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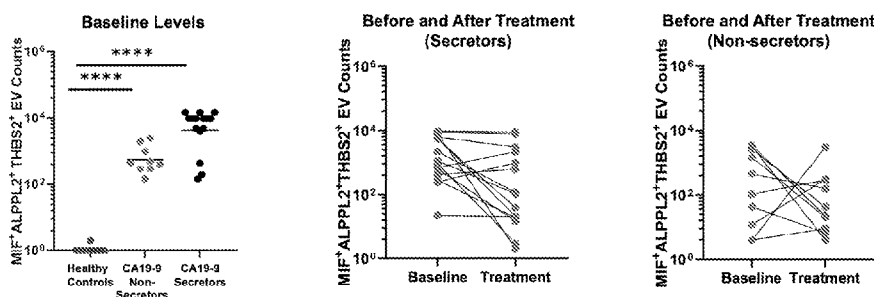


FIG. 6B

(57) Abstract: The disclosure concerns methods and kits of diagnosis of pancreatic cancer and monitoring disease burden in patients diagnosed with pancreatic cancer, the method comprising quantitative determination of the concentration of extracellular vesicles that are positive for one, two or three markers selected from thrombospondin-2 (THBS2), alkaline phosphatase placental-like 2 (ALPPL2), and macrophage migration inhibitory factor (MIF) in the patients' fluid samples.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/73989

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G01N 33/574 (2023.01)

ADD. G01N 33/68, A61P 35/00, C07K 14/47, C07K 14/705 (2023.01)

CPC - INV. G01N 33/57438

ADD. G01N 33/57488, G01N 33/6854, C07K 14/705

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/010919 A2 (CORNELL UNIVERSITY) 13 January 2022 (13.01.2022) Claim 1; Claim 11; para [0009]; [0064]; [0070]; [0071]; [0085]; [0086]; [0105]; [0120]; [0124]; [0159]; [0162]; Table 5; [0182]; [0231]; [0235]	1, 13-15, 18, 29
A	WO 2021/211460 A1 (CITY OF HOPE) 21 October 2021 (21.10.2021) Abstract	1, 13-15, 18, 29

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/73989

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.: 6-12, 16-17, 21-23, 27-28
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:

----Please see continuation in first 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.:
claims 1, 13-15, 18, 29, limited to THBS2

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 23/73989

Continuation of 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 searched, the appropriate additional search fees must be paid.

Group I+, Claims 1, 13-15, 18-20, 29, directed to a method of diagnosing pancreatic cancer or monitoring a disease burden in a patient already diagnosed with pancreatic cancer, and use of one or more markers in diagnosing pancreatic cancer. The method and use will be searched to the extent that the method and use encompasses quantitatively determining the concentration of a single selected marker in the sample, the marker being selected from the group consisting of: (i) thrombospondin-2 (THBS2) positive extracellular vesicles (note, this is the first claimed marker for the inventive method and use). The first named invention was determined based on the first claimed marker (claim 1) for the inventive method and use embodiment. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1, 13-15, 18, 29 encompass this first named invention, and thus these claims will be searched without fee to the extent that the method and use encompasses quantitatively determining the concentration of a single selected marker in the sample, the marker being selected from the group consisting of: (i) thrombospondin-2 (THBS2) positive extracellular vesicles. Additional method(s) and use(s) comprising additional marker(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected method(s) and use(s) comprising additional marker(s). 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. An exemplary election would be a method and use comprising quantitatively determining the concentration of a single selected marker in the sample, the marker being selected from the group consisting of: (i) macrophage migration inhibitory factor (MIF) positive extracellular vesicles (claims 1, 13-15, 20, 29).

Group II+, claims 2-5, 24-26, 30, directed to a method of treating pancreatic ductal adenocarcinoma in a patient, determining continuation of pancreatic cancer treatment or dosage of pancreatic cancer treatment agent, and use of one or more markers in the treatment of pancreatic cancer by surgery. Group II+ will be searched upon payment of additional fees. The method and use may be searched, for example, to encompass quantitatively determining the concentration of a single selected marker in the sample, the marker being selected from the group consisting of: (i) thrombospondin-2 (THBS2) positive extracellular vesicles for an additional fee and election as such. It is believed that claims 2-5, 24-25, 30 read on this exemplary invention. Additional method(s) and use(s) comprising additional marker(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected method(s) and use(s) comprising additional marker(s). 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. Another exemplary election would be method and use comprising quantitatively determining the concentration of a single selected marker in the sample, the marker being selected from the group consisting of: (i) macrophage migration inhibitory factor (MIF) positive extracellular vesicles (claims 2-5, 24-25, 30).

The inventions listed as Groups I+ and II+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of a method of diagnosing pancreatic cancer, that is not required by Group II+. Group I+ does not require any treatment of a patient such as by administering neoadjuvant therapy or by surgical resection, required for Group II+.

Group II+ has the special technical feature of treating pancreatic cancer in a patient, that is not required by Group I+. Group II+ does not require diagnosing a patient, required for Group I+.

Common technical features

Groups I+ and II+ share the technical features of including:

- a) obtaining a sample from the patient, wherein the sample is selected from the group consisting of: plasma, serum, and pancreatic ductal fluid;
- b) quantitatively determining the concentration of one or more selected markers in the sample, the markers being selected from the group consisting of: (i) one or both of thrombospondin-2 (THBS2) positive extracellular vesicles and macrophage migration inhibitory factor (MIF) extracellular vesicles or (ii) a combination of alkaline phosphatase placental-like 2 (ALPPL2) positive extracellular vesicles and THBS2 positive extracellular vesicles or (iii) a combination of ALPPL2 positive extracellular vesicles and MIF positive extracellular vesicles or (iv) a combination of ALPPL2 positive extracellular vesicles, THBS2 positive extracellular vesicles, and IF positive extracellular vesicles;
- c) comparing the quantitatively determined concentration of the one or more selected markers to a healthy control value or the patient's past concentration of the selected marker.

However, these shared technical features are previously disclosed by prior art, as discussed below.

Continuation of Box No. III. Observations where unity of invention is lacking.

Group I+ inventions additionally share the technical features of including:

method of monitoring appearance, disappearance, regression, or progression of disease in patients diagnosed with or being screened for pancreatic cancer, the method comprising:

- a) obtaining a sample from the patient, wherein the sample is selected from the group consisting of: plasma, serum, and pancreatic ductal fluid;
- b) quantitatively determining the concentration of EVs positive for one or more selected markers in the sample, the markers being selected from the group consisting of: (i) one or both of thrombospondin-2 (THBS2) positive extracellular vesicles and macrophage migration inhibitory factor (MIF) positive extracellular vesicles or (ii) a combination of alkaline phosphatase placental-like 2 (ALPPL2) positive extracellular vesicles and THBS2 positive extracellular vesicles or (iii) a combination of ALPPL2 positive extracellular vesicles and MIF positive extracellular vesicles or (iv) a combination of ALPPL2 positive extracellular vesicles, THBS2 positive extracellular vesicles, and IF positive extracellular vesicles;
- c) comparing the quantitatively determined concentration of the one or more selected markers to a healthy control value or the patient's past concentration of the selected marker; and
- d) monitoring appearance, disappearance, regression, or progression of disease in patients diagnosed with or being screened for pancreatic cancer based on the comparison of step c), wherein a concentration above the healthy control value or the patient's past concentration is indicative of the appearance or progression of pancreatic cancer.

Group II+ inventions additionally share the technical features of including:

a method of treating pancreatic ductal adenocarcinoma in a patient, the method comprising:

- a) obtaining a first sample from the patient before neoadjuvant therapy wherein the sample is selected from the group consisting of: plasma, serum, and pancreatic ductal fluid;
- b) quantitatively determining the concentration of one or more selected markers in the first sample, the markers being selected from the group consisting of: (i) one or both of thrombospondin-2 (THBS2) positive extracellular vesicles and macrophage migration inhibitory factor (MIF) dual positive EV extracellular vesicles or (ii) a combination of alkaline phosphatase placental-like 2 (ALPPL2) positive extracellular vesicles and THBS2 positive extracellular vesicles or (iii) a combination of ALPPL2 positive extracellular vesicles and MIF dual positive EV extracellular vesicles or (iv) a combination of ALPPL2 positive extracellular vesicles, THBS2 positive extracellular vesicles, and IF positive extracellular vesicles;
- c) administering neoadjuvant therapy to the patient;
- d) obtaining a second sample from the patient after administration of the neoadjuvant therapy;
- e) quantitatively determining the concentration of EVs positive for the one or more selected markers in the second sample;
- f) comparing the quantitatively determined concentration of EVs positive for the one or more selected markers between the first sample and the second sample; or
- d) administering to the patient a neoadjuvant therapy;
- e) assessing the extent of pancreatic cancer in the patient using computed tomography (CT) or ultrasound of the patient's chest, abdomen, and pelvis after neoadjuvant therapy, thereby identifying a surgery plan for the patient; and
- g) resecting the patient's pancreatic tumor when the concentration of the one or more selected markers is decreased in the second sample compared to the first sample thereby treating the patient's pancreatic ductal adenocarcinoma; or
- (b) correlating the amount of the one or more markers with treatment effectiveness by comparing amount of the one or more selected markers to a healthy control value or the patient's past concentration of the selected marker.

However, these shared technical features are made obvious by WO 2022/010919 A2 to Cornell University, (hereinafter 'Cornell Univ').

Cornell Univ teaches a) obtaining a sample from the patient, wherein the sample is selected from the group consisting of: plasma, serum, and pancreatic ductal fluid (Claim 11 - 'The method of claim 1, wherein the liquid biopsy sample is selected from the group consisting of whole blood, blood serum, blood plasma, urine, saliva, sputum, breast milk, ascites fluid, synovial fluid, amniotic fluid, semen, cerebrospinal fluid, follicular fluid and tears.');

b) quantitatively determining the concentration of one or more selected markers in the sample, the markers being selected from the group consisting of: (i) one or both of thrombospondin-2 (THBS2) positive extracellular vesicles and macrophage migration inhibitory factor (MIF) extracellular vesicles or (ii) a combination of alkaline phosphatase placental-like 2 (ALPPL2) positive extracellular vesicles and THBS2 positive extracellular vesicles or (iii) a combination of ALPPL2 positive extracellular vesicles and MIF positive extracellular vesicles or (iv) a combination of ALPPL2 positive extracellular vesicles, THBS2 positive extracellular vesicles, and IF positive extracellular vesicles (para [0009] - 'In another aspect, the present disclosure is directed to a method for screening a subject for the presence of cancer that involves obtaining a tissue sample from a subject. Extracellular vesicles and particles are separated from the tissue sample, and protein from the separated extracellular vesicles and particles is isolated to form an extracellular vesicle and particle protein sample. The extracellular vesicle and particle protein sample is subjected to a detection assay suitable for detecting: (i) a protein selected from the group consisting of thrombospondin 2'); c)

c) comparing the quantitatively determined concentration of the one or more selected markers to a healthy control value or the patient's past concentration of the selected marker (para [0064] - 'FIG. 10A is a heatmap showing proteins exclusively found in more than 30% of PaCa patient plasma-derived EVP samples but never found in 28 healthy control plasma-derived EVP samples (left)'; para [0070] - 'FIG. 16 is heat map showing proteins expressed in plasma exosomes of patients having various stages of pancreatic lesions including benign cysts, intraductal papillary mucinous neoplasm (IPMN), and pancreatic ductal adenocarcinoma (PDAC).');

Cornell Univ further teaches determining a treatment regimen and resection of tumor with curative intent (Claim 97 - 'A method of determining a treatment regimen for a subject having a tumor, said method comprising: obtaining, from the subject having the tumor, a biopsy of tumor tissue and a biopsy of tissue adjacent to the tumor; separating extracellular vesicles and particles from the obtained samples; isolating protein from the separated extracellular vesicle and particles to form extracellular vesicle and particle protein samples; subjecting the extracellular vesicle and particle protein samples to a detection assay suitable for detecting proteins differentially expressed in exosomes from the tumor tissue versus adjacent, non-tumor tissue; and treating said subject based on said subjecting.'; para [0296] - 'Tissue samples. Fresh tumor and peritumoral adjacent tissue were collected from patients with localized PaCa undergoing resection with curative intent (either pancreaticoduodenectomy or distal pancreatectomy) at MSKCC.');

Continuation of Box No. III. Observations where unity of invention is lacking.

Cornell Univ further teaches utility of exosome biomarkers for prognosis, and for monitoring treatment responses (para [0333] - 'Liquid biopsies based on simple blood tests show promise as non- invasive approaches for early detection, differentiating tumor type, and for monitoring treatment responses. Circulating EVs, present in the order of billions in blood plasma and other bodily fluids, could represent an essential component of the liquid biopsy test on which clinical care decisions would be based'; para [0004] - 'As such, characterization of EVP proteins obtained from blood liquid biopsies can offer valuable information for cancer diagnosis, prognosis and for monitoring therapeutic outcomes.').

Cornell Univ does not expressly teach c) administering neoadjuvant therapy to the patient;

d) obtaining a second sample from the patient after administration of the neoadjuvant therapy;

e) quantitatively determining the concentration of EVs positive for the one or more selected markers in the second sample;

f) comparing the quantitatively determined concentration of EVs positive for the one or more selected markers between the first sample and the second sample; or

d) administering to the patient a neoadjuvant therapy;

e) assessing the extent of pancreatic cancer in the patient using computed tomography (CT) or ultrasound of the patient's chest, abdomen, and pelvis after neoadjuvant therapy, thereby identifying a surgery plan for the patient; and

g) resecting the patient's pancreatic tumor when the concentration of the one or more selected markers is decreased in the second sample compared to the first sample thereby treating the patient's pancreatic ductal adenocarcinoma; or

(b) correlating the amount of the one or more markers with treatment effectiveness by comparing amount of the one or more selected markers to a healthy control value or the patient's past concentration of the selected marker. However, since administering neoadjuvant therapy to the patient was a well known treatment for prostate cancer, and Cornell Univ teaches use of exosome biomarkers for monitoring and prognosis, and Cornell Univ teaches the subject could be undergoing treatment for cancer (Claim 25 - 'The method of claim 15, wherein the subject is undergoing treatment for cancer.'), it would have been obvious to one of ordinary skill in the art that the method of Cornell Univ could be used to compare the exosome biomarker level in patient sample before and after administration of treatment, to determine efficacy of neoadjuvant treatment. Additionally, it would have been obvious that the assessment of disease could be based on using computed tomography (CT) or ultrasound of the patient's chest, abdomen, and pelvis after neoadjuvant therapy, thereby identifying a surgery plan for the patient according to established clinical practice for prostate cancer.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

Continuation of item 4 above: claims 6-12, 16-17, 21-23, 27-28 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).