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(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, CL, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: CROWN-LIKE STRUCTURES AS A BIOMARKER FOR CANCER RISK AND CANCER PROGNOSIS

(57) Abstract: Chronic inflammation increases the risk of several epithelial malignancies. The present invention provides methods for determining cancer risk in a patient which comprises detecting the presence of crown-like structures (CLS), methods for treating cancer associated with CLS presence, methods for determining cancer risk in a patient by quantifying the number of CLS in a sample of adipose tissue of the patient, methods of determining prognosis of a patient with breast cancer or other cancers by detecting CLS, and screening methods for anti-cancer agents or agents that inhibit or reduce CLS formation or consequences thereof.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24777

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/52; G01N 1/30 (2012.01)

USPC - 436/64, 435/40.52

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- 436/64, 435/40.52

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 436/63, 435/4, 29, 40.5, 40.51 (keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google Patents; Google Scholar:
Crown-like structures, necrotic adipose tissue macrophages, head and neck carcinoma, adipocyte, dead adipocytes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y*	US 2009/0258025 A1 (Godowski et al.) 15 October 2009 (15.10.2009) para [0014], [0016], [0132], [0148], [0166], [0307], [0325]	1-33, 53-55
Y	US 2010/0239589 A1 (Woods et al.) 23 September 2010 (23.09.2010) para [0015], [0073], [0132], Fig. 5D, 5F	1-33, 53-55
Y	Rutkowski et al. 'Mechanisms of obesity and related pathologies: the macro- and microcirculation of adipose tissue' FEBS J vol 276 pg 5738-5746 (2009) Author Manuscript pg 1-13; pg 4, para 2,	1-8, 10, 17-25, 53/(2,19), 54-55
Y	Kusmartsev et al. 'Role of immature myeloid cells in mechanisms of immune invasion in cancer' Cancer Immunol Immunother vol 55 pg 237-245 (2006) Author Manuscript pg 1-13; abstract, pg 8, para 1	5-6, 13-14, 22-23, 54-55

☒ 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

27 July 2012 (27.07.2012)

Date of mailing of the international search report

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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	Park et al. 'Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression' Cell vol 140 pg 197-208, 22 January 2010 (22.01.2010), Author Manuscript pg 1-23, abstract	7, 15, 24
Y	US 2006/0030608 A1 (Nelson et al.) 09 February 2006 (09.02.2006) para [0006]-[0008]	27, 32
Y	Kolak et al. 'Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity' Diabetes vol 56 pg 1960-1968 (2007) abstract	53(11,29), 53(2,19)
A	Murano et al. 'Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice' J Lipid Research vol 49 pg 1562-1568 (2008) entire doc.	1-33, 53-55

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24777

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.: 51-52
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 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.: 1-33, 53-55

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/US 12/24777

Continuation of:

Box NO III. Observations where unity of invention is lacking

Group I: claims 1-33, 53-55, drawn to a method for determining cancer risk or prognosis in a patient which comprises detecting the presence of crown-like structures (CLS) in adipose tissue in a tissue sample from said patient, said adipose tissue being in or around an epithelial-derived tissue or organ or said adipose tissue being associated with a lymph node metastasis arising from an epithelial-derived cancer, wherein the presence of CLS indicates poor prognosis or an increased cancer risk.

Group II: claims 34-50, drawn to a method to screen a compound for anti-cancer activity.

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:

The inventions of Group II do not include the inventive concept of a method for determining cancer risk or prognosis in a patient by detecting the presence of crown-like structures (CLS) in adipose tissue in a tissue sample from said patient, said adipose tissue being in or around an epithelial-derived tissue or organ or said adipose tissue being associated with a lymph node metastasis arising from an epithelial-derived cancer, wherein the presence of CLS indicates poor prognosis or an increased cancer risk, as required by Group I.

The inventions of Group I do not include the inventive concept of a method to screen a compound for anti-cancer activity, as required by Group II.

In addition, crown-like structures (CLS) in adipose tissue were known in the art at the time of the invention, as evidenced by a paper titled "Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically-obese mice" by Murano, et al. (J Lipid Res 2008, 49:1562-1568) (hereinafter "Murano") (Abstract, "We previously showed that .90% of macrophages infiltrating the adipose tissue of obese animals and humans are arranged around dead adipocytes, forming characteristic crown-like structures (CLS). In this study we quantified CLS in visceral and subcutaneous depots from two strains of genetically obese mice, db/db and ob/ob").

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