0043], [0045], [0046], [0047], [0053], [0054], [0055], [0058], [0060], and [0064]	1-15, 20-21
Y	RAY et al. Epithelial Tissues Have Varying Degrees of Susceptibility to KrasG12D-Initiated Tumorigenesis in a Mouse Model. PLoS One. 2011 Feb, Vol. 6(2):e16786. PDF file: pg 1-8. Abstract; pg 3, col 1, last para, and col 2, para 1; pg 4, col 1, para 1; and pg 7, col 1, last para	1-15, 20-21
A	US 2009/0042810 A1 (Chung) 12 February 2009 (12.02.2009), para [0005], [0047], and [0055]	1-15, 20-21
A	JI et al. Exogenous cell-permeable C6 ceramide sensitizes multiple cancer cell lines to doxorubicin-induced apoptosis by promoting AMPK activation and mTORC1inhibition. Oncogene, 2010, Vol. 29(50), p. 6557-68. Abstract	1-15, 20-21
A	VLERKEN et al. Augmentation of Therapeutic Efficacy in Drug-Resistant Tumor Models Using Ceramide Coadministration in Temporal-Controlled Polymer-Blend Nanoparticle Delivery Systems. AAPS J. 2010, Vol.12(2), p. 171-80. Entire documentation, especially Abstract	1-15, 20-21
A	ZHU et al. C6-ceramide synergistically potentiates the anti-tumor effects of histone deacetylase inhibitors via AKT dephosphorylation and a-tubulin hyperacetylation both in vitro and in vivo. Cell Death Dis. 2011 Jan, Vol. 2:e117. pg 1-12. Entire documentation, especially Abstract	1-15, 20-21
A	GUO et al. The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. Proc Natl Acad Sci U S A. 2009, Vol. 106(31), p. 12932-7. Entire documentation, especially Abstract	1-15, 20-21

☒ 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
06 September 2012 (06.09.2012)	21 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

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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.
A	ROTH et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010, Vol. 28(3), p. 466-74. Epub 2009 Dec. Abstract; pg 467, col 2, para 4; and pg 468, Table 1	1-15, 20-21
A	GARASSINO et al. Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. Ann Oncol. 2011 Jan, Vol. 22(1), Vol. 235-7. pg 236, Fig 1; and pg 237, col 1, para 3-5	1-15, 20-21
A	ROOCK et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA. 2010, Vol. 304(16), p. 1812-20. Entire documentation, especially Abstract	1-15, 20-21

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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:
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 1-21, drawn to a method for treating cancer, wherein the method comprises contacting a cancer cell having an activating KRAS mutation with (a) an effective amount of C6-ceramide; and (b) an effective amount of at least one anti-cancer agent, thereby treating cancer. The first named invention (claims 1-15, 20-21), is limited to wherein the anti-cancer agent is a chemotherapeutic molecule, selected from the group consisting of taxol, paclitaxel, gemcitabine, oxaliplatin, and cisplatin (claim 15). Applicant is invited to elect (an) additional anti-cancer agent(s) including: wherein the anti-cancer agent is an inhibitor of EGFR signaling selected from the group consisting of cetuximab, panitumumab, and erlotinib (claims 16-17), or/and wherein the anti-cancer agent is an inhibitor of RAS signaling selected from the group consisting of salirasib, tipifamib, pre-let 7a microRNA, Lin28 siRNA, and an anti-mutant KRAS siRNA (claims 18-19), to be searched by paying additional fee for each election.
**********Continued in the extra sheet**********

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.:
1-15, 20-21

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.

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Continuation of:
Box No III (unity of invention is lacking)

Group II+, claims 22-41, drawn to a method for treating cancer, wherein the method comprises contacting a cancer cell with (a) an effective amount of C6-ceramide; (b) an effective amount of an anti-cancer agent; and (c) an effective amount of an agent selected from the group consisting of an enhancer of the AMPK signaling pathway, an inhibitor of the PI3K/AKT/mTORC1 signaling pathway, and an inhibitor of the MEKIERK signaling pathway, thereby treating cancer. The first named invention (claims 22-35, 38-41) is limited to wherein agent (c) enhances the AMPK signaling pathway by modulating the activity of one or more polypeptides selected from the group consisting of AMPK and ACC (claim 35). Applicant is invited to elect (an) additional agent(s) including: wherein agent (c) inhibits the PI3K/AKT/mTORC1 signaling pathway by modulating the activity of one or more polypeptides selected from the group consisting of PI3K, AKT, Gabl, TSC2, mTORCl, mTORC2, S6K, S6, and 4EBP1 (claim 36), or/and wherein agent (c) inhibits the MEK/ERK signaling pathway by modulating the activity of one or more polypeptides selected from the group consisting of MEK and ERK (claim 37), to be searched by paying additional fee for each election.

The inventions listed as Groups I+-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:

Groups I+ do not include the inventive concept of an effective amount of an agent selected from the group consisting of an enhancer of the AMPK signaling pathway, an inhibitor of the PI3K/AKT/mTORC1 signaling pathway, and an inhibitor of the MEK/ERK signaling pathway, as required by Groups II+.

Groups II+ do not include the inventive concept of a cancer cell having an activating KRAS mutation, as required by Groups I+.

Among Group I+, a chemotherapeutic molecule in claim 15, each is structurally and functionally different from an inhibitor of EGFR signaling in claims 16-17, or an inhibitor of RAS signaling in claims 18-19, and vice versa.

Among Group II+, an agent that enhances the AMPK signaling pathway by modulating the activity of AMPK or ACC in claim 35, each is structurally and functionally different from an agent that inhibits the PI3K/AKT/mTORC1 signaling pathway by modulating the activity of PI3K, AKT, Gabl, TSC2, mTORC1, mTORC2, S6K, S6, or 4EBP1 in claim 36, or an agent that inhibits the MEK/ERK signaling pathway by modulating the activity of MEK or ERK in claim 37, and vice versa.

The inventions of Groups I+ through II+ share the technical feature of treating cancer comprising contacting a cancer cell with (a) an effective amount of C6-ceramide; and (b) an effective amount of at least one anti-cancer agent, thereby treating cancer; the inventions of Group I+ further share the technical feature of a method for treating cancer, wherein the method comprises contacting a cancer cell having an activating KRAS mutation with (a) an effective amount of C6-ceramide; and (b) an effective amount of at least one anti-cancer agent, thereby treating cancer. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2009/0246271 A1 to Wanebo, in view of an article entitled 'Epithelial Tissues Have Varying Degrees of Susceptibility to KrasG12D-Initiated' by RAY et al. (hereinafter 'Ray'; PLoS One. 2011 Feb, Vol. 6(2):e16786. PDF file: pg 1-8) as follows:

Wanebo discloses a method for treating cancer (Abstract - 'a method for treating a subject afflicted with cancer'), wherein the method comprises
--- contacting a cancer cell with (a) an effective amount of C6-ceramide (Abstract - 'contacting the cancer cell with ... C6-ceramide'; para [0031] - 'an "effective amount," when used with respect to ... C6-ceramide'), and
---(b) an effective amount of at least one anti-cancer agent (Abstract - 'contacting the cancer cell with (a) gemcitabine and (b) C6-ceramide, sequentially or concomitantly', wherein 'gemcitabine' is an anti-cancer agent; para [0047] - 'C6-ceramide is first administered and gemcitabine is subsequently administered'; para [0046] - 'gemcitabine is first administered and C6-ceramide is subsequently administered'; para [0031] - 'an "effective amount," when used with respect to the combination of gemcitabine and C6-ceramide'; para [0058] - 'C6 ceramide significantly inhibited tumor growth and enhanced survival of all the major classes of chemotherapeutic agents tested'),
--- thereby treating cancer (para [0058] - 'C6 ceramide significantly inhibited tumor growth and enhanced survival of all the major classes of chemotherapeutic agents tested against the aggressive human pancreatic cancer cell line L3.6 growing in ... SCID mouse'; para [0060] - 'Combination with C6-ceramide augmented the tumor reduction obtained by chemotherapy alone by 57%'; para [0064] - 'Combination therapy with apoptotic signal C6 Ceramide significantly enhanced the anti-tumor regression and survival induced by Oxaliplatin and Gemcitabine'; para [0009] - 'the apoptosis in the subject's cancer cells induced by the combination of gemcitabine and C6-ceramide is greater than the apoptosis in the subject's cancer cells induced by contacting the cancer cells with either gemcitabine alone or C6-ceramide alone, thereby treating the subject afflicted with cancer').

Wanebo further discloses the method can be used for treating different types of cancers (para [0037] - 'the above-mentioned methods, the cancer cell ... is selected from ... a breast carcinoma cell, a melanoma cell, ... a colon carcinoma cell, ... a lung carcinoma cell,... a pancreatic cancer cell').

Wanebo does not specifically teach wherein the cancer cells having an activating KRAS mutation. Ray discloses activating KRAS mutation induces tumorigenesis in different types of cancers (Abstract - 'Activating mutations in the Kras gene are commonly found ... epithelial cancers. ...we introduced one of the most common Kras mutations, KrasG12D, broadly in epithelial tissues. ... controlled by inducible, Cre-mediated recombination ?. many hyperplasias, metaplasias and adenomas were observed in the oral cavity, stomach, colon and lungs, suggesting that exposure to products of the outside environment promotes KrasG12D-initiated tumorigenesis'; pg 3, col 1, last para - 'KrasG12D mutation induced lung adenoma formation'; pg 4, col 1, para 1 - 'KrasG12D mutation induced metaplasia in the colon...capable of giving rise to serrated carcinoma in a mouse model').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Wanebo and Ray, to obtain a method for treating cancer, wherein the method comprises contacting a cancer cell with (a) an effective amount of C6-ceramide; and (b) an effective amount of at least one anti-cancer agent, thereby treating cancer, as taught by Wanebo, and further wherein the cancer cells having an activating KRAS mutation, based on the combination of Ray and Wanebo, in order to use the method of Wanebo for treating different types of cancers in combination of monitoring the KRAS mutation for better understanding the mechanism of different types of cancer formation for facilitating choosing the combination therapy agents comprising C6-ceramide for achieving a synergistic effect on treating different types of cancers. Without a shared special technical feature, the inventions lack unity with one another.

**********Continued in the next extra sheet**********

Continuation of:
The previous extra sheet - Box No III (unity of invention is lacking)

The inventions of Groups II+ further share the technical feature of contacting a cancer cell with an additional agent for modulating a specified signaling pathway including enhancing the AMPK signaling pathway by modulating the activity of AMPK. However, this shared technical feature does not represent a contribution over prior art. Specifically, US 2009/0042810 A1 to Chung discloses a method for treating cancer comprising administering an AMPK activator (para [0047] - 'that AMPK as an important target of cancer treatment .... provides for the use of activators of AMPK (e.g., meformin) to treat cancer'; para [0055] - 'a patient is administered an AMPK activator (e.g., metformin or derivative thereof) to treat a ... cancer'; para [0005] - 'an AMPK-activating drug... comprising ... metformin... AICAR'). One of ordinary skill in the art at the time the invention was made would have known to combine the teaching of Wanebo, discussed above, with the teaching of Chung by including one or more agent that modulating a specified signaling pathway such as administering an AMPK activator in a combination therapy for enhancing the AMPK signaling pathway for further achieving a synergistic effect on treating different cancers based on the combination of Chung and Wanebo. Furthermore, an article entitled 'Exogenous cell-permeable C6 ceramide sensitizes multiple cancer cell lines to doxorubicin-induced apoptosis by promoting AMPK activation and mTORC1inhibition' by JI et al. (Oncogene, 2010, Vol. 29(50), p. 6557-68) discloses activation of AMPK by AMPK activator AICAR enhances Doxorubicin-induced cancer cell death, which is further enhanced by C6-ceramide in multiple progressive cancer cell lines (Abstract - 'exogenous cell-permeable C6 ceramide may be a useful adjunct to the anti-tumor effects of chemotherapeutic agents (such as Taxol) against multiple cancers. ... C6 ceramide largely sensitizes multiple progressive cancer cell lines to Doxorubicin-induced cell death and apoptosis. ... Doxorubicin induces AMP-activated protein kinase (AMPK) activation.... Activation of AMPK contributes to Doxorubicin-induced cancer cell death and apoptosis.... AMPK activator AICAR enhances it.... C6 ceramide largely enhances Doxorubicin-induced activation of AMPK, which leads to mTOR complex 1 inhibition and chemo-sensitization'). Without a shared special technical feature, the inventions lack unity with one another.

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